SARS-CoV-2 variants as super cell fusers: cause or consequence of COVID-19 severity?

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The severity of COVID-19 correlates closely with lung damage, and syncytia are often observed in the lungs of patients who have developed fatal pneumonia (Braga et al., 2021). Although syncytia have been reported to form in cell monolayers in vitro upon SARS-CoV-2 infection, their contribution to lung injury and disease development in patients infected with any emerging SARS-CoV-2 variants remains largely uncharacterized (Buchrieser et al., 2020). Rajah et al. (2021a) examined the syncytia-forming potential of the SARS-CoV-2 alpha, beta, and delta strains. The authors also assessed the contribution of the individual spike-associated mutations to the fusogenicity of the alpha and beta variants, their binding to ACE2, and their evasion from the humoral immune response.

Rajah and colleagues first followed productive infection by the alpha and beta variants in cell lines and primary human airway epithelial cells and found that replication did not differ from that of a D614G strain, one of the earliest variants isolated with increased transmission. The authors then measured syncytia formation upon infection using split green fluorescent protein (GFP) technology combined with image-based analysis (Foglieni et al., 2017). The method involves splitting GFP into two nonfluorescent fragments, each expressed in a distinct cell population; in this system, GFP does not fluoresce until infected cells fuse and the two fragments assemble. Syncytia sizes were normalized against the number of nuclei to allow a quantitative characterization of the differences in fusogenicity. With this elegant approach, the authors demonstrated that the alpha and beta variants produce increasingly larger syncytia in infected cells than both the original Wuhan strain and the D614G variant.

These results suggested a higher fusogenicity and syncytia-forming potential of the spike proteins of emerging variants. To pursue this possibility, the authors overexpressed the spike protein from plasmids in their split GFP system. In this model, the spikes of the alpha and delta variants were the fastest to produce syncytia, followed by that of the beta strain. Based on these experiments, spike mutations appear to confer an advantage to SARS-CoV-2 not only for cell-free infection and spread in cell monolayers but also for syncytia formation. During natural infection, syncytia may contribute to virus persistence, viral spread, and immune evasion (Rajah et al., 2021b).

Next, Rajah and colleagues evaluated the contribution of all individual mutations associated with the spike protein to the ability of alpha and beta variants to bind to cellular receptors, escape neutralizing antibodies (nAbs), and induce cell–cell fusion. To this end, the authors genetically engineered and then overexpressed the spike proteins of the D614G strain with each mutation of alpha and beta variants on the cell surface. The soluble ACE2 ectodomain showed the highest affinity for spike proteins with an RBD bearing the N501Y mutation. Of the two variants carrying

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N501Y, only ACE2 binding to the alpha spike was significantly enhanced, whereas that to the beta spike was increased to a much lesser extent. In addition, although it had no mutations at position 501, the delta variant showed increased binding to ACE2.

Similar results were obtained when the spike mutants were assessed for either antibody escape or cell-cell fusion. For instance, the K417N mutation prevented detection of the beta variant spike by mAb48, but not by mAb98, while both antibodies targeted the RBD, and more mutations had a negative rather than a positive effect on syncytia formation. No clear correlation could be established between syncytia formation and the impact of spike mutations on receptor binding or antibody escape. Collectively, these results provide important evidence that combinations of mutations, not single mutations, underlie enhanced receptor binding and immune escape. This phenomenon presumably occurs through conformational structural changes in the spike, the details of which remain to be explored. Although the authors did not test nAbs for their ability to prevent syncytia formation, their work suggests an evolutionary trade-off of the spike protein between receptor binding, immune escape, and fusogenicity.

This study provides significant insights into the fusogenic potential of the alpha, beta, and delta variants of SARS-CoV-2, and as is often the case in science, has raised new questions: How the variants elicit a higher rate of transmissibility globally remains to be addressed. TMPRSS2 expression was found to abolish the apparent enhanced fusogenicity of the variants compared to the original Wuhan isolate. Beyond the question about the role of

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**Figure 1. Emerging SARS-CoV-2 variants induce enhanced syncytia formation.**

Lung injury correlates closely with COVID-19 severity. The original SARS-CoV-2 strain isolated in Wuhan in 2019 has been supplanted by many variants, most often with numerous mutations in the viral envelope glycoprotein spike. In infected cells, a fraction of the spike protein is expressed on the surface and can induce fusion between neighboring cells, leading to syncytia formation. In this study, Rajah and colleagues showed that spike proteins of emerging SARS-CoV-2 variants produce more and larger syncytia (shown in green) than the original Wuhan strain upon infection of cell monolayers. Spike mutations with a positive, negative, or no effect appear in green, red, and blue, respectively. Mutations in black were not tested in this study.
TMPRSS2 in producer and target cells in activating variant spikes on the cell surface, future work should address whether syncytia formation upon infection with the emerging SARS-CoV-2 variants contributes to disease severity or is a consequence (Fig 1).

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