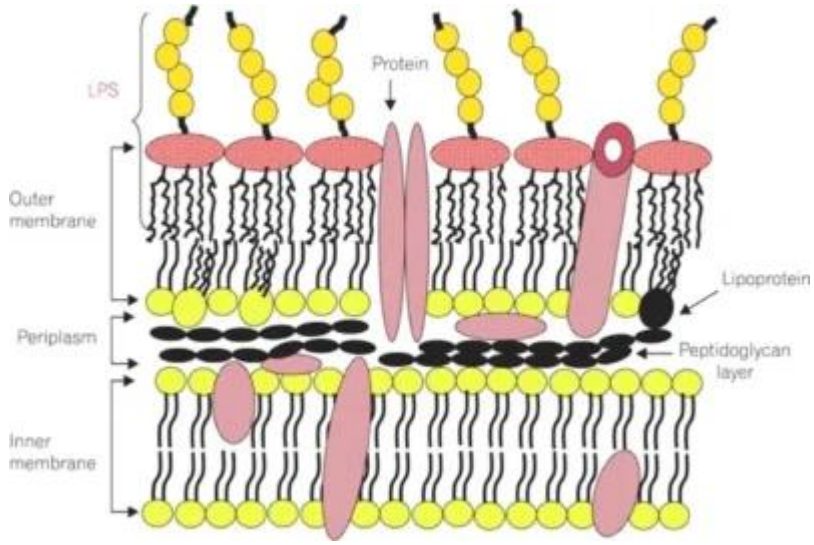
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The S protein appears to be a major pathogenic factor that contributes to the unique pathogenesis of SARS-CoV-2. The consortium of authors from Sweden, Singapore, and Denmark, in several studies, investigated the specific interaction between the SARS-CoV-2 spike (S) protein and bacterial lipopolysaccharide (LPS).

The LPS is the main component of the outer membrane of Gram-negative bacteria. The toll-like receptor 4 (TLR4) recognizes LPS; this interaction activates the TLR4 pathway and massive release of cytokines. Overstimulation of the TLR4 pathway by LPS triggers a hyperinflammatory state that can lead to sepsis and acute respiratory distress syndrome (ARDS).



The outer membrane of Gram-negative bacteria

The S protein, a major pathogenic factor in the unique pathogenesis of SARS-CoV-2, is a glycosylated homotrimer with each monomer composed of subunits S1 and S2. The S1 subunit is composed of the N-terminal domain (NTD), the receptor binding domain (RBD) with a receptor binding motif, and two C-terminal domains (CTD).

Previous studies have shown that patients with severe COVID-19 and non-survivors had significantly elevated levels of bacterial LPS during hospitalization. Also, according to previous data, patients with elevated levels of bacterial LPS due to gut dysbiosis and translocation of bacterial components into the systemic circulation are at higher risk of

developing severe COVID-19 with sepsis and ARDS.

About the Studies and Results

The interaction between the SARS-CoV-2 S protein and lipopolysaccharide

In 2020, Petruk G et al. showed a previously unrecognized interaction between the SARS-CoV-2 S protein and bacterial LPS. They have found that a combination of low concentrations of LPS and the SARS-CoV-2 S protein boosted inflammatory responses in human peripheral blood mononuclear cells (PBMC) and in monocytic THP-1 cells, an immortalized monocyte-like cell line derived from the peripheral blood of a childhood case of acute monocytic leukemia.

The S protein alone did not increase the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates multiple aspects of innate and adaptive immune functions as a pivotal mediator of inflammatory responses. But, a combination of the S protein and extremely low concentrations of LPS significantly enhanced the activation of NF-κB in monocytic THP-1 cells and induced significant boosting of cytokines directly dependent on NF-κB activation, such as tumor necrosis factor-alpha and interleukin-6 in human PBMCs.

The S protein preparations were contaminated with LPS, and researchers took this into account. The incubation of constant concentrations of SARS-CoV-2 S1 or S2 subunits with increasing concentrations of LPS for 20 hours in monocytic THP-1 cells demonstrated that the NF-κB levels and proinflammatory activity of the S2 subunit correlated with the presence of LPS contaminants. This effect was not seen for the S1 subunit.

