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Neutralizing *ebolavirus*: structural insights into the envelope glycoprotein and antibodies targeted against it

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The *ebolavirus* (EBOV) envelope glycoprotein (GP) is solely responsible for viral attachment to, fusion with, and entry of new host cells, and consequently is a major target of vaccine design efforts. Recently determined crystal structures of key antibodies in complex with their EBOV epitopes have provided insights into the molecular architecture of GP and defined likely hotspots for viral neutralization. In this review, we discuss the structural basis for antibody-mediated neutralization of *ebolavirus* and its implications for novel therapeutic or vaccine strategies.

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Introduction

Ebolavirus (EBOV) is a filamentous, pleiomorphic virus in the family *filoviridae*. Infection with *ebolavirus* causes a severe hemorrhagic fever, with 50–90% lethality. Disturbingly, outbreak frequency has increased fourfold in the past decade. Five different species of *ebolavirus* have been identified: *Zaire*, *Sudan*, *Cote d'Ivoire*, *Reston* and *Bundibugyo*, each named after the location in which the species was first described. All species are lethal to humans, with the possible exception of the rare *Cote d'Ivoire* species, for which only a single human case has been reported, and the *Reston* species, which thus far, appears to be non-pathogenic to humans [1,2]. Among these species, *Zaire ebolavirus* is the most common and the most lethal.

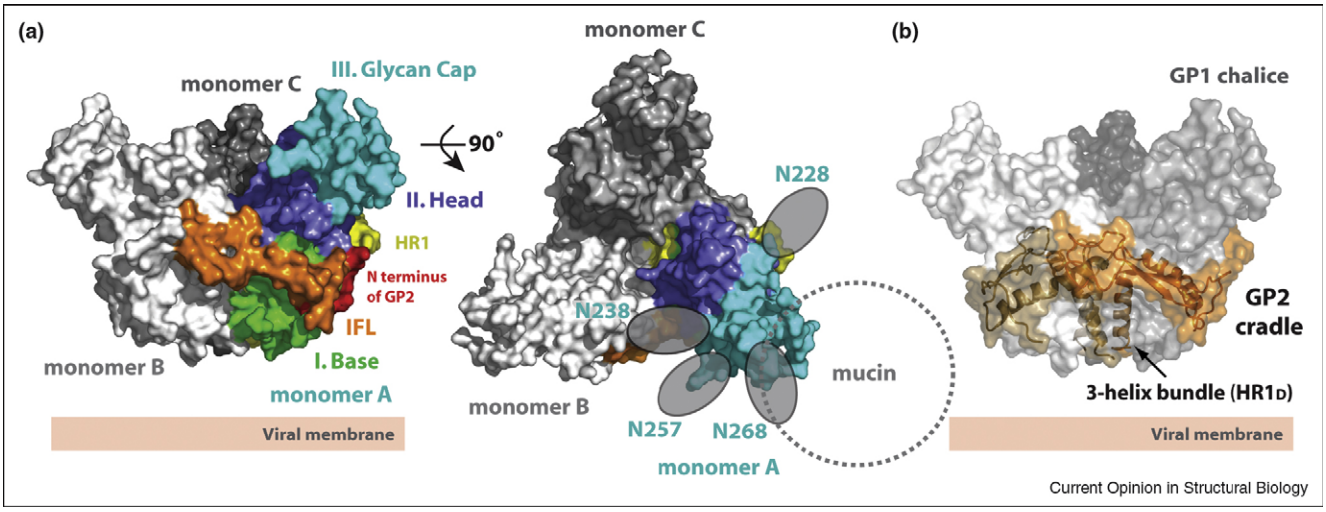
The negative-stranded genome of *ebolavirus* encodes just seven genes. However, the fourth gene, *GP*, actually encodes two unique proteins: a non-structural, dimeric secreted glycoprotein, termed sGP, and a trimeric, virion-attached, envelope glycoprotein, termed GP. These two glycoproteins share the first 295 amino acids, but have unique C termini as a result of transcriptional editing. The unique C termini confer different patterns of disulfide bonding, different structures, and different roles in pathogenesis. Approximately 80% of the mRNA transcripts direct synthesis of sGP [3], which is secreted abundantly early in infection [4]. The remaining 20% of the mRNA transcripts direct synthesis of GP. The unique C terminus of GP encodes a heavily glycosylated mucin-like domain, a transmembrane region, and a short cytoplasmic tail. Trimeric GP is thus embedded in the viral surface, in contrast to the secreted sGP. Indeed, GP is the only virally encoded protein on the virion surface, and is solely responsible for recognition and entry of new host cells [5–7].

Survival of *ebolavirus* infection appears to depend on the ability of the host to mount an early and strong immune response. Studies in three separate outbreaks suggest that fatal infection is associated with a poor immune response as measured by low levels of interferon-γ, CD8+ T cells and antibodies [8,9]. By contrast, non-fatal cases have been associated with a strong inflammatory response and higher levels of antibody [8–11]. Furthermore, in a murine model, short-term control of the virus can be achieved by CD8+ T cells alone, but long-term control requires the presence of antibodies and CD4+ T cells [12].

