

Human antibody pieces together the puzzle of the trimeric Lassa virus surface antigen

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The envelope glycoprotein spike is the sole antigen on the Lassa virus surface and, as such, constitutes the focal point of the host neutralizing immune response. A recent high resolution structure of the trimeric Lassa virus glycoprotein in an antibody-bound form illuminates the molecular architecture of the antigen and reveals the mode of action of the most abundant class of Lassa-specific human neutralizing antibodies.

The World Health Organization has included Lassa virus (LASV), a rodent-borne haemorrhagic fever agent, in the list of top emerging diseases likely to cause severe outbreaks in the near future¹. LASV is an arenavirus responsible for thousands of deaths each year in western Africa and there is a paucity of medical countermeasures to treat infection. The envelope-displayed LASV glycoprotein spike complex (GPC) drives host cell entry and constitutes a key target for antiviral and vaccine development. Each protomer of the mature trimeric spike consists of a membrane-inserted stable signal peptide (SSP), a host cell receptor attachment subunit (GP1), and a transmembrane fusion subunit (GP2)². A major consideration for immunogen and antiviral design is the higher order assembly of this multi-subunit complex. Until now, the construction and characterization of an intact GPC ectodomain has been hindered by metastability of the complex, a property important for driving energetically favourable conformational changes within and between non-covalently associated subunits during pH-mediated host cell entry.

In a manner reminiscent of the genetic stabilization and enhancement of proteolytic maturation of the HIV-1 envelope glycoprotein³, Hastie *et al.*⁴ have executed a comprehensive mutagenesis approach to lock the LASV GPC ectodomain in a pre-fusion conformation, creating a soluble homogeneous sample suitable for structural analysis. This approach includes the introduction of a disulphide bond to covalently link the GP1 and GP2 subunits, a ‘helix-breaking mutation’ (E329P) in the GP2, and the replacement of the native site-1 protease cleavage site between the GP1 and GP2 subunits with a RRRR furin site to facilitate efficient processing of the overexpressed protein. Although, this so-called ‘GPCysR4’ construct is recognized by human-derived neutralizing antibodies specific to the pre-fusion GP1-GP2 complex, unlike the optimized HIV-1 construct³, the resulting LASV GPCysR4 only forms monomers of the GP1-GP2 heterodimer in solution and not the native trimer.

Hastie *et al.*⁴ circumvent this conundrum by piecing the trimeric LASV GPC ectodomain together using a multi-subunit-targeting Fab fragment from a recently isolated human neutralizing antibody (37.7H)⁵. Consistent with a previous low resolution electron microscopy analysis of virion-displayed GPC⁶, the ensuing crystallographic investigation confirms that the heavily glycosylated LASV glycoprotein spike forms a tripodal organization (**Fig. 1**). Interestingly, Hastie’s high resolution analysis also reveals features not observed in the glycoproteins of viruses which also have class-1 fusion machinery, such as Ebola virus, HIV-1, and Influenza virus⁷⁻⁹. For example, LASV GPC lacks a central three-helix fusion core and the GP1 subunits contribute extensively to the trimeric interface. This structure thus not only clarifies the higher-order assembly of arenaviral glycoproteins but also enhances our appreciation of the structural diversity amongst class-1 fusion glycoprotein-bearing viruses.

Prior to pH-induced conformational changes of the LASV GPC in endosomal compartments during viral fusion, host cell entry is instigated by an initial attachment interaction with the cell surface receptor, α -dystroglycan (α -DG)¹⁰. Mapping analysis reveals

that residues involved in α -DG binding are located near the trimeric interfaces of the GP1 subunit, rationalizing the observation that recombinantly produced LASV GP1 monomers are incapable of interacting with α -DG¹¹ and that an intact trimer is required for receptor recognition (**Fig. 1**)¹². Interestingly, this contrasts with the mode of cell attachment by transferrin receptor-binding arenaviruses from the Americas (e.g. Bolivian haemorrhagic fever virus), which encode the entire receptor-binding site on a single GP1 monomer¹³. The differential use of the GP1 glycoprotein, as revealed by this investigation, thus provides an initial structural rationale for the differential host receptor entry pathways utilized by these geographically and genetically distinct groups of arenaviruses.

LASV also requires an intracellular receptor, the lysosome-associated membrane protein 1 (LAMP1), during endocytic uptake of the virus¹². Previous crystallographic analysis of monomeric LASV GP1¹¹ revealed a conformational state distinct from the GP1 of the genetically related lymphocytic choriomeningitis virus solved in the context of a GP1-GP2 protomer¹⁴, an observation attributed to the isolated LASV GP1 forming a low pH-induced conformation capable of binding LAMP1¹¹. The LASV GPC structure by Hastie *et al.* enables an molecular-level comparison of the pre-fusion and low pH-induced states of LASV GP1, confirming that not only the GP2 subunit undergoes extensive pH-driven rearrangements to achieve host cell entry^{4,11,14-16}. Although GP1-specific rearrangements have been suggested for other arenaviruses, particularly those found in the Old World¹⁷, it will be of interest to determine whether these are a conserved feature across the entire family.