The experiments were repeated with polymyxin B, a neutralizer of LPS. The administration of polymyxin B suppressed the activation of NF-κB by the S2 subunit alone or the S2 subunit mixed with LPS, indicating an LPS-mediated effect by the S2 preparation. This result was not seen for the S1 subunit alone or S1 mixed with LPS, indicating that the S1 subunit did not display a boosting effect on LPS-induced inflammation *in vitro.*

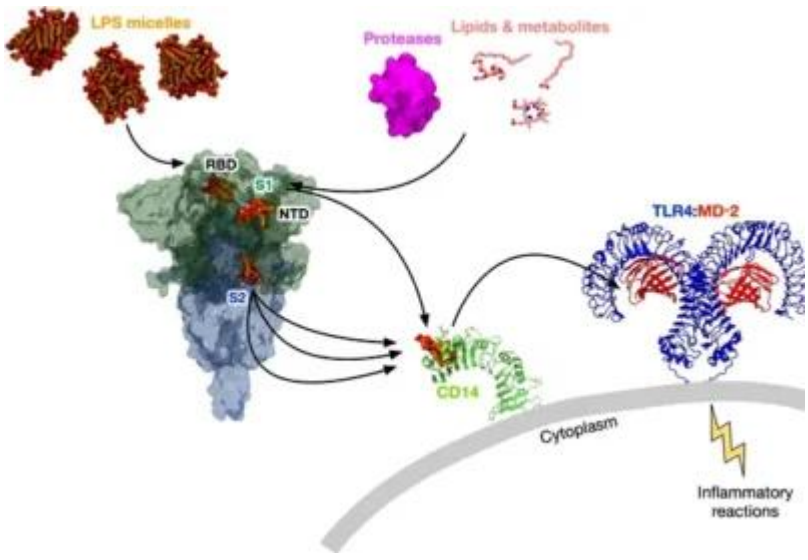
In vivo administration of the S1 subunit alone did not result in any measurable boosting of NF-κB activation. However, the administration of the S1 in combination with subcutaneously administered LPS resulted in a significant proinflammatory response. These results show that the S1 subunit displayed a boosting effect on LPS-induced inflammation *in vivo* but not *in vitro*, suggesting some additional mechanisms in the former.

Interestingly, the LPS-binding capability and proinflammatory boosting effect of the Omicron S protein were reduced compared to those of the Wuhan strain both *in vivo* and *in vitro*.

Complexes of the S protein and LPS can form stable aggregates

Electron and fluorescence microscopy, used to examine the size of the S protein aggregates before and after the LPS challenge, showed significantly larger S protein aggregates formed in the presence of LPS than those formed by the S protein alone. Administration of TCP-25 peptide, which blocks the LPS-triggering effect, reversed the observed S protein aggregation. According to the authors, these results confirmed the role of LPS in this process and showed that complexes of the S protein and LPS can form stable aggregates.

The investigation of regions with positive aggregation propensity scores (indicating a high propensity for aggregation) revealed two LPS-binding pockets with positive aggregation propensity scores on the S protein, loop 246–250 on the NTD, and loop 621–624 near the C-terminal domain 2 (CTD2). Importantly, the previous *in vitro* studies demonstrated that the S protein forms amyloid fibrils, and peptide 601–620, which is one of three peptides (192–211, 601–620, and 1166–1185) that meet the criteria for amyloid fibrils, is adjacent to the LPS-binding pocket 621–624.
https://febs.onlinelibrary.wiley.com/doi/full/10.1002/1873-3468.14490



The original figure from the article by Samsudin F et al. Journal of Molecular Cell Biology (2022)

Conclusion

These studies have shown that binding of bacterial LPS at multiple sites of the SARS-CoV-2 S protein enhanced proinflammatory responses *in vitro* and *in vivo* and led to S protein aggregate formation. According to the authors, the S protein acts as a mediator rather than a direct cause of hyperinflammation.

These findings have established a significant link between excessive inflammation during SARS-CoV-2 infection and comorbidities associated with increased levels of bacterial endotoxins. This synergism between LPS and the S protein is of clinical and therapeutic importance.

In addition, as some recent computational studies showed that the S protein could bind to several aggregation-prone amyloid proteins, it is imperative to investigate whether LPS, under certain conditions, may trigger *in vivo* aggregation of the S proteins and the formation of amyloids through the interaction between the S protein/LPS complexes and other amyloidogenic proteins.

These articles were published in the Journal of Molecular and Cell Biology and FEBS Letters.

Journal References

Petruk GPM et al. SARS-CoV-2 spike protein binds to bacterial lipopolysaccharide and boosts proinflammatory activity. Journal of Molecular Cell Biology 2020;12 (12):916–32. (Open Access) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7799037/

Petrlova J, et al. SARS-CoV-2 spike protein aggregation is triggered by bacterial lipopolysaccharide. FEBS Letters 596 (2022) 2566–2575. (Open Access) https://febs.onlinelibrary.wiley.com/doi/full/10.1002/1873-3468.14490

Samsudin F et al. SARS-CoV-2 spike protein as a bacterial lipopolysaccharide delivery system in an overzealous inflammatory cascade. Journal of Molecular Cell Biology (2022), 14(9), mjac058 (Open Access) https://academic.oup.com/jmcb/article/14/9/mjac058/6761401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