Recombinant SARS-CoV-2 spike protein is not sufficient to initiate an inflammatory response in human alveolar epithelial cells in vitro

Despite an overwhelming body of research already produced in the wake of the COVID-19 pandemic, there is still much more required to understand the acute respiratory failure and hyperinflammatory state that can result from infection with SARS-CoV-2. One significant barrier to further elucidating these mechanisms is the requirement for biosafety level-3 (BSL-3) facilities to perform experiments with the complete SARS-CoV-2 virus. The aim of this research was to create an *in vitro,* non-infectious model of COVID-19, using recombinant SARS-CoV-2 spike protein (SP) to initiate an inflammatory response in human alveolar epithelial cells. Such a model would allow for the investigation of various pathways involved in the pulmonary inflammatory response that occurs in COVID-19. We hypothesized that treating A549 cells (a human alveolar adenocarcinoma cell line) engineered to express human angiotensin converting enzyme-2 (ACE2) with recombinant SP would be sufficient to initiate an inflammatory response.

To this end, full-length, recombinant SP was added to ACE2 expressing A549 cells at a dosage range of 100 ng/mL to 2 ug/mL. Cells were allowed to incubate with SP for 24 hrs., at which point mRNA was isolated from the samples. To observe the response induced by SP, qPCR was used to measure the relative expression of inflammatory cytokines IL-1β, IL-6, and TNF-α.

Our results show that recombinant SP does not cause a statistically significant change in the expression of the selected inflammatory markers at a 24-hour time point. This suggests that, in alveolar epithelial cells engineered to express the ACE2 receptor, the full-length SARS-CoV-2 SP alone may not be sufficient to trigger an innate immune response.

While we did not achieve the initial purpose of our investigation, to create a non-infectious model of COVID-19 that could be efficiently replicated by other groups, these results uncover important insights into the SARS-CoV-2 virus and will help guide future research into COVID-19.