Rapid Reviews COVID-19

Review 1: "SARS-CoV-2 spike protein as a bacterial lipopolysaccharide delivery system in an overzealous inflammatory cascade"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

The study claims are very well-described through the different analytical methods used. There is little room for doubt for the study. It shows very similar results and conclusions as compared with the hypothetical ideal study.

The paper entitled "SARS-CoV-2 spike protein as a bacterial lipopolysaccharide delivery system in an overzealous inflammatory cascade" is an interesting piece of article. The research suggests the lipopolysaccharide (LPS) bound in the different hydrophobic pockets of the S-glycoprotein. In this study, researchers stated that LPS binds with the S1 pocket with a higher affinity compared to the S2 pocket of the S-glycoprotein. Consecutively, it can interact with the TLR4, and finally, TLR4 activation triggers the nuclear factor-kappa B (NF-κB) cascade. The study concluded that the S-glycoprotein of SARS-CoV-2 might assist in delivering the LPS to activate the TLR4 cascade. In general, the data presented supports the conclusion that the S-glycoprotein might act as a delivery system during the S-glycoprotein mediated the LPS -TLR4 interaction.

The study developed a hypothesis that LPS binds TLR4, which activates related immune responses. The study is fascinating and illustrates a novel research finding.

The study performs different analytical methods such as S protein expression and purification study design, deuterium labeling and quenches conditions, mass spectrometry, and peptide identification, SDS-PAGE, blue native (BN)-PAGE, microscale thermophoresis, unbiased molecular dynamics simulations, potentials of mean force (PMF) calculations, MTT assay, mouse inflammation model and in vivo imaging to make the claim.

The HDXMS (hydrogen-deuterium exchange mass spectrometry) assay confirms LPS binding activity with the S2 pocket of the S-glycoprotein and detects the binding event to the RBD pocket and NTD pocket. Using native gel electrophoresis, Samsudin et al. illustrated the interaction of LPS with both the subunits of the S-glycoprotein (S1 and S2 subunits) in the analysis. PMFs calculation was supported inside an MD (molecular dynamics) simulation set-up, which was again supported by MST (microscale thermophoresis binding) assay. The study design showed that LPS binds with the NTD pocket and RBD pocket on the S1 subunit. At the same time, the S2 was noted to be a weaker binding pocket in contrast with the LPS receptor (CD14). It might play a vital role as an intermediate agent of the cascade to the LPS receptor. Moreover, mice experiment NF- κ B reporter assay was performed to understand the enhanced inflammatory response with both the subunits of the S-glycoprotein. Finally, the researchers tried to illustrate a molecular mechanism of hyper-inflammation events. It describes that LPS-mediated hyper-inflammation emerges due to the LPS delivery to the LPS receptor (CD14), and finally to the TLR4:MD-2 complex where the S-glycoprotein act as a delivery agent.

Conclusion: The research is very significant as it tried to describe the LPS-mediated hyper-inflammation in SARS CoV-2 infection and great interest to future researchers. The study attempted to open a novel view to describe the hyper-inflammation among severe COVID-19 patients in the current pandemic.

However, some questions are still unknown, and I urge future researchers to understand more about the hypothesis and comprehend the molecular mechanisms. The questions are:

1. It has been illustrated that LPS binds with the different hydrophobic pockets of the S-glycoprotein. Understanding more about the dynamic events of LPS dissociation from the S-glycoprotein and other interaction events with the LPS receptor (CD14) should be needed.
2. At the same time, future researchers should illustrate the factors which will help to dissociate the LPS from the S-glycoprotein and further interact with TLR4 /MD-2 complex directly.
3. Researchers should explain any synergetic effect during the lipid A/LPS binding to S-glycoprotein (both the S1 pocket and the S2 pocket) of SARS-CoV-2 compared to the LPS binding with the S-glycoprotein.
4. At the same time, PMF calculations and other assays should be performed to illustrate more about both the binding events of the S1 pocket and S2 pocket and

compare and comprehend more regarding both of the binding events.

5. Scientists should explain the minimum and maximum concentration /amount and their dynamic state of lipid A/LPS to intermediate events to interact with TLR4 /MD-2 complex and, finally, to trigger the nuclear factor-kappa B (NF- κ B) cascade for LPS-mediated hyper-inflammation.
6. Simultaneously, different interaction models should be developed, such as LPS- S-glycoprotein interaction, LPS- LPS receptor (CD14), and LPS- TLR4 /MD-2 interaction.
7. Binding pocket modeling is a critical study for drug discovery. Therefore, future researchers should perform experiments illustrating the binding pocket modeling during the different interactions such as LPS- S-glycoprotein, LPS- LPS receptor (CD14), and LPS- TLR4 /MD-2. At the same time, future studies should be developed different 3D models, which can provide an inside understanding of the structural interface for interaction. This understanding will help to develop flawless therapeutic molecules, especially for the LPS-mediated hyper-inflammation through the structure-based drug development for SARS-CoV-2 infection.