



## Perspective

## The structure of Crimean-Congo hemorrhagic fever virus Gc is revealed; many more still need an answer

Wei Ye<sup>a,\*</sup>,<sup>1</sup>, Chuantao Ye<sup>b,1</sup>, Yongliang Hu<sup>a,c</sup>, Yangchao Dong<sup>a</sup>, Yingfeng Lei<sup>a,\*</sup>, Fanglin Zhang<sup>a,\*</sup><sup>a</sup> Department of Microbiology, School of Preclinical Medicine, Airforce Medical University: Fourth Military Medical University, Xi'an, 710032, China<sup>b</sup> Department of Infectious Diseases, Tangdu Hospital, Airforce Medical University: Fourth Military Medical University, Xi'an, 710038, China<sup>c</sup> Department of Dermatology, The Eighth Medical Center of PLA General Hospital, Beijing, 100091, China

Crimean-Congo hemorrhagic fever virus (CCHFV) is regarded as one of the most deadly viruses, with mortality of up to 40%. Currently, no licensed vaccine with validated efficacy against CCHFV is available. CCHFV uses Gn and Gc glycoproteins to bind and penetrate the cell, like other bunyaviruses (Hulswit et al., 2021). Thus, the desired vaccines are needed, to elicit a potent neutralizing antibody (NAb) response to counteract this process. The only target for NAb recognition is Gc, a typical type II membrane fusion protein like flavivirus E protein (Modis et al., 2003). However, the administration of NAb targeting CCHFV Gc after one day post-challenge cannot provide protection in a lethal murine model (Fels et al., 2021).

The structure of CCHFV Gc was recently solved by two groups using X-ray diffraction (PDB: 7A5A, 7A59) and cryo-electron microscopy (PDB: 7FGF), respectively, providing informative insights into the CCHFV structural protein for the first time (Li et al., 2022; Mishra et al., 2022).

Both pieces of research adopted the *Drosophila* S2 cells to express the Gc ectodomain of IbAr10200 strain, with similar residues; both groups were failed to obtain the crystals due to the poor stability of Gc ectodomain. To solve the problem, research by Mishra et al. chose to mutate the amino acid residues in the fusion loop (W1191H, W1199A, and W1197A) to make the “W3” mutant, which was commonly adopted to obtain the crystals of other class II fusion proteins in post-fusion form (Klein et al., 2013; Mishra et al., 2022). The study by Li et al. utilized a C-terminal trimeric motif (GCN4), which was used to generate HIV and other viral envelope glycoprotein trimers, and successfully got the stable trimer crystals for structural analysis (Yang et al., 2002; Li et al., 2022). Though resolved by different methods, both groups revealed that CCHFV Gc adopted a typical class II glycoprotein structure comprising domains I, II, and III (Fig. 1). Overall, the structures determined by the two studies are pretty similar, except for the fusion loop in domain II, in which Mishra et al. adopted the “W3” mutant. Meanwhile, the CCHFV Gc shares structural similarities to other bunyaviruses, the fusion loops and domain

III of which are similar to that of hantavirus and Rift valley fever virus (RVFV), respectively. Both groups depicted the epitopes on the structure. Mishra et al. solved X-ray structure of CCHFV Gc in complex with antigen-binding fragments (Fabs) of two potent NABs, ADI-37801 and ADI-36121 (Fels et al., 2021).

ADI-37801 binds to the fusion-loop of the Gc, while ADI-36121 binds to domain II of the Gc and possibly prevents Gc from forming the post-fusion trimer. Neither each alone nor their combination affords the protective effect against CCHFV *in vivo* (Fels et al., 2021). Bispecific antibody DVD-121-801 is an engineered four dual-variable-domain Igs (DVD-Igs), combining the vH and vL of ADI-36121 on the top of ADI-37801. DVD-121-801 could provide potent therapeutic protection (Fels et al., 2021). Since NABs exert a synergistic effect against other hemorrhagic fever viruses, it's interesting to figure out why ADI-37801 and ADI-36121 fail to protect mice after the challenge of CCHFV.

