

# Significance of the SARS-CoV-2 Spike Protein 'Superantigen' Sequence in the mRNA Vaccines

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By March 2020 the heart was recognized to be a frequent target organ in SARS-CoV-2 infection, as evidenced by the increased cardiac troponin levels observed in many late-stage COVID-19 patients.<sup>1</sup> The mechanisms for this cardiac damage appear multifactorial, involving both a myocarditis initiated by the direct viral binding to the normal angiotensin converting enzyme 2 (ACE-2) receptors on the cardiac pericytes, as well as extra-cardiac processes involving myocardial ischemia.<sup>2</sup> Pericarditis/myocarditis post COVID-19 mRNA vaccination is also an increasing concern.

## Vaccine-Induced Cardiac Injury

New data concerning COVID-19 mRNA vaccine injury comes from a recent report on the autopsy of two adolescent deaths shortly after the administration of a second Pfizer BioNTech COVID-19 mRNA injection.<sup>3</sup>

One autopsy revealed global myocardial injury with areas of coagulative myocytolysis and contraction bands, with a perivascular pattern of inflammation consisting of predominantly neutrophils with histiocytes, scant lymphocytes, and occasional eosinophils. The second autopsy showed predominant subepicardial myocyte injury, while in other sections it was patchy and transmural.

Polymerase chain reaction (PCR) tissue testing performed by the Centers for Disease Control and Prevention (CDC) on heart and lung found no molecular evidence of SARS-CoV-2 infection. Molecular testing on postmortem blood detected two heterozygous DOLK gene variants of uncertain significance and a heterozygous MAP2K2 gene (Intronic). There were no acute or organizing thrombi.

The overall pattern of injury was consistent with a norepinephrine-mediated stress (toxic) cardiomyopathy involving a direct injury to the myocardium.<sup>4</sup> This has some commonalities with patients diagnosed with Takotsubo cardiomyopathy.<sup>5</sup>

## Positive Norepinephrine-Cytokine Feedback Loop

Both septic shock and the hyperinflammatory side effects associated with several new cancer therapies have revealed the existence of a self-amplifying positive feedback loop between an excess cytokine release by macrophages and the progressive abnormal elevation of circulating norepinephrine levels in patients. In some instances, this expanding positive feedback loop may progress into an uncontrolled severe lethal inflammatory response called a "cytokine storm" or cytokine release syndrome.<sup>6</sup>

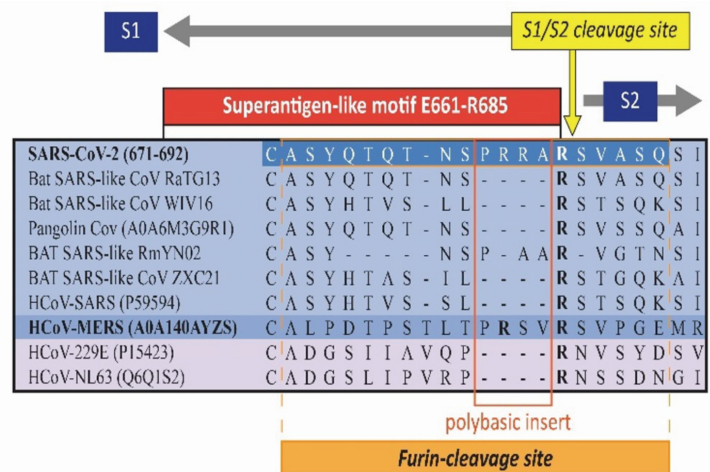
The fact that cytokine storms may appear in some late-stage COVID-19 patients prompted a search for a causative component common to both a natural SARS-CoV-2 infection

and the COVID-19 mRNA vaccines.<sup>1</sup> This commonality appears to be located in a short amino acid sequence in the S1 spike protein of the virus.

## Toxic "Superantigen" Motif in the mRNA Vaccines

Extensive writings about the furin cleavage site (FCS) of the spike protein of the SARS-CoV-2 virus largely ignore a preceding curious stretch of 20 amino acids (segment T678 to Q690) embedded in the S1 monomer of the trimeric spike protein. This segment harbors a unique sequence motif that is not present in other known SARS-related  $\beta$ -coronaviruses.

