In the majority of cases, a life-threatening toxic shock syndrome (TSS) is triggered by toxin-producing strains of *Staphylococcus aureus* or *Streptococcus pyogenes* that overstimulate the adaptive immune system. Clinical cases of TSS involving other bacteria have also been reported. Bacterial superantigens (SAgs) constitute a family of exotoxins that bind directly to major histocompatibility complex (MHC) class II and T cell receptors (TCRs) to drive extensive T cell activation and cytokine release. SAgs can activate up to 20% of the body's T-cells. Although these toxins have been implicated in serious diseases, including TSS, the specific pathological mechanisms remain unclear. TSS leads to rapid and severe shock, multiple organ failure, and death. Given the similarity between the TSS and severe COVID-19, the authors from Canada investigated the similarities between sequences of SARS-CoV2 spike (S) protein and bacterial superantigens. They also evaluated whether regions of the S protein can directly stimulate T-cells like SAgs.

In 2020, Cheng MH et al. were the first to discover, by structure-based computational modeling, a sequence motif unique to SARS-CoV-2 (not present in other SARS-related coronaviruses), which is highly similar in both sequence and 3-dimensional structure to the bacterial superantigen staphylococcal enterotoxin B. They reported that a high-affinity SAg-like sequence motif is located near the S1/S2 cleavage site. This region containing the SAg-like motif also exhibited a high affinity binding to TCRs. Staphylococcal enterotoxin B is known to interact with the TCR and CD28, a differentiation antigen expressed on thymocytes and most mature T-cells. Staphylococcal enterotoxin B triggers a large-scale T-cell activation, resulting in massive production of proinflammatory cytokines, typical for TSS. The authors hypothesized that exposure of the SAg-like region of the SARS-CoV-2 S protein leads to T-cell activation, cytokine storm, and hyperinflammation in children with MIS-C and adult patients with severe COVID-19. (Cheng MH et al. Superantigenic character of an insert unique to SARS-CoV-2 spike supported by skewed TCR repertoire in patients with hyperinflammation. PNAS 2020: 117 (41) 25254-25262.)
https://doi.org/10.1073/pnas.2010722117

In addition, Dakal TC applied an integrative computational approach in 2021 to determine the molecular similarity of antigenic sites predicted in the receptor binding domain (RBD) of the SARS-CoV-2 S protein with proteins/antigens of other pathogens. He found that seven antigenic sites in the S protein RBD showed molecular similarity with 54 antigenic determinants of fifteen pathogenic bacteria, parasites, and viruses, including the enterotoxin A from *Staphylococcus aureus,* and M protein from *Streptococcus pyogenes.* The author stated that antigenic sites of the SARS-CoV-2 RBD, which display molecular

similarity with highly potent antigenic determinants of other pathogens, are expected to induce exacerbated innate and adaptive immune responses with hyperactivation of B-cells, T-cells, DCs, NK-cells, and macrophage/monocyte lineage cells, and the excessive release of cytokines that adversely affect numerous vital organs. https://discovermednews.com/molecular-similarities-between-sars-cov-2-rbd-and-pathogens/

