Review 2: "The glycosylated extracellular domain of MUC1 protects against SARS-CoV-2 infection at the respiratory surface"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

Review:

Chatterjee *et al.* investigates an important topic of the possible role of mucins in SARS-CoV-2 infection of lung cells. By analyzing publicly available single-cell RNA sequencing data, the authors determine that several secreted and transmembrane mucins are co-expressed in ACE2-expressing lung cell subsets—with the most prominent expression of MUC1. They then test the protein expression of several of these mucins in lung cell line Calu-3 and determine that only MUC1 is expressed on the surface of the cells. The authors show that the extracellular domain of MUC1 can be cleaved off by mucinase StcE, which is used in various experimental settings throughout the rest of the manuscript. The authors conclusively show that treatment with StcE dramatically enhances the infectivity of Calu-3 cells with SARS-CoV-2 S pseudotyped VSV particles, and a less pronounced effect is seen with the authentic virus. Upon investigating co-expression of MUC1 and ACE2, the latter is found in all cells, whereas only a fraction of the cells express MUC1. In cells co-expressing the two molecules, MUC1 extracellular domain signal appears to be localized above the ACE2 signal. The authors conclude that MUC1 might be shielding ACE2 from the virus and removal of MUC1 extracellular domain enhances infection.

While the pseudotyped viral particle infectivity data is very striking, I do find the link to MUC1 problematic. First, based on presented immunofluorescence data, no more than 25 % of Calu-3 cells express MUC1, while most cells express ACE2. Thus, it is not very likely that cleaving off MUC1 on that fraction of cells would be the only cause for the dramatic increase in the proportion of cells infected with the pseudotyped virus. Second, the authentic virus infection experiments are performed at a very low multiplicity of infection, leading to only every one hundredth or so cell being infected,

making it even less likely for the cells previously presenting MUC1 to be solely responsible for the increase in infectivity.

Importantly, while clearly preferential to mucins, StcE can recognize and cleave a number of other O-glycosylated proteins containing shorter regions of dense glycosylation, as shown in recent glycoproteomic studies. Thus, it is possible that StcE treatment will reduce the complexity of the glycocalyx in general by cleaving off a number of other surface molecules in addition to Muc1, making easier viral access to ACE2, also on cells not expressing Muc1. While there is a clear increase in susceptibility to viral infection after StcE treatment, the presented data is not conclusive enough to support the main claim of the paper and assign the effect to the removal of the MUC1 extracellular domain. Experiments more exclusively addressing MUC1, such as gene knock-out experiments, would improve the plausibility of the claims.