University of Colorado Anschutz Medical Campus

SARS-CoV-2 Mediates TGF-β Hijacking and Immune Dysregulation Through a Novel Gain of Function Mutation in its NSP15 Protein

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Objective

- After discovering the SARS-CoV-2 genome had a novel TGF-β activating sequence in its NSP15 protein, we hypothesized that the NSP15 protein causes immune dysregulation by activation of latent TGF-β and subsequent activation of immunosuppressive T-regulatory (Treg) cells
- We also hypothesized that substantial TGF-β is present in the lungs of COVID-19 acute respiratory distress syndrome (ARDS) patients

Methods

- In silico search for canonical latent TGF-β1 activation domains (KRFK, WXXW)
- Produced recombinant SARS-CoV-2 NSP15 protein in *E. coli* using artificial genes for NSP15
- Assessed recombinant NSP15 protein's ability to activate latent TGF-β1 (Luciferase signal) using Mink lung epithelial cell bioassay
- Evaluated TGF-β1 concentrations in endotracheal aspirates (ETA) of 32 COVID-19 ARDS patients and 23 non-COVID ARDS patients (ELISA)
- Assessed TGF-β inhibitors' ability to block NSP15 effects in Mink lung epithelial cell bioassay (smad-driven Luciferase reporter)
- Obtained blood mononuclear cells from healthy subjects and enriched for Tregs
- Assessed Tregs activation via intracellular smad-2 phosphorylation (pSMAD2) with flow cytometry

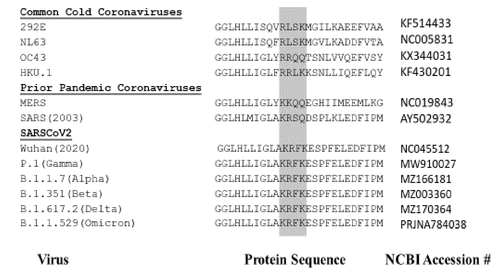
Demographics

	COVID	Non-COVID
Age (avg)	53.5	56
Gender		
Female	4	12
Male	27	11
BMI		
BMI (avg)	35.4	29
Race/Ethnicity		
Non-Hispanic White	9	15
Hispanic White	16	4
Black	3	2
Other	3	2
Other		
P/F Ratio (avg)	127.6	117.9
Hospital Days** (avg)	53.6	30.0
Vent Free Days* (avg)	2.0	7.4
Died	32.26%	43.48%

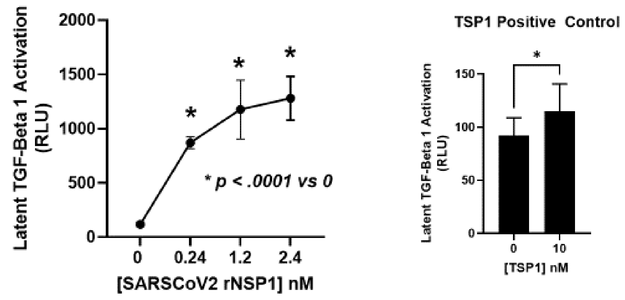
* Indicates p < 0.05, ** Indicates p < 0.01

Results

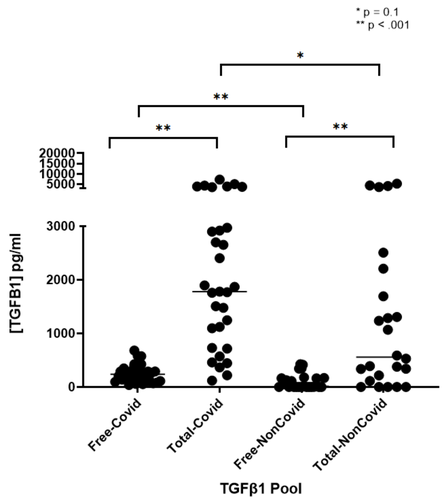
1. SARS-CoV-2 Has a Novel Latent TGF-β Activation Motif (Amino Acids KRFK) in its NSP15 Protein (absent in other Coronaviruses)