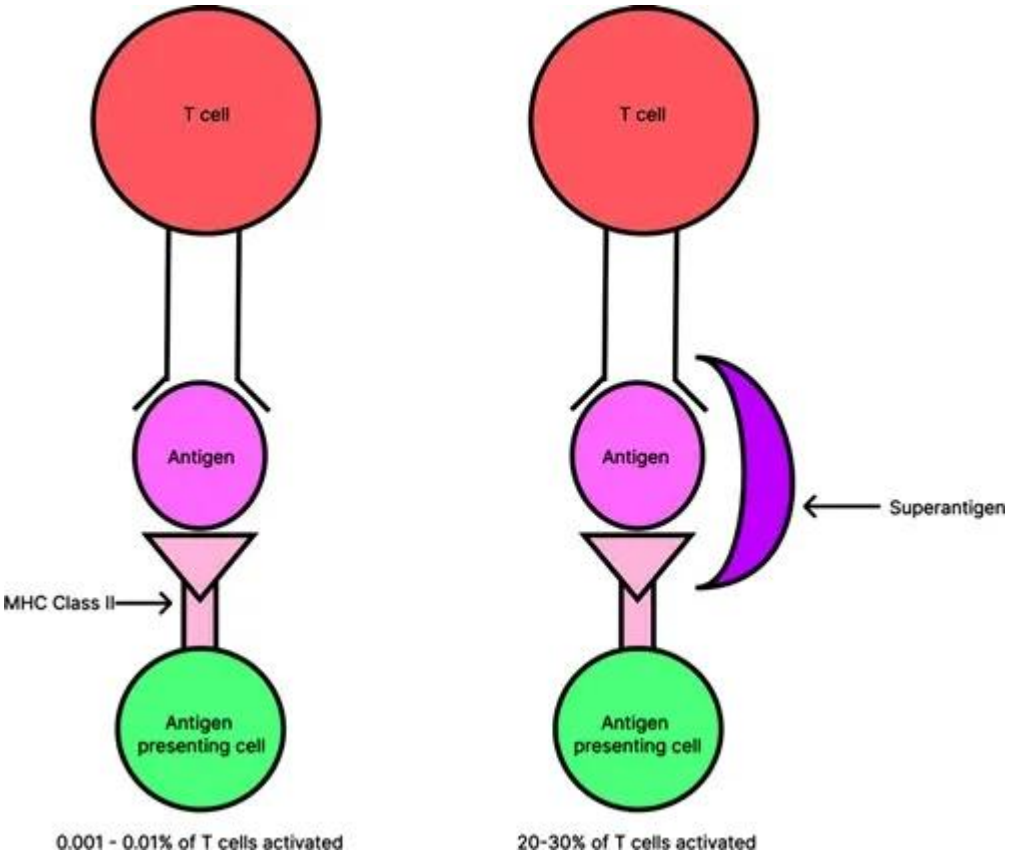
About the study

The authors initially compared the sequences of the SARS-CoV2 S protein, in particular the regions in the S2 subunit of the SARS-CoV2 S protein with staphylococcal enterotoxins B (SEB) and H (SEH) that induce septic shock syndrome. Focusing on P3, the scientists also investigated whether this peptide can activate human T-cells from the peripheral blood of healthy donors and induce the activation markers CD69 and Ki67. CD69 is an activation antigen induced by the TCR ligation, and Ki67 is a nuclear protein expressed by cycling and recently divided cells, but not by naïve or resting cells.

Results

The first comparison identified a region in the S2 subunit of the S protein (referred to as P3) with sequence similarity to bacterial superantigens SEB and SEH. Computational docking analysis revealed that P3 binds to overlapping sites on MHC class I and class II antigens and the TCR, that are also bound by staphylococcal enterotoxins B and H. For example, P3 and staphylococcal enterotoxin H shared three binding residues on TCRα and two on TCRβ.

While the initial search identified the P3 peptide, later analysis showed the sequence termed P3b. Both peptides bound to a similar region between MHC class II and I and the TCR. Consistent with its superantigen-like properties, P3 and P3b induced the proliferation of 25–35% of CD4+ and CD8+ T-cells, higher than the expected 0.0001-0.001% induced by conventional peptide antigens. The P3b was less effective in the activation of T-cells. This stimulation resulted in the upregulation of various activation markers and the increased transcription of the pro-inflammatory cytokine interleukin (IL)-6.



Further, stimulation with P3 resulted in an expansion of the focused TCR repertoire. This selective expansion of specific TCR Vα and Vβ chains is consistent with the superantigenic nature of P3, as reported for the bacterial superantigen SEH.

The P3 stimulated multiple rounds of cellular division (≥5 cycles) in both CD4+ and CD8 + T lymphocytes, accompanied by upregulation of the proliferation marker Ki67. Notably, the P3 showed a superior CD8 + T-cell stimulatory capacity compared to the smaller peptides SEB and SEH.

Lastly, *in vivo* studies in mice revealed that P3 administration led to elevated levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α.

Since a small peptide, P3 from the S2 subunit of the SARS-CoV2 S protein was able to induce unexpected proliferation of T-cells, the scientists also investigated a homology of P3 and P3b in the gene databank. The results showed that P3 shares sequence similarities with several endogenous proteins in mammalian cells, including cancer-associated gene 1 protein, E3 ubiquitin ligase TRIM35 isoform 2, MAP3K7 C-terminal-like protein isoforms 2, 3, X1, 7, 6, and 1, prolactin-releasing peptide receptor, and others. According to the authors, the peptides derived from endogenous proteins and related to the P3 protein may contribute to human inflammatory or autoimmune responses.

Conclusion

This study showed similarities between SARS-CoV2 spike protein and bacterial superantigens. The results identified a small peptide P3 in the S2 subunit of the SARS-CoV2 S protein with sequence homology to staphylococcal enterotoxins B and H and the capacity to induce unexpected proliferation of T-cells. Computational modeling predicts the binding of P3 to sites on the MHC class I/II and the TCR that partially overlap with the binding sites of staphylococcal enterotoxins B and H. While the clinical significance of P3 in COVID-19 remains unclear, its homology to other mammalian proteins suggests a potential role for these peptides in human inflammation and autoimmunity.

This article was published in Communications Biology.

Journal Reference

Hien Tu T, Ezzahra Bennani F, Masroori N etal. The identification of a SARs-CoV2 S2 protein derived peptide with super-antigen-like stimulatory properties on T-cells. Communications Biology 2025; 8:14. https://doi.org/10.1038/s42003-024-07350-8