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Viewpoint

Blocking Coronavirus 19 Infection via the SARS-CoV-2 Spike Protein: Initial Steps

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ABSTRACT: Recent crystal structure data for protein−protein interactions featuring the SARS-CoV-2 spike protein will inevitably trigger a new wave of research in this area that was not possible before. This *Viewpoint* outlines a few of the ways that it is already happening.

KEYWORDS: *SARS-CoV-2, spike protein, peptide, monoclonal antibody*

It is inspiring to see how quickly the scientific community has mobilized to address a new threat to human health. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, the COVID-19 virus) contains a spike protein that mediates entry into the host's cells by binding a cell surface receptor there called angiotensin converting enzyme 2 (ACE2).[1] This *Viewpoint* focuses on factors that influence the SARS-CoV-2-spike protein·ACE2 protein−protein interaction and how it may be targeted for therapeutic gain.

A milestone in this area has arisen from three groups who separately elucidated the crystal structure of SARS-CoV-2 spike protein·ACE2.[2−4] These structures reveal ACE2 at the interface contributes almost exclusively a ∼24 amino acid extended helix. Correlations have been made between the dissociation constants for SARS-CoV S proteins from the ACE2 receptor and rates of transmissibility, viral infection, and disease severity.[1] SARS-CoV-2 S protein may have a lower K_d than the one from SARS-CoV; hence, the virus may have evolved to maximize this interaction.[3] To virologists, these structural insights into the SARS-CoV-2-spike protein·ACE2 interface reveal a potentially important epitope that vaccines might be engineered to bind. Peptide chemists, on the other hand, will view that helix as an inviting target to mimic with short peptide sequences or with helical peptide mimics, to retard uptake of the virus particles by the host cells. In fact, peptide chemists are already "on the case".

Initial steps in mimicry of the key helical peptide motif in ACE2 must involve synthesis of the native peptide and assays to determine its effects on SARS-CoV-2 spike protein·ACE2 *in vitro*; Pentelute's group at MIT might be first to publish this.[5] Their 23-mer peptide bound SARS-CoV-2 spike protein gave a K_d value of 47 nM, whereas that for full length ACE2 was 7 nM (measured using biolayer interferometry, BLI). Consequently, even this unmodified peptide, consisting of only natural amino acids, might outcompete ACE2 for binding the virus, provided it is present in excess. These observations will cause excitement among the community preparing stapled helical peptides and other types of peptidomimetics, and it has already stimulated computational chemists from the University of Michigan to predict helical sequences comprised of naturally encoded amino acids that might bind more effectively.[6]

When COVID-19 enters the mouth there are two immediate destinations: the lungs and the intestines. Intestines present

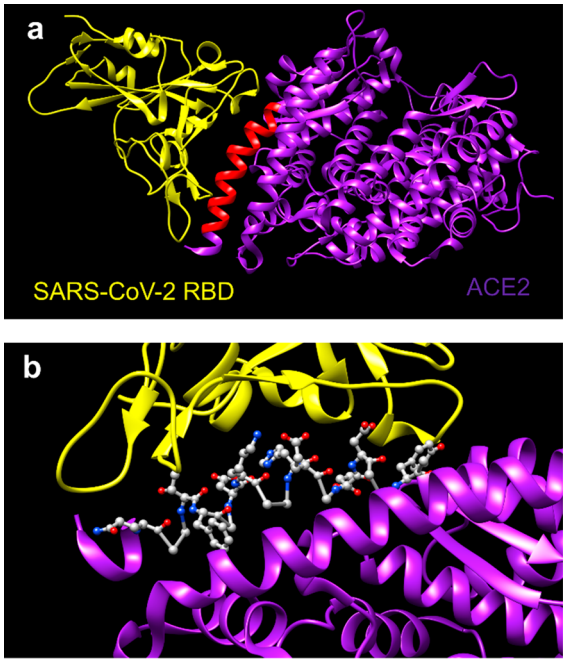

Figure 1. (a) Receptor binding domain of SARS-CoV-2 spike protein·ACE2 (PDB 6M0J). (b) Residues of the red helix in ACE2 that interact with SARS-CoV-2 spike protein at the interface.