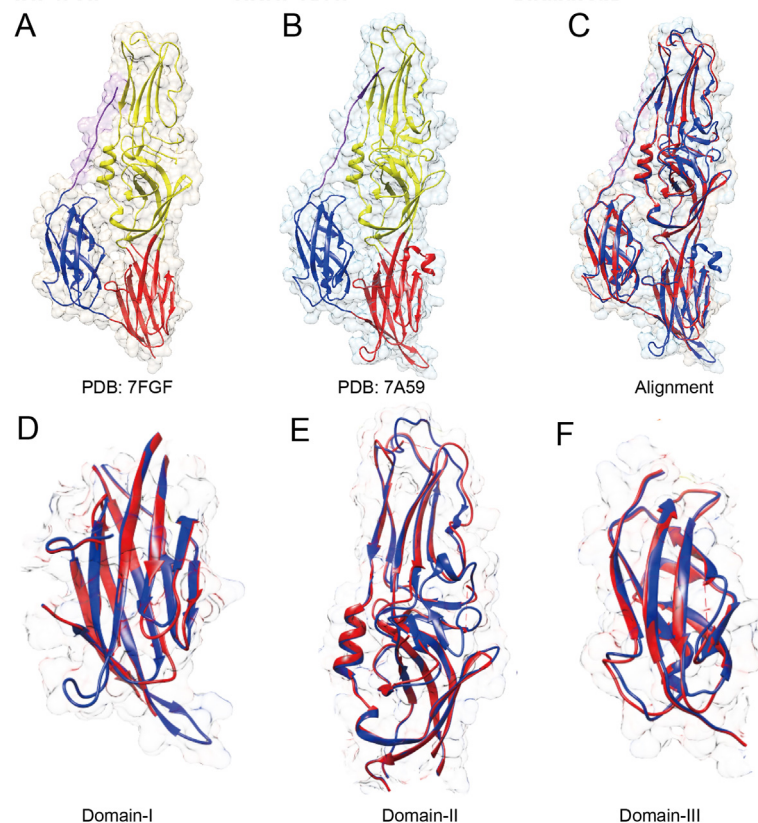
Two studies provided the post-fusion trimer structure of the CCHFV Gc ectodomain. The structure of the ternary complex with monomeric Gc and two Fabs of potent NABs was also determined by Mishra et al. The epitope information they offered may guide the design of protective vaccines and antibodies against CCHFV in the future. However, the native structural information of Gc and how Gc is placed on the virion is more valuable to help design the effective vaccine immunogen.

As for other structural glycoprotein, Gn-induced antibodies are unable to neutralize CCHFV infection. This phenomenon is quite different from other bunyaviruses. For instance, the Gn of the RVFV and hantaviruses are protective antigens and can elicit NABs (Hulswit et al., 2021). Uncovering CCHFV Gn's structure, and revealing how the Gn interacts with Gc, and how Gn-Gc is arranged on the virion surface may facilitate cracking these mysteries.

Additionally, aside from Gn and Gc, CCHFV glycoproteins also generate other nonstructural peptides, including mucin-like protein (MLD), double-membrane-spanning protein NSm, and GP38. Although

\* Corresponding authors.

E-mail addresses: [virologyw@fmmu.edu.cn](mailto:virologyw@fmmu.edu.cn) (W. Ye), [yflei@fmmu.edu.cn](mailto:yflei@fmmu.edu.cn) (Y. Lei), [flzhang@fmmu.edu.cn](mailto:flzhang@fmmu.edu.cn) (F. Zhang).<sup>1</sup> Wei Ye and Chuantao Ye contributed equally to this work.



**Fig. 1.** Structural comparisons of CCHFV Gc from different PDB structures. **A, B** Ribbon diagram of Gc monomer in PDB: 7FGF and PDB: 7A59 colored according to the domain (domain I: red, domain II: yellow, domain III: blue). **C** Superposition of Gc monomer in 7FGF and 7A59. The Gc monomer of 7FGF is colored in red, whereas the Gc monomer of 7A59 is colored in blue. **D–F** Superposition of the domains of Gc in 7FGF and 7A59. Domain I and domain III of Gc showed considerable similarities, whereas the differences mainly appeared in the fusion loop of domain II.

GP38 was not a structural protein, a specific antibody against GP38 (13G8) could provide partial protection *in vivo*, even in therapeutic settings (Golden et al., 2019). A recent study provided the possible roles of MLD, GP38, and NSm in CCHFV glycoprotein trafficking, assembly, and virion secretion (Freitas et al., 2020). Meanwhile, the GP38 structure shares homology with the Gn ectodomain and is presumed as the result of a gene duplication of Gn, which has not been observed in other viruses (Mishra et al., 2020). So, how GP38 affects Gc's maturation and proper processing is an intriguing issue to be investigated.

In short, current NABs elicited by Gc are not effective in protecting the CCHFV-infected animals. The structure of CCHFV Gc provides the potential information for antigen design of the vaccine. To further elucidate the structure of Gn and the native form of Gc, and to reveal how the Gn and Gc are arranged on the virion surface may facilitate the development of next-generation CCHFV vaccine.

Questions still need an answer for CCHFV.

- 1) Though the post-fusion structure of Gc was solved, the structure of native Gc still remains elusive, and how it is arranged on the surface of virion along with Gn is an intriguing issue to be pursued.
- 2) The glycosylation site on the Gn (N577) was regarded as necessary for Gc to transport from endoplasmic reticulum (ER) to Golgi, while the sites on the Gc (N1054, N1563) were not (Erickson et al., 2007). Moreover, Mishra et al. found that N1563 of Gc only minimally affected the low-pH-triggered cell-cell fusion. So, how the glycosylation sites affect the glycoprotein transport and function still needs the additional structure information of the Gn and Gn-Gc complex.
- 3) As a nonstructural protein, how does GP38 mimic the structure of Gn? And why do antibodies against Gn lack neutralizing effect? What is

the mechanism of a single antibody against GP38 (13G8) is protective?

