Achilles' Heel of SARS-CoV-2 Structure

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ABSTRACT: Guided by evolutionarily signaled vulnerabilities in the structure of SARS-CoV-2, we identify epitopes in free monomers of the spike protein that steer the generation of induced or administered antibodies geared at promoting destabilization of the virus quaternary structure, thereby hampering infectivity.

Achilles' heel of SARS-CoV-2

antigen 603–690

KEYWORDS: SARS-CoV-2, COVID19, G614 dominant strain, protein structure, structural defect, antigen, antibody, epitope, virus transmission

1. RECENT EVOLUTIONARY HISTORY POINTS TO THE ACHILLES' HEEL OF SARS-COV-2 STRUCTURE

Most current immunotherapies to mitigate the impact of COVID19 aim at impairing the cellular uptake of culprit SARS-CoV-2 mediated by human receptor ACE2.^{1,2} Vaccine-induced or administered antibodies focus on epitopes on the surface of the trimeric viral spike (S) protein that has proven to be significantly antigenic. These epitopes typically include RBD (receptor-binding domain), NTD (N-terminal domain), and intra- or intersubunit combinations thereof.¹ Here we advocate a different therapeutic approach that emerges as we examine the recent evolutionary history of the virus and identify structural defects that have been corrected by dominant mutations that entail selective advantages. The goal is to engineer therapeutic agents that amplify such vulnerabilities. In this regard, a remarkable event in the recent evolutionary history of SARS-CoV-2 has taken place. In a few months, the D614G mutation in the S protein has become dominant, as revealed by epidemiological evidence.³ This dominance implies a fitness advantage. Efforts to track down this advantage are underway and reveal that the G614 strain presents a more stable phenotype yielding higher transmission efficacy.^{4–6} More specifically, the D614G substitution results in less S1 shedding⁶ following the preactivation phase of S1/S2 junction cleavage, the latter being a necessary step in the process of membrane fusion.^{7,8} Recent structural analysis of the impact of the D614G substitution is consistent with these findings.⁹ The D614G mutation destabilizes the free S protein monomers, thus stabilizing the S-protein quaternary structure (a trimer) after S1/S2 junction

cleavage. More specifically, the D614G substitution creates a packing defect in S1, right at the S1/S2 association interface, by exposing the backbone to the structure-disruptive effects of hydration of the amide and carbonyl paired by the 614–647 backbone hydrogen bond (BHB). Thus, in the G614 strain, S1 becomes more reliant on its association with S2 (within the binding partner monomer) to maintain its structural integrity, especially the BHB 614–647, after S1/S2 junction cleavage. In thermodynamic (free energy) terms, the D614G mutation entails an additional stabilization of the S protein assemblage estimated at $\Delta\Delta G = -5.73$ kcal/mol.⁹ When interpreted in terms of selective advantage, we may state that the dominant mutation D614G mitigates a structural defect in the virus. The vulnerability is given by the fragility of the S1/S2 interface in the S protein quaternary structure right at the BHB 614–647 which, in the G614 strain, is solvent-exposed in the free monomer.⁹

2. VIRUS-DESTABILIZING ANTIBODIES

This analysis prompts us to design immunotherapies to amplify the vulnerability of the SARS-CoV-2 assemblage that surfaced upon examination of its recent evolutionary history. Because we aim to destabilize the S protein quaternary structure at the

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preactivation juncture, we need to identify antigenic regions and epitopes in the free monomeric S subunits, rather than in the trimer assembly, as done in current therapeutic approaches. To be disruptive of the S protein quaternary structure upon antibody recognition, these epitopes should be adjacent to the evolutionarily signaled BHB D(G)614-A647, which in the G614 strain, becomes exposed to structure-disruptive hydration in the free monomeric S subunit.

We have identified B-cell epitopes to be targeted for virus destabilization using a linear epitope predictor from the public domain.¹⁰ A practical hurdle arises as the PDB and FASTA files in PDB entry 6VXX for the SARS-CoV-2 S protein have different sequence numbering because disordered terminal residues are excluded in the structural file. Key residues D(G)614 and A647 become D(G)633 and A666 in the FASTA file for the amino acid sequence of the free monomeric S subunit inputted into the BepiPred Linear Epitope Prediction 2.0 accessible at <http://tools.iiedb.org/main/bcell/>. With this caveat, the predicted epitopes (underlined) adjacent to BHB D(G)633-A666 (BHB-paired residues in bold) are subsumed in the minimal S-protein-derived antigen to induce S1 shedding:

622NTSNQVAVLYQDVNCTEVPVAIHADQLTPTW-
RVYSTGSNVFQTRAGCLIGAHEVNNNSYECDIPIGA-
GICASYQTQTNSPSGAGSVASQ₇₀₉

The strength of the signal (residues scoring above 0.6) and the length ($n > 20$) of the two last epitopes 643–663, 674–709 flanking A666 attest to the reliability of the specific predictions.

These B-cell epitopes are to be targeted for destabilization of the quaternary structure of the spike protein in SARS-CoV-2, thereby overwriting the stabilizing effect of the dominant D614G (D633G) mutation. The virus-destabilizing monoclonal antibodies (mAbs) will need to be isolated from affinity selection using the free monomeric S subunit or the antigen derived thereof as bait for specific memory B-cells from COVID-19 patients.

As part of the S1/S2 association interface, the minimal antigen containing the reliably identified epitopes is not subject to immunosurveillance while the virus maintains the intact S protein quaternary structure. The antigen becomes exposed to immunosurveillance with a significant probability after the S1/S2 junction cleavage, as partial dismantling of the spike quaternary structure takes place during membrane fusion.^{7–9} Thus, the antigen described may be exploited to select mAbs that block the reassemblage of the quaternary structure of the S protein. The proposed antigen with full coverage of the S1-shedding target region is also suitable for the vaccine-induced immune response geared at destabilizing the virus.

3. CONCLUSIONS

This work offers a concrete pathway to develop a COVID19 therapy totally different from current efforts underway. We describe COVID19 immunotherapies geared at destabilizing the virus quaternary structure and promote S1 shedding, thus greatly reducing transmission efficacy. The intervention occurs after hACE2 recognition, during pre-fusion activation. The approach draws from an analysis of the recent evolutionary history of SARS-CoV-2 and from an assessment of the selective advantages that result from the mutational mitigation of key structural defects. This observation inspired the proposed therapeutic modality based on the amplification of evolutionarily signaled virus vulnerabilities. Given the reliability of the predicted epitopes to tackle the “Achilles’ heel of SARS-CoV-2”,

the proposed therapeutic intervention has a chance to be successful and it may not be far-fetched to state that, within a suitable vaccine platform, the proposed antigen may save millions of lives.

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Notes

The author declares no competing financial interest.

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