In September 2020, a computational molecular modeling study revealed that this amino acid region is similar both in function and three-dimensional structure to the functional motif of staphylococcal enterotoxin B (SEB) (amino acid sequence T150NKKKATVQELD161), a potent bacterial "superantigen" (SAg) toxin produced by Gram-positive *Staphylococcus aureus* bacteria (Figure ).<sup>7,8</sup> It is of note that the SEB toxin itself is potent enough to be classified as a category B select agent and that it was manufactured on a large scale as a biological weapon during the 1960s (William C. Patrick III, personal communication, Jul 11, 1998).



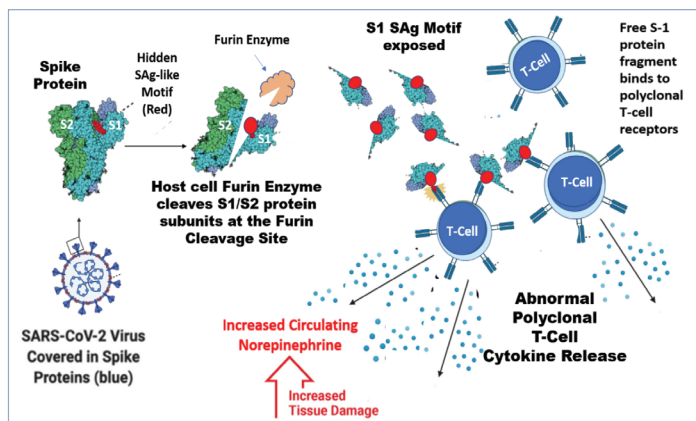
**Figure 1.** Sequence Alignment of SARS-CoV-2 and Related Strains Near the S1/S2 Cleavage Site. Viruses belonging to the same lineage are shown by the same color shade. The only other strain with a furin-like cleavage site is the distantly related MERS coronavirus highlighted in bold font. So far, the polybasic insert PRRA and the upstream SAg-like motif of SARS-CoV-2 has not been found in any closely related SARS-like coronavirus. (Illustration courtesy of Brian Scaglione at the P-Value Group)

The spike protein of SARS-CoV-2 is a trimeric trans-membrane protein responsible for host cell recognition, attachment, and entry of the virus. It features two extremely large monomers termed S1 and S2, each containing roughly 1,300 amino acid residues. These are fixed to a protein backbone to form the complete trimeric three-dimensional spike protein structure.<sup>9</sup>

### Furin Cleavage Site Enclosed within the SAg-like Motif of the S1 Protein Subunit

Upon the cleavage of the viral PRRA polybasic insert by the furin enzyme located on the host cell surface, the S1 protein is released into the extracellular space and the 20-amino acid SAg-like sequence similar to SEB, is available to undergo a high-affinity general binding to T-cell receptors (TCRs) largely irrespective of their antigenic clonality. This polyclonal TCR binding ability is common to both SARS-CoV-2 and the spike protein-based mRNA vaccines when the S1 protein is cleaved.

After intracellular viral replication, the new daughter viruses are transported back to the surface of the cell where they are released to infect more normal cells. As part of this process, the virus's S1 monomer in the new daughter viruses is cleaved at the FCS, and it changes its three-dimensional conformation from a closed into an open configuration (Figure 2). This now-open conformation exposes the amino acid sequences forming the SEB SAg-like motif of the cleaved S1 subunit to the outside where they are accessible for binding to TCRs found on circulating cytotoxic T-cell lymphocytes to cause large-scale polyclonal T-cell activation and proliferation. This leads to a massive production of circulating proinflammatory cytokines (cytokine storm) that includes IFN $\gamma$ , TNF $\alpha$ , and IL-2 from T cells, as well as IL-1 and TNF $\alpha$  from macrophages.<sup>8</sup> This can cause a progressive positive feedback loop between elevated cytokine levels and circulating norepinephrine levels, leading to severe cardiac damage.<sup>9</sup>



**Figure 2.** Conformation Changes in the Free-floating Fragment of the S1 Protein after Furin Cleavage of the SARS-CoV-2 Spike Protein

### Clinical Effects of Superantigen Exposure

SEB is one of the most potent bacterial superantigens known to science, and it can generate severe clinical symptoms in humans at concentrations as low as 0.0004  $\mu\text{g}/\text{kg}$  with death from multi-organ failure and toxic shock at 0.02  $\mu\text{g}/\text{kg}$  of body weight.<sup>10</sup>

