SARS-COV-2 MEDIATES TGF-β HIJACKING AND IMMUNE DYSREGULATION THROUGH A NOVEL GAIN OF FUNCTION MUTATION IN ITS NSP15 PROTEIN. Lauren Miller, Kelsey Lesterberg, PhD; Adela Cota-Gomez, PhD; Kimberly Jordan, PhD; Jennifer McWilliams, PhD; J. David Beckham, MD; and James P. Maloney, MD. From the Departments of Medicine and Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, CO.

Rationale: The coronavirus disease of 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has killed millions. COVID-19 mortality remains high for those hospitalized with severe disease. The early immune suppression of SARS-CoV-2 and subsequent inflammation suggests its ability to cause host immune dysregulation is a key mechanism. Host Transforming Growth Factor β (TGF-β) is an immune-suppressing and profibrotic cytokine frequently exploited by microbes to evade immune detection. We discovered a KRFK amino acid domain in the SARS-CoV-2 nonstructural 15 (NSP15) protein, which is an activating motif for latent TGF-β, potentially explaining immune evasion features of SARS-CoV-2. We hypothesized that the SARS-CoV-2 NSP15 protein causes immune dysregulation by activation of latent TGF-β and subsequent activation of immunosuppressive T-regulatory (Treg) cells, and that substantial TGF-β is present in the lungs of COVID-19 acute respiratory distress syndrome (ARDS) patients.

Methods: We evaluated TGF-β1 concentrations in endotracheal aspirates (ETA) of 27 COVID-19 ARDS patients by Enzyme Linked Immunoassay (ELISA). We produced recombinant SARS-CoV-2 NSP15 protein in *E. coli* and tested its ability to activate latent TGF-β1 using *in vitro* assays. TGF-β inhibitors were assessed for their ability to block any NSP15 effects. We obtained blood mononuclear cells from healthy subjects and isolated T regulatory cells (Tregs) to assess their activation via intracellular smad-2 phosphorylation (pSMAD2) with flow cytometry.

Results: High concentrations of both active and total TGF-β1 were detected in ETA of COVID-19 ARDS patients (150 +/- 34 pg/ml active; 1,819 +/- 304 pg/ml total); these free TGF-β1 concentrations were in a range previously shown to affect T cell function. NSP15 at 2.4 nM increased activation of latent TGF-β (0.5 nM) 12-fold (vs. vehicle) ($p < .001$ vs. vehicle), compared to an 11% activation with the positive control thrombospondin-1 (TSP1; 10 nM). TGF-β inhibitors blocked NSP15 effects on latent TGF-β activation and intracellular TGF-β1 signaling in a bioassay by over 95% ($p < .01$). At tested concentrations (25, 50, 100 nM) NSP15 increased Treg pSMAD2 levels via activation of 2 nM latent TGF-β1, exceeding levels seen in Tregs stimulated with 400 pM of active TGF-β1 (positive control) (pSMAD2 + cells: vehicle 1.1%, active TGF-β1 43%, NSP15/latent TGF-β1 49-56%).

Conclusions: High concentrations of active and total TGF-β1 are present in the ETA of COVID-19 ARDS patients, suggesting SARS-CoV-2 uses host TGF-β hijacking as a mechanism for immune evasion. The NSP15 protein of SARS-CoV-2 potently activates latent TGF-β in vitro, leading to Treg activation as one mechanism of immune suppression and host evasion in early COVID-19 infection, while immune dysregulation and increased TGF-β1 airway levels may contribute to later fibroproliferative stages of ARDS. Current TGF-β inhibitors are potent inhibitors of NSP15 effects. A strategy to block NSP15-mediated effects with TGF-β inhibitors is an innovative therapy worthy of testing in COVID-19 prevention and treatment trials.