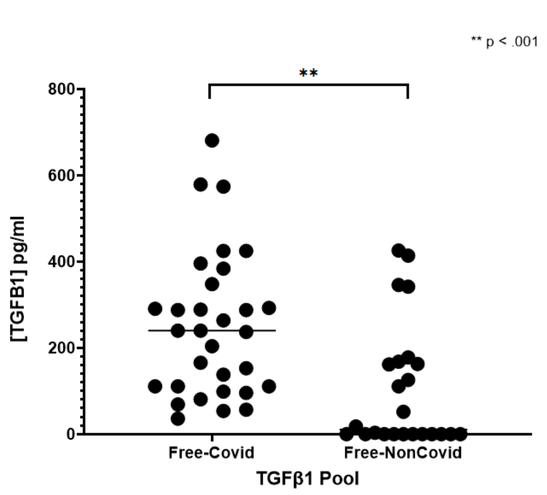


2. Recombinant SARS-CoV-2 NSP15 is a Potent Activator of Latent TGF-β1

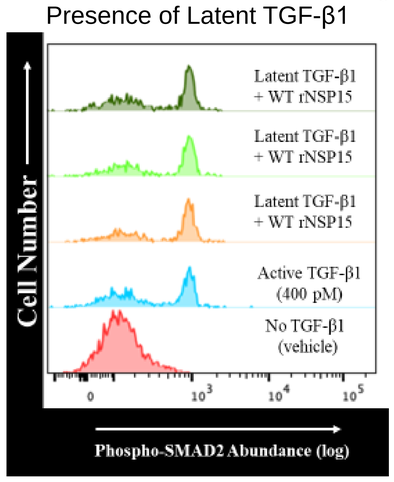


3. Total and Free TGF-β1 are Increased in COVID-19 ARDS ETA 2-3x Higher than in Non-COVID ARDS ETA



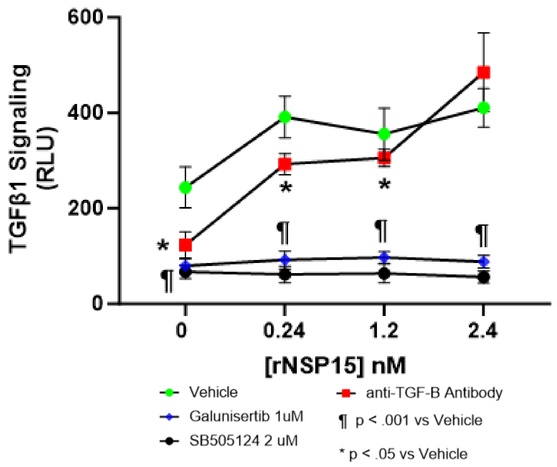


4. SARS-CoV-2 NSP15 Activates Tregs in Presence of Latent TGF-β1



25 nM, 50 nM, 100 nM rNSP15 (ascending panes)

5. TGF-β Inhibitors Block NSP15 Induced TGF-β1 Activity (Mink Lung Cell Bioassay)



Discussion and Conclusions

- **Mutations in the SARS-CoV-2 NSP15 protein (an endonuclease) created a gain-of-function KRFK latent TGF-β activation domain**
- **TGF-β1 concentrations in ETA of COVID-19 patients are 2-3x > vs. non-COVID** patients, suggesting SARS-CoV-2 uses host **TGF-β hijacking** as a mechanism for immune evasion
- **NSP15 protein of SARS-CoV-2 potently activates latent TGF-β1 in vitro**, leading to **Treg activation** as a mechanism of immune suppression & host evasion in early COVID-19
- Immune dysregulation and **increased TGF-β1 airway levels** may contribute to later fibroproliferative stages of ARDS
- **Blocking NSP15-mediated effects with TGF-β inhibitors** is an innovative therapy worthy of testing in COVID-19 prevention and treatment trials (> 10 TGF-β inhibitors exist for human use)

Future Directions

- Test activation of TGF-β on the surface of human Tregs in the presence of wild-type and mutant rNSP15 while evaluating subsequent autocrine (Treg phospho-SMAD2 levels) and paracrine (T effector cell inhibition) immunosuppressive effects of rNSP15
- Test the effects of current TGF-β inhibitors on NSP15 effects
- Test TGF-β inhibitors in animal models of COVID-19 Illness

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- COMIRB #21-3537
- COI: JPM is the inventor on a patent owned by the Univ. of Colorado titled "TGFB1 Inhibitors for Preventing and Treating SARS-CoV-2"

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