Severe pulmonary exposure to the purified SEB superantigen causes fever, chills, headache, and muscle pain, with reactive interstitial and later pulmonary edema. This is similar to late-phase COVID-19 infection in adults who may progress into an adult respiratory distress syndrome (ARDS) and septic shock.<sup>1</sup>

The inflammatory cytokine release from T-cells and macrophages induced by the bacterial SEB SAg toxin has a profile similar to the cytokine profile in COVID-19 patients with a prognosis of severe infection and death.<sup>12</sup> This includes elevated levels of IL-6, TNF $\alpha$ , IL-8, and IL-1 $\beta$ , which lead to multiorgan tissue damage.<sup>12</sup>

Such an abnormal cytokine profile is also observed in infected children that develop COVID-19-associated multisystem inflammatory syndrome (MIS-C),<sup>13,14</sup> and in COVID-19 "long hauler syndrome."<sup>15</sup> A cohort analysis of adult COVID-19 patients reveals that cases with severe hyperinflammatory disease also exhibit a marked skewing of the TCR repertoire that is consistent with superantigen activity.<sup>7</sup>

Another study indicates that the rare SARS-CoV-2 spike protein mutation (D839Y/N/E) detected in a European strain of the COVID virus may potentially further enhance TCR binding in late-phase COVID-19 patients.<sup>7</sup>

It is therefore reasonable to assume that the hyperinflammatory syndrome of COVID-19 originates from the superantigen sequence embedded in the S1 monomer of the spike protein in both SARS-CoV-2 and the mRNA vaccines.

It is also reasonable to propose that in some cases the superantigen motif in the spike protein of SARS-CoV-2 can trigger an uncontrolled positive feedback loop between excessive cytokine release and excessive norepinephrine release, causing both cytotoxic and ischemic myocardial damage. This is expected to occur in natural COVID-19 infections and in some mRNA vaccine recipients, as well as in rare cases of the multisystem inflammatory syndrome (MIS-C) observed in COVID-19-infected European and American children.<sup>3, 14</sup>

### More Studies of Myocardial Injury Urgently Needed

More data on norepinephrine-induced myocardial injury in mRNA vaccine recipients are urgently needed. Studies must focus on new immunizations involving the original mRNA vaccines, and they should not include Omicron and its subvariant clinical cases or recipients of the new Omicron mRNA vaccine, because of the extensive mutational changes that have occurred in the spike protein of these new variants.<sup>16</sup> Omicron exhibits mutations in the P681RRAR685 putative SAg-like segment of the S1 subunit with possible changes in spike protein cleavage and exposure of the C-terminal end,

with changes in viral fitness, infectivity, and apparently a lower virulence.<sup>17</sup>

Repeated flow-cytometry assessments and measurements of circulating catecholamine levels, particularly norepinephrine, should be performed on new recipients of the original Moderna and Pfizer vaccines. Existing autopsy material could also be re-examined and partially characterized via immunohistochemical studies.

Early clinical use of  $\beta$ 2-receptor antagonists to diminish any developing positive feedback loop between catecholamine-driven signaling and hyperinflammation in active COVID-19 infection, long-hauler syndrome, and non-Omicron post-vaccine injuries should also be studied.<sup>18</sup>

Potential therapeutic agents, already long approved by the Food and Drug Administration (FDA), include anti-inflammatory drugs such as the antibiotic minocycline<sup>19</sup> and the inexpensive drug indomethacin (which has shown value in SARS1 infection).<sup>20</sup> These should be tested in combination in animal models of SEB-induced toxic shock syndrome. Other agents to test include CTLA4-Ig, which can inhibit CD28 costimulation of cytotoxic T-cells,<sup>21</sup> and the mTOR inhibitor rapamycin.<sup>22</sup>

### Origin of the Superantigen Sequence

The SAg sequence embedded in the SARS-CoV-2 virus lacks any defined viral evolutionary lineage. No other SARS-related  $\beta$ -coronaviruses outside of SARS-CoV-2 is known to have this SEB-analog sequence. As noted above, the SEB toxin has been manufactured on a large-scale as a biological weapon.<sup>23</sup>

Lacking a defined natural evolutionary lineage for the SEB-like motif in the S1 monomer, we must consider a laboratory origin for SARS-CoV-2. The presence of the SAg-like motif was not detected by normal protein or nucleotide Basic Local Alignment Search Tool (BLAST Search) efforts. It was only discovered after a structure and dynamics analysis. With respect to an artificial origin of SARS-CoV-2, the Wuhan Institute of Virology is often mentioned. However, the creation of the mRNA for this S1 SEB SAg-like sequence would have required full structure and dynamics analyses necessitating the cooperation of a well-resourced advanced research laboratory with a computational protein modeling capability. This would include an experienced proteomics facility with specialized equipment and expertise not generally available in a dedicated virology laboratory.