The neutralizing monoclonal antibody, 37.7H, was derived from a human survivor and demonstrates promising protective properties *in vivo*^{5,18}. Hastie's analysis provides a structural rationale for neutralization⁴, revealing that 37.7H stabilizes the GPC by locking the complex in the pre-fusion conformation, preventing the conformational rearrangements required for both LAMP1 binding and membrane fusion. The 37.7H epitope is located at the membrane-

proximal base of the trimer and includes two neighbouring GP2s, as well as minor contacts with a single GP1 (**Fig. 1**). Given the importance of quaternary multi-subunit epitopes in antibody-mediated neutralization of LASV⁵, it becomes clear that future monoclonal antibody isolation and structural characterization will rely heavily on structure-guided stabilization of a trimeric and ligand-free soluble LASV glycoprotein.

In summary, the article by Hastie *et al.*⁴ signifies a major milestone in our understanding of the architecture of the LASV surface antigen. Ingeniously, this breakthrough was achieved by gluing together genetically stabilized GP1-GP2 protomers into a native trimeric state with a multi-subunit-targeting human neutralizing antibody. This work establishes a foothold in understanding the structure and function of the LASV spike and provides insights into one of the several mechanisms by which the humoral immune system may target this deadly virus. Significantly, this work also provides a blueprint for the generation of structurally optimized arenaviral immunogens, which can be further used for the isolation of therapeutically promising mAbs. While future structural studies will undoubtedly investigate the contribution of the GP2 transmembrane region and SSP to the functionality of the trimeric complex^{2,19} as well as the mechanisms by which LASV GPC recognizes host cell receptors, this work pieces together the formerly elusive puzzle of the LASV glycoprotein spike architecture.

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References

1. World Health Organization. WHO publishes list of top emerging diseases likely to cause major epidemics (2015). <http://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/> (accessed 8 June 2017).
2. Nunberg, J.H. & York, J. The curious case of arenavirus entry, and its inhibition. *Viruses* **4**, 83-101 (2012).
3. Sanders, R.W. et al. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS Pathog* **9**, e1003618 (2013).
4. Hastie, K.M. et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
5. Robinson, J.E. et al. Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits. *Nat Commun* **7**, 11544 (2016).
6. Li, S. et al. Acidic pH-Induced Conformations and LAMP1 Binding of the Lassa Virus Glycoprotein Spike. *PLoS Pathog* **12**, e1005418 (2016).
7. Lee, J.E. et al. Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. *Nature* **454**, 177-182 (2008).
8. Julien, J.P. et al. Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science* **342**, 1477-83 (2013).
9. Gamblin, S.J. et al. The structure and receptor binding properties of the 1918 influenza hemagglutinin. *Science* **303**, 1838-42 (2004).
10. Cao, W. et al. Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. *Science* **282**, 2079-2081 (1998).
11. Cohen-Dvashi, H., Cohen, N., Israeli, H. & Diskin, R. Molecular Mechanism for LAMP1 Recognition by Lassa Virus. *J Virol* **89**, 7584-92 (2015).
12. Jae, L.T. et al. Virus entry. Lassa virus entry requires a trigger-induced receptor switch. *Science* **344**, 1506-10 (2014).

13. Abraham, J., Corbett, K.D., Farzan, M., Choe, H. & Harrison, S.C. Structural basis for receptor recognition by New World hemorrhagic fever arenaviruses. *Nat Struct Mol Biol* **17**, 438-44 (2010).
14. Hastie, K.M. et al. Crystal structure of the prefusion surface glycoprotein of the prototypic arenavirus LCMV. *Nat Struct Mol Biol* **23**, 513-21 (2016).
15. Igonet, S. et al. X-ray structure of the arenavirus glycoprotein GP2 in its postfusion hairpin conformation. *Proc Natl Acad Sci U S A* **108**, 19967-72 (2011).
16. Parsy, M.L., Harlos, K., Huiskonen, J.T. & Bowden, T.A. Crystal structure of Venezuelan hemorrhagic fever virus fusion glycoprotein reveals a class 1 postfusion architecture with extensive glycosylation. *J Virol* **87**, 13070-5 (2013).
17. Israeli, H., Cohen-Dvashi, H., Shulman, A., Shimon, A. & Diskin, R. Mapping of the Lassa virus LAMP1 binding site reveals unique determinants not shared by other old world arenaviruses. *PLoS Pathog* **13**, e1006337 (2017).
18. Cross, R.W. et al. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral Res* **133**, 218-22 (2016).
19. Messina, E.L., York, J. & Nunberg, J.H. Dissection of the role of the stable signal peptide of the arenavirus envelope glycoprotein in membrane fusion. *J Virol* **86**, 6138-45 (2012).

Figure Legend:

Figure 1 Organization of the trimeric LASV GPC spike in complex with the Fab fragment of a multi-subunit-targeting neutralizing antibody. The protein surface is based upon the recently determined crystal structure by Hastie *et al.*⁴ (PDB ID 5VK2). GP1 and GP2 subunits are colored purple and blue respectively, and the heavy and light chains of the Fab are colored dark and light grey, respectively. N-linked glycans (mint green) surround most of the protein surface. Putative α -dystroglycan and LAMP1 binding sites are labelled in lime green and orange, respectively. Blue cylinders show the expected location of the stable signal peptide (SSP) and GP2 stalk.