AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

The Nucleocapsid Protein of SARS–CoV-2: a Target for Vaccine Development

Noton K. Dutta,[a] Kaushiki Mazumdar,[b] James T. Gordy[c]

[a]Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[b]Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, Maryland, USA
[c]The W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA

KEYWORDS coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS–CoV-2), nucleocapsid protein, vaccine

During the current coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS–CoV-2), there has been an unprecedented level of global collaboration that has led to a rapid characterization of SARS–CoV-2 (1). Its sequence shares 79.6% identity to SARS–CoV (1, 2), the infectious virus that caused an epidemic in 2003 (2, 3). SARS–CoV-2 has a single-stranded, plus-sense, RNA genome of approximately 30 kb, which includes five major open reading frames encoding nonstructural replicase polyproteins and structural proteins (1), namely, spike (S) (4–6), envelope (E), membrane (M), and nucleocapsid (N) (7), and they are in the same order and of approximately the same sizes as those in SARS-CoV.

The SARS–CoV-2 S protein is being used as the leading target antigen in vaccine development (8, 9). However, the complex molecular details of viral entry may lead to complications with the vaccine response, similar to those seen with HIV type 1 (HIV-1) Env protein vaccine efforts (10). The SARS–CoV-2 S gene has 76% amino acid similarity to the SARS-CoV S gene (11), and nonsynonymous mutations developed in the S protein as the SARS-CoV epidemic progressed (12, 13). In contrast, the N gene is more conserved and stable, with 90% amino acid homology and fewer mutations over time (2, 3, 11, 14–16). N proteins of many coronaviruses are highly immunogenic and are expressed abundantly during infection (17). High levels of IgG antibodies against N have been detected in sera from SARS patients (18), and the N protein is a representative antigen for the T-cell response in a vaccine setting, inducing SARS-specific T-cell proliferation and cytotoxic activity (19, 20). We have already shown that the middle or C-terminal region of the SARS-CoV N protein is important for eliciting antibodies against SARS-CoV during the immune response (21–23).

New reports have additionally shown that the crystal structure of the SARS–CoV-2 nucleocapsid protein is similar to those of previously described coronavirus N proteins, but their surface electrostatic potential characteristics are distinct (7). Sheikh et al. studied the factors influencing N gene variations among 13 coronaviruses and how these affect virus-host relationships, reporting a high AT% and low GC% in the nucleotide contents of SARS coronavirus (24). In this issue, Cong et al. (17) used a mouse hepatitis virus (MHV) model to show that the viral nucleocapsid (N) protein contributes to forming helical ribonucleoproteins during the packaging of the RNA genome, regulating viral RNA synthesis during replication and transcription and modulating metabolism in infected subjects. This study complements others that have shown N to have multiple functions (25). It is becoming more evident just how critical this protein is for multiple steps of the viral life cycle. These reports offer important and timely insights relevant to the SARS–CoV-2 N protein, a vaccine target that has some distinct advantages over other potential SARS–CoV-2 antigens. Because of the conservation of the N protein sequence, the expanding

Citation Dutta NK, Mazumdar K, Gordy JT. 2020. The nucleocapsid protein of SARS–CoV-2: a target for vaccine development. J Virol 94:e00647-20. https://doi.org/10.1128/JVI.00647-20.

Editor Rebecca Ellis Dutch, University of Kentucky College of Medicine

Address correspondence to Noton K. Dutta, ndutta1@jhmi.edu

Published 16 June 2020

knowledge of its genetics and biochemistry, and its strong immunogenicity, the N protein of SARS–CoV-2 should be strongly considered as a vaccine candidate for SARS–CoV-2.

ACKNOWLEDGMENTS

We received no funding.

We report no conflicts of interest. We have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