## Footnotes

The present study was supported in part by grants from the National Natural Science Foundation of China (No. 82072268), the University supporting grants (Nos. 2018JSTS03 and 2020SWAQ09), and the Open Fund of the State Key Laboratory of Pathogenic Microbial Biosafety (No. SKLPBS1834). The funders had no role in the study design, data collection, analysis, publication decision, or manuscript preparation. We thank Dr. Xinzhen Zhang for a thorough discussion of this manuscript. We declare no competing interests.

## References

- Erickson, B.R., Deyde, V., Sanchez, A.J., Vincent, M.J., Nichol, S.T., 2007. N-linked glycosylation of gn (but not gc) is important for crimean Congo hemorrhagic fever virus glycoprotein localization and transport. *Virology* 361, 348–355.
- Fels, J.M., Maurer, D.P., Herbert, A.S., Wirchnianski, A.S., Vergnolle, O., Cross, R.W., Abelson, D.M., Moyer, C.L., Mishra, A.K., Aguilan, J.T., Kuehne, A.I., Pauli, N.T., Bakken, R.R., Nyakatura, E.K., Hellert, J., Quevedo, G., Lobel, L., Balinandi, S., Lutwama, J.J., Zeitlin, L., Geisbert, T.W., Rey, F.A., Sidoli, S., McLellan, J.S., Lai, J.R., Bornholdt, Z.A., Dye, J.M., Walker, L.M., Chandran, K., 2021. Protective neutralizing antibodies from human survivors of crimean-Congo hemorrhagic fever. *Cell* 184, 3486–3501 e3421.
- Freitas, N., Enguehard, M., Denolly, S., Levy, C., Neveu, G., Lerolle, S., Devignot, S., Weber, F., Bergeron, E., Legros, V., Cosset, F.L., 2020. The interplays between crimean-Congo hemorrhagic fever virus (cchfv) m segment-encoded accessory proteins and structural proteins promote virus assembly and infectivity. *PLoS Pathog.* 16, e1008850.
- Golden, J.W., Shoemaker, C.J., Lindquist, M.E., Zeng, X., Daye, S.P., Williams, J.A., Liu, J., Coffin, K.M., Olschner, S., Flusin, O., Altamura, L.A., Kuehl, K.A.,

- Fitzpatrick, C.J., Schmaljohn, C.S., Garrison, A.R., 2019. Gp38-targeting monoclonal antibodies protect adult mice against lethal crimean-Congo hemorrhagic fever virus infection. *Sci. Adv.* 5, eaaw9535.
- Hulswit, R.J.G., Paesen, G.C., Bowden, T.A., Shi, X.H., 2021. Recent advances in bunyavirus glycoprotein research: precursor processing, receptor binding and structure. *Viruses-Basel* 13, 353.
- Klein, D.E., Choi, J.L., Harrison, S.C., 2013. Structure of a dengue virus envelope protein late-stage fusion intermediate. *J. Virol.* 87, 2287–2293.
- Li, N., Rao, G., Li, Z., Yin, J., Chong, T., Tian, K., Fu, Y., Cao, S., 2022. Cryo-em structure of glycoprotein c from crimean-Congo hemorrhagic fever virus. *Virol. Sin.* 37, 127–213.
- Mishra, A.K., Hellert, J., Freitas, N., Guardado-Calvo, P., Haouz, A., Fels, J.M., Maurer, D.P., Abelson, D.M., Bornholdt, Z.A., Walker, L.M., Chandran, K., Cosset, F.L., McLellan, J.S., Rey, F.A., 2022. Structural basis of synergistic neutralization of crimean-Congo hemorrhagic fever virus by human antibodies. *Science* 375, 104–109.
- Mishra, A.K., Moyer, C.L., Abelson, D.M., Deer, D.J., El Omari, K., Duman, R., Lobel, L., Lutwama, J.J., Dye, J.M., Wagner, A., Chandran, K., Cross, R.W., Geisbert, T.W., Zeitlin, L., Bornholdt, Z.A., McLellan, J.S., 2020. Structure and characterization of crimean-Congo hemorrhagic fever virus gp38. *J. Virol.* 94, e02005–e02019.
- Modis, Y., Ogata, S., Clements, D., Harrison, S.C., 2003. A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6986–6991.
- Yang, X., Lee, J., Mahony, E.M., Kwong, P.D., Wyatt, R., Sodroski, J., 2002. Highly stable trimers formed by human immunodeficiency virus type 1 envelope glycoproteins fused with the trimeric motif of t4 bacteriophage fibrin. *J. Virol.* 76, 4634–4642.