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about 200 m^2 of epithelial cell surface per individual for the virus to work on, but relatively few patients with life-threatening cases of COVID-19 infection develop diarrhea; most show only severe respiratory symptoms. This outcome seems inconsistent with the fact that ACE2 is abundant on small intestine enterocytes as well as lung alveolar epithelial cells. COVID-19 seems to tend to lose the battle waged in the intestines because of defensins. Defensins are a subset of amphiphilic antimicrobial peptides of which the abundant HD5 (human defensin 5) is illustrative. In fact, HD5 also plays a key role in protecting cells in the intestine from the virus, as shown by a group from Third Military Medical University, Chongqing.[7] HD5 is secreted by Paneth cells in the crypts of the small intestine; it is a 32-residue peptide constrained by three disulfide bonds (Figure 2). Abundance of HD5 in the intestine suggests that it may effectively compete with SARS-CoV-2 spike protein to bind ACE2 even though it has a higher K_d (39.3 nM) than the viral protein (2.68 nM also via BLI; K_d value slightly different to that quoted above probably due to different protein source and, perhaps, glycosylation state). Modeling indicates HD5 binds the *N*-terminal region of the critical red helix shown in Figure 1, and this is the interaction

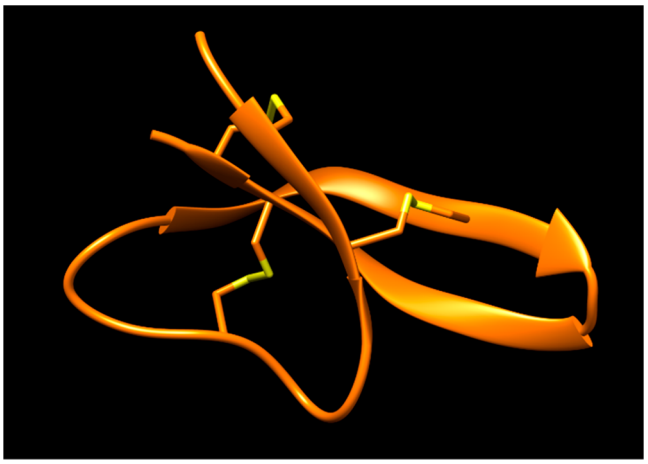

Figure 2. Structure of human defensin 5 (HD5) from 1AMP.

that gives the blocking effect. SARS-CoV-2 spike protein also binds HD5 (K_d, 82 nM) though that interaction does not prevent it binding ACE2. The Chongqing group assayed their compounds by BLI for binding, by immunofluorescence (with and without HD5) of Paneth cells exposed to SARS-CoV-2 spike protein and a monoclonal antibody (mAb) that binds this on the cell surface, and by Western blot of SARS-CoV-2 spike protein that makes its way into cells. As expected, HD5 suppresses uptake of SARS-CoV-2 spike protein into the colon cells, and it is in the colon where this defensin is excreted by Paneth cells.

In *Science*,[8] researchers from Scripps and The University of Hong Kong report the structure of mAb CR3022 bound to SARS-CoV-2 spike protein. CR3022 is a neutralizing mAb for SARS-CoV (from "SARS", K_d 1 nM), but it also binds SARS-CoV-2 spike protein (K_d 115 nM) in the same region, where the two spike proteins are 86% homologous. Six CR3022 turns, three on the light chain and three on the heavy, bind the SARS-CoV-2 spike protein (Figure 3, turns in red), and the region where they bind does *not* overlap with that which contacts ACE2. CR3022 neutralizes SARS-CoV but not SARS-