Development of neutralizing antibodies in the context of natural infection may be difficult. Even those people that survive *ebolavirus* infection often have low to insignificant titers of such antibodies [7,10]. It has been suggested that sGP and shed GP may act as decoys by binding to any neutralizing antibodies [4,13,14]. Indeed, antibodies found in survivor sera appear to preferentially recognize secreted sGP over virion surface GP [15•]. Antibodies specific to sGP are probably non-neutralizing, as they do not recognize the virus itself. Antibodies that cross-react between sGP and GP may neutralize, but may not be as effective *in vivo*, as they may be absorbed by the much more abundant sGP. It is possible that those antibodies specific for viral surface GP may offer the best protection, and hence, structural analysis of GP-specific epitopes has a particular importance.

It is clear that when such antibodies are elicited by vaccination, they do neutralize *ebolavirus in vitro* and contribute to protection against lethal *ebolavirus* challenge [16–19]. Further, transfer of sera containing neutralizing antibodies has, anecdotally, conferred some protection,

Figure 1



Overall structure of EBOV GP. **(a)** Molecular surface of the GP trimer viewed on its side and down its threefold axis. Monomer A is colored according to its subdomains: GP1 base, green; GP1 head, blue; GP1 glycan cap, cyan; GP2 N terminus, red; GP2 internal fusion loop, orange; and GP2 HR1, yellow. **(b)** Molecular surface of the EBOV GP chalice and cradle. Three lobes of GP1, shown in shades of gray, form the GP chalice, and three subunits of GP2 (orange) wrap around the base of the chalice to form the cradle. Adapted from [22••].

but other explanations for recipients' survival have also been proposed [20,21]. It is not yet clear which epitopes on GP (or sGP) are targeted by these successful polyclonal sera. However, several monoclonal antibodies against GP have been described. Completion of the crystal structure of *ebolavirus* GP has now provided a framework for analysis of the epitopes of these monoclonal antibodies, and has suggested new epitopes that could be targeted in immunotherapeutic development [22••]. In this review, we describe the structural basis of antibody recognition of trimeric *Zaire ebolavirus* GP and we mapped known epitopes across its surface.

Overall EBOV glycoprotein structure

The *ebolavirus* glycoprotein (EBOV GP) is synthesized as a 676 amino acid precursor that is post-translationally cleaved by furin to yield two subunits, termed GP1 and GP2. The two subunits remain covalently attached through a disulfide bond between Cys53 in GP1 and Cys609 in GP2. GP1 is responsible for viral attachment and contains the putative receptor-binding site, as well as a heavily glycosylated mucin-like domain. GP2 contains the protein machinery responsible for the fusion of the viral and host cell membranes as well as a hydrophobic internal fusion loop and two heptad repeat regions (HR1 and HR2). After post-translational modification, each EBOV GP monomer (a complex between GP1 and GP2) is ~150 kDa in size. Three monomers oligomerize to form a non-covalently attached trimer (~450 kDa) on the viral surface. During infection, the metastable, prefusion conformation of GP transforms into a low energy, stable, six-helix bundle, post-fusion conformation. The post-fusion, six-helix bundle structure of GP2 was crystallographically defined in 1998 [23,24].

We have recently determined the crystal structure of the prefusion conformation of *ebolavirus* GP. Here, trimeric GP was crystallized [25] in complex with a neutralizing antibody derived from a human survivor of the 1995 Kikwit, Zaire outbreak [22••]. The overall EBOV GP trimer adopts a chalice-like shape (95 × 95 × 70 Å), composed of three non-covalently attached monomers (A, B and C) (Figure 1a). In the trimer, the three GP1 subunits together form a bowl-like chalice and the three GP2 subunits wrap around GP1 to form a cradle (Figure 1b).

EBOV GP1 can be divided into three subdomains: (I) base, (II) head and (III) glycan cap (Figure 1a). The base subdomain (I) forms a hydrophobic concave surface that clamps GP2, probably preventing the GP2 $HR1_A$ helix from springing into its fusion-active state prematurely. The head subdomain (II), centrally located between the base and glycan cap, contains the putative receptor-binding site (RBS). This subdomain forms a four-stranded, mixed β-sheet flanked by an α-helix and a smaller, two-stranded anti-parallel β-sheet. Two intramolecular disulfide bonds stabilize the head subdomain. The glycan cap (III) is furthest from the viral surface (closest to the target host cell) and contains four clustered *N*-linked glycosylation sites (N204, N238, N257 and N268) in an α/β dome over the GP1 head subdomain.

Our structure, combined with carbohydrate sequence analysis, predict that the 12 clustered glycans, of the