During the 1990s, the former Soviet Biopreparat offensive biological weapons program was experimenting with the genetic insertion of peptide toxins and immunogenetic peptides (myelin basic protein) into known biological warfare pathogens (Kanatjan Alibekov, personal communication, 1998).<sup>24</sup> Also note that beginning in 2003 a number of senior Chinese officials started to advocate that China continue this type of gain-of-function Soviet offensive biological warfare research.<sup>25,26</sup>

Although the majority of SARS-CoV-2 infections to date have been accompanied by a very low mortality rate, if the SEB SAg-like motif present in the virus proves to be an intentional genetic insert, this technology represents a dramatic advancement in offensive biological warfare using

incapacitating agents. As has now been witnessed, this constitutes a direct, severe strategic threat to the U.S. and its allies.

### Unconscionable Delay

Although the discovery of the SEB-like functional superantigen motif in the spike protein of SARS-CoV-2 was published as an open-access report in the *Proceedings of the National Academy of Sciences* on Sept 28, 2020,<sup>7</sup> the clinical significance of the SAg-like motif in the spike protein of the virus is only now receiving attention and debate. Yet millions of Americans and Europeans have already been injected with this mRNA sequence as part of the global mRNA vaccination program. The mRNA produced by these injections can remain in the body for months.

When published in 2020, the in-silico computational modeling report should have triggered an immediate vaccine review by Dr. Anthony Fauci and the COVID-19 Task Force, Dr. Francis Collins at the National Institutes of Health, Professor Arnold Monto at the University of Michigan (chairman of the outside panelists at the FDA Center for Biologics Evaluation), FDA Commissioner Dr. Stephen Hahn, Dr. Janet Woodcock, and Dr. Peter Marks. It also should have included Moncef Slaoui, Ph.D., who was tasked to manage the Operation Warp Speed project, as well as representatives from the vaccine manufacturers.

As far as I can determine, no such meeting concerning the COVID-19 superantigen motif ever occurred, yet questions over mRNA vaccine safety had appeared as early as February 2021. By March 2021, more than 20 European countries had temporarily stopped using AstraZeneca's COVID-19 vaccine pending a safety review. In April 2021, an 11-day hold was placed on the Johnson & Johnson mRNA vaccine. The extensive serious adverse events associated with the Moderna and Pfizer vaccines are currently the subject of continuing discussion, with many countries now moving away from their ineffective mRNA vaccination mandates.

### Vaccine Adverse Events

Throughout 2021 and into 2022, the number of recorded serious adverse post-injection clinical events has dramatically increased, even as hesitant Americans have been under intensifying duress to receive these still highly experimental, non-FDA-approved mRNA vaccines. Additionally, clinically unnecessary childhood vaccinations using these preparations are being intensely promoted.

In contrast to the reported annual 39 deaths attributed to the influenza vaccine, with roughly 43 percent of the U.S. population vaccinated,<sup>27</sup> thousands of COVID-19 vaccinations-associated deaths have been reported to the Vaccine Adverse Event Reporting System (VAERS),<sup>28</sup> with roughly 66 percent of the total population vaccinated.

### Conclusion

The mechanism of severe adverse reactions, especially myocarditis, in both COVID-19 patients and in COVID mRNA



vaccine recipients may be related to the superantigen motif in the spike protein of the virus, for which the vaccine mRNA codes. Yet, the reported presence of this SAg has not been openly discussed, and its origin is not clear. Urgent study is needed of this or any other potential mechanism of cardiac injury in COVID-19 and COVID-19 vaccinated patients. Officials responsible for failure to act on information pertaining to potentially disastrous vaccine-induced damage should be held accountable.

**Steven J. Hatfill, M.D.,** served as a daily outside senior medical advisor to the Executive Office of the President throughout 2020.

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