Review 2: "SARS-CoV-2 Spike protein promotes hyper-inflammatory response that can be ameliorated by Spike-antagonistic peptide and FDA-approved ER stress and MAP kinase inhibitors in vitro"

Christoph Hölscher¹

¹Research Center Borstel, Infection Immunology, Germany

Published on: Nov 18, 2020

DOI: 10.1162/2e3983f5.c8bc6b2d

License: [Creative Commons Attribution 4.0 International License \(CC-BY 4.0\)](https://creativecommons.org/licenses/by/4.0/)

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 preprint "SARS-CoV-2 Spike protein promotes hyperinflammatory response that can be ameliorated by Spike-antagonistic peptide and FDA-approved ER stress and MAP kinase inhibitors in vitro" provides evidence that blocking of SARS-CoV-2 spike protein subunit S1 (CoV2-S1) interaction with ACE2 on human bronchial epithelial cells and inhibition of downstream endoplasmic reticulum (ER) stress and mitogen-activated protein (MAP) kinase-mediated inflammatory signals controls excess inflammation and prevents progressive lung tissue destruction in the context of COVID-19.

Severe COVID-19 is associated with an uncontrolled systemic hyperinflammation ("cytokine storm") and subsequent acute lung injury and acute respiratory distress syndrome. Hence, understanding the cellular and molecular mechanisms leading to the SARS-CoV-2-mediated inflammation is a prerequisite to therapeutically interfere with the development of severe COVID-19. This investigation finds that compared to counterparts of SARS-CoV-1, CoV2-S1 and the receptor-binding domain of CoV2-S1 (RBD) induces an ACE2-dependent enhanced release of pro-inflammatory cytokines and markers of cellular damage in bronchial epithelial cells. Blockade of CoV2-S1/ACE2 interaction by an antagonistic peptide impairs inflammatory responses. Because CoV2-S1 and RBD promotes early ER stress and MAP kinase activation inhibition by approved ER stress and MAP kinase inhibitors also ameliorate the hyperinflammatory response of epithelial cells. These results are consistent with findings in severe COVID-19 patients treated with the broad-spectrum immunosuppressant dexamethasone. This research, however, gives new insights into the inflammatory signaling cascade induced by CoV2-S1 and RBD and suggests more specific anti-inflammatory treatment options.

The strength of this manuscript lies in the classical but also consistent analysis of signal transduction events after stimulation of bronchial epithelial cells with and without inhibition of individual cellular signal transduction components. These analyses culminate in a valid concept in which the binding of CoV2-S1 to ACE2 via the induction of ER stress leads to the activation of MAP kinases and subsequently to the NF- κ B-mediated expression of pro-inflammatory cytokines. Based on this concept, three

possible interventions to control inflammation in the context of a severe COVID-19 disease are developed and were convincingly tested experimentally by the use of antagonizing peptides and FDA-approved inhibitors: the blocking of the CoV2-S1/ACE2 interaction, the reduction of ER stress, and the inhibition of MAP kinases.

Although this investigation makes an important contribution to the understanding of SARS-CoV-2-mediated hyperinflammation and resulting treatment concepts, there are some limitations that need to be addressed either in the scope of the present manuscript or in subsequent studies. (1) It remains unclear how CoV2-S1 and RBD were produced. However, this information is necessary with regard to a possible cell activation through endotoxin contaminations. (2) Even if CoV2-S1 present in soluble form *in vivo*, the question arises to what extent the here-described signal transduction cascade and cell activation is similar in terms of quality and quantity after infection with intact viruses. (3) The question also arises what effect an inhibition of ER stress and MAP kinase activation has on virus replication. (4) Since the manuscript essentially deals with treatment strategies for severe COVID-19 courses, a more extensive subsequent study in laboratory animals would be of great interest. (5) Finally, in this context, the here-presented effects and the resulting therapy options should be weighed against the now clinically established treatment of COVID-19 patients with dexamethasone.