REFERENCES

1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579:270–273. https://doi.org/10.1038/s41586-020-2012-7.
2. Marra MA, Jones SJM, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YSN, Khattra J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS, Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM, Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K, Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R, Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A, Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S, Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M, Skowronski DM, Upton C, Roper RL. 2003. The genome sequence of the SARS-associated coronavirus. Science 300:1399–1404. https://doi.org/10.1126/science.1085953.
3. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348:1967–1976. https://doi.org/10.1056/NEJMoa030747.
4. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367:1260–1263. https://doi.org/10.1126/science.abb2507.
5. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181:281–292. https://doi.org/10.1016/j.cell.2020.02.058.
6. Jaimes JA, André NM, Millet JK, Whittaker GR. 2020. Structural modeling of 2019-novel coronavirus (nCoV) spike protein reveals a proteolytically-sensitive activation loop as a distinguishing feature compared to SARS-CoV and related SARS-like coronaviruses. bioRxiv https://doi.org/10.1101/2020.02.10.942185.
7. Kang S, Yang M, Hong Z, Zhang L, Huang Z, Chen X, He S, Zhou Z, Zhou Z, Chen Q, Yan Y, Zhang C, Shan H, Chen S. 2020. Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. bioRxiv https://doi.org/10.1101/2020.03.06.977876.
8. Pang J, Wang MX, Ang IYH, Tan SHX, Lewis RF, Chen JI, Gutierrez RA, Gwee SXW, Chua PEY, Yang Q, Ng XY, Yap RK, Tan HY, Teo YY, Tan CC, Cook AR, Yap JC, Hsu LY. 2020. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): a systematic review. J Clin Med 9:E623. https://doi.org/10.3390/jcm9030623.
9. Chen WH, Strych U, Hotez PJ, Bottazzi ME. 3 March 2020. The SARS-CoV-2 vaccine pipeline: an overview. Curr Trop Med Rep https://doi.org/10.1007/s40475-020-00201-6.
10. Kwong PD, Doyle ML, Casper DJ, Cicala C, Leavitt SA, Majeed S, Steenbeke TD, Venturi M, Chaiken I, Fung M, Katinger H, Parren PW, Robinson J, Van Ryk D, Wang L, Burton DR, Freire E, Wyatt R, Sodroski J, Hendrickson WA, Arthos J. 2002. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature 420:678–682. https://doi.org/10.1038/nature01188.
11. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. 8 April 2020. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. Cell Host Microbe https://doi.org/10.1016/j.chom.2020.03.002.
12. Ruan YJ, Wei CL, Ee AL, Vega VB, Thoreau H, Su ST, Chia JM, Ng P, Chiu KP, Lim L, Zhang T, Peng CK, Lin EO, Lee NM, Yee SL, Ng LF, Chee RE, Stanton LW, Long PM, Liu ET. 2003. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 361:1779–1785. https://doi.org/10.1016/S0140-6736(03)13414-9.
13. Yang ZY, Werner HC, Kong WP, Leung K, Traggiai E, Lanzavecchia A, Nabel GJ. 2005. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. Proc Natl Acad Sci U S A 102:797–801. https://doi.org/10.1073/pnas.0409065102.
14. Holmes KV, Enjuanes L. 2003. Virology. The SARS coronavirus: a post-genomic era. Science 300:1377–1378. https://doi.org/10.1126/science.1086418.
15. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH, Tong S, Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman DD, Peret TC, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Gunther S, Osterhaus AD, Drosten C, Pallansch MA, Anderson LJ, Bellini WJ. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300:1394–1399. https://doi.org/10.1126/science.1085952.
16. Zhu Y, Liu M, Zhao W, Zhang J, Zhang X, Wang K, Gu C, Wu K, Li Y, Zheng C, Xiao G, Yan H, Zhang J, Guo D, Tien P, Wu J. 2005. Isolation of virus from a SARS patient and genome-wide analysis of genetic mutations related to pathogenesis and epidemiology from 47 SARS-CoV isolates. Virus Genes 30:93–102. https://doi.org/10.1007/s11262-004-4586-9.
17. Cong Y, Ulasli M, Schepers H, Mauthe M, V'Kovski P, Kriegenburg F, Thiel V, de Haan CAM, Reggiori F. 2020. Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle. J Virol 94:e01925-19. https://doi.org/10.1128/JVI.01925-19.
18. Leung DT, Tam FC, Ma CH, Chan PK, Cheung JL, Niu H, Tam JS, Lim PL. 2004. Antibody response of patients with severe acute respiratory syndrome (SARS) targets the viral nucleocapsid. J Infect Dis 190:379–386. https://doi.org/10.1086/422040.
19. Gao W, Tamin A, Soloff A, D'Aiuto L, Nwanegbo E, Robbins PD, Bellini WJ, Barratt-Boyes S, Gambotto A. 2003. Effects of a SARS-associated coronavirus vaccine in monkeys. Lancet 362:1895–1896. https://doi.org/10.1016/S0140-6736(03)14962-8.
20. Okada M, Takemoto Y, Okuno Y, Hashimoto S, Yoshida S, Fukunaga Y, Tanaka T, Kita Y, Kuwayama S, Muraki Y, Kanamaru N, Takai H, Okada C, Sakaguchi Y, Furukawa I, Yamada K, Matsumoto M, Kase T, Demello DE, Peiris JS, Chen PJ, Yamamoto N, Yoshinaka Y, Nomura T, Ishida I, Morikawa S, Tashiro M, Sakatani M. 2005. The development of vaccines against SARS corona virus in mice and SCID-PBL/hu mice. Vaccine 23:2269–2272. https://doi.org/10.1016/j.vaccine.2005.01.036.
21. Lee HK, Lee BH, Dutta NK, Seok SH, Baek MW, Lee HY, Kim DJ, Na YR, Noh KJ, Park SH, Kariwa H, Nakauchi M, Mai Le Q, Heo SJ, Park JH. 2008. Detection of antibodies against SARS-coronavirus using recombinant truncated nucleocapsid proteins by ELISA. J Microbiol Biotechnol 18:1717–1721.
22. Dutta NK, Mazumdar K, Lee BH, Baek MW, Kim DJ, Na YR, Park SH, Lee HK, Kariwa H, Mai Le Q, Park JH. 2008. Search for potential target site of nucleocapsid gene for the design of an epitope-based SARS DNA vaccine. Immunol Lett 118:65–71. https://doi.org/10.1016/j.imlet.2008.03.003.
23. Lee HK, Lee BH, Seok SH, Baek MW, Lee HY, Kim DJ, Na YR, Noh KJ, Park SH, Kumar DN, Kariwa H, Nakauchi M, Heo SJ, Park JH. 2010. Production of specific antibodies against SARS-coronavirus nucleocapsid protein without cross reactivity with human coronaviruses 229E and OC43. J Vet Sci 11:165–167. https://doi.org/10.4142/jvs.2010.11.2.165.
24. Sheikh A, Al-Taher A, Al-Nazawi M, Al-Mubarak AI, Kandeel M. 2020. Analysis of preferred codon usage in the coronavirus N genes and their implications for genome evolution and vaccine design. J Virol Methods 277:113806. https://doi.org/10.1016/j.jviromet.2019.113806.
25. Chang CK, Hou MH, Chang CF, Hsiao CD, Huang TH. 2014. The SARS coronavirus nucleocapsid protein—forms and functions. Antiviral Res 103:39–50. https://doi.org/10.1016/j.antiviral.2013.12.009.