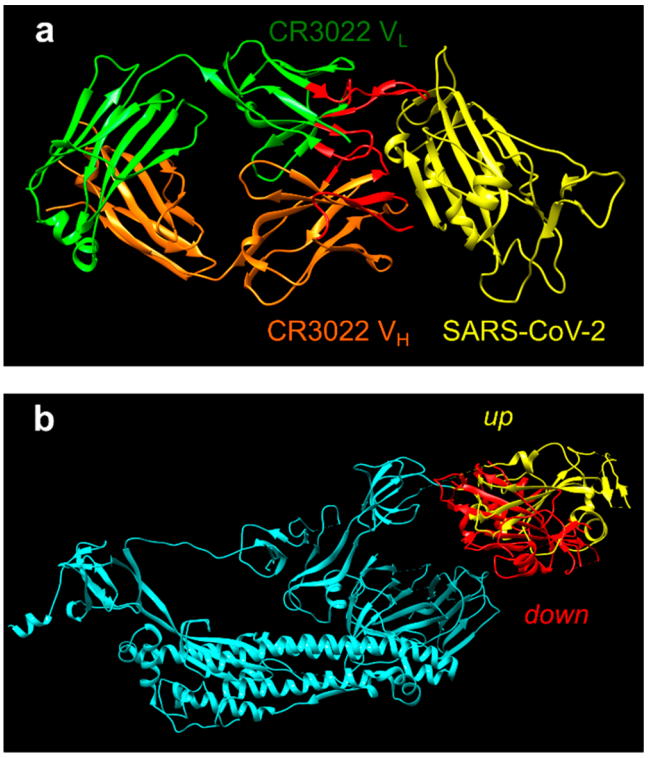

Figure 3. (a) SARS-CoV-2 spike protein RBD bound to the heavy and light chains of CR3022 via the six loops highlighted in red. (b) Structure of the SARS-COV-2 S glycoprotein. Yellow and red segments represent the receptor binding domain in *up* and *down* conformation, respectively, and the cyan is the truncated S protein.

CoV-2 (from COVID-19). A mechanism of inactivation other than competition for ACE2 must be involved for SARS-CoV·CR3022 that is not applicable to SARS-CoV-2. On that subject the authors note the receptor binding domains of coronaviruses undergo hinge-like transitions between *up* and *down* conformations (Figure 3b, hinge in blue and CR3022 binding site in red/yellow); only the *up* is accessible to CR3022, and ACE2 only binds to the *up* form. These observations speak to the potential importance of mAbs and eventually small molecules that bind the spike protein, including ones that do not bind the PPI interface.

Overall, it is an exciting time to be working on SARS-CoV-2 in an otherwise stressful period. "It was the best of times. It was the worst of times." (Charles Dickens).

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■ ABBREVIATIONS

BLI, biolayer interferometry; PPI, protein−protein interaction

■ REFERENCES

(1) Walls, A. C.; Park, Y.-J.; Tortorici, M. A.; Wall, A.; McGuire, A. T.; Veesler, D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **2020**, *181* (2), 281−292.e6.

(2) Shang, J.; Ye, G.; Shi, K.; Wan, Y.; Luo, C.; Aihara, H.; Geng, Q.; Auerback, A.; Li, F. Structural basis of receptor recognition by SARS-CoV-2. *Nature* **2020**, *581*, 221−224.

(3) Wang, Q.; Zhang, Y.; Wu, L.; Niu, S.; Song, C.; Zhang, Z.; Lu, G.; Qiao, C.; Hu, Y.; Yuen, K.-Y.; Wang, Q.; Zhou, H.; Yan, J.; Qi, J. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* **2020**, *181*, 1−11.

(4) Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; Wang, X. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **2020**, *581*, 215.

(5) Zhang, G.; Pomplun, S.; Loftis, A. R.; Loas, A.; Pentelute, B. L. The first-in-class peptide binder to the SARS-CoV-2 spike protein. *bioRxiv* **2020**, DOI: 10.1101/2020.03.19.999318.

(6) Huang, X.; Pearce, R.; Zhang, Y. Computational design of peptides to block binding of the SARS-CoV-2 spike protein to human ACE2. *bioRxiv* **2020**, DOI: 10.1101/2020.03.28.013607.

(7) Wang, C.; Wang, S.; Li, D.; Zhao, X.; Han, S.; Wang, T.; Zhao, G.; Chen, Y.; Chen, F.; Zhao, J.; Wang, L.; Sun, W.; Huang, Y.; Su, Y.; Wei, D.; Zhao, J.; Wang, J. Lectin-like intestinal defensin inhibits 2019-nCoV spike binding to ACE2. *bioRxiv* **2020**, DOI: 10.1101/2020.03.29.013490.

(8) Yuan, M.; Wu, N. C.; Zhu, X.; Lee, C.-C. D.; So, R. T. Y.; Lv, H.; Mok, C. K. P.; Wilson, I. A. A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV. *Science* **2020**, *368*, 1−16.

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