UNIVERSITY of VIRGINIA

280

ACE2 SUBSTRATE CLEAVAGE EFFECT ASSOCIATED WITH COVID-19 PATIENT PLASMA IMMUNOGLOBULIN

Yufeng Song, Regan Myers, Lila Murphy, Bailey Brooks, Jeffrey M. Wilson, Alexandra Kadl, Judith Woodfolk, **Steven L .Zeichner**
University of Virginia, Charlottesville, Virginia, USA

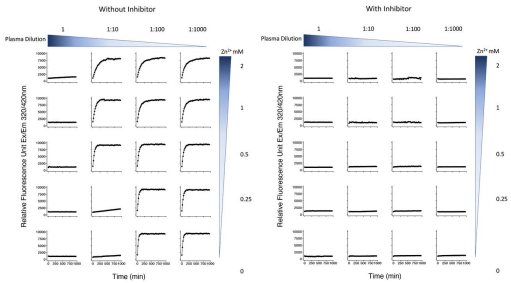
BACKGROUND

Many mechanisms responsible for COVID-19 pathogenesis are well-known, but COVID-19 includes features with unclear pathogenesis, like autonomic dysregulation, coagulopathies, and high levels of inflammation. The SARS-CoV-2 spike protein receptor binding domain (RBD) receptor is angiotensin converting enzyme 2 (ACE2). **We hypothesized that some COVID-19 patients may develop immunoglobulins (Igs) that have a negative molecular image of RBD sufficiently similar to ACE2 to yield ACE2-like catalytic activity – ACE2-like 'abzymes'.**

METHODS

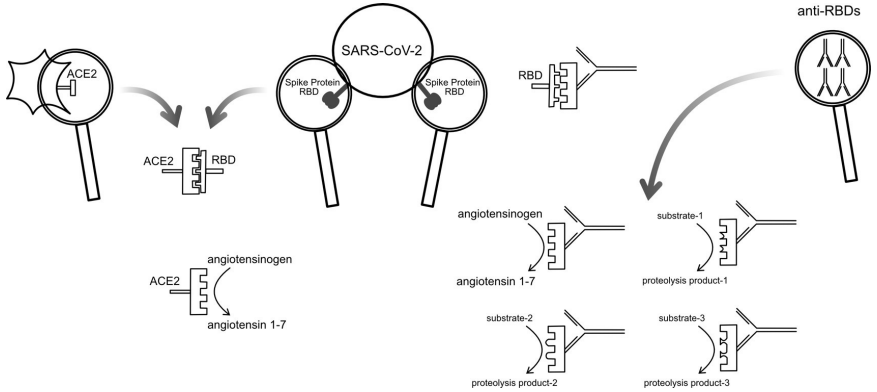
We studied 67 patients hospitalized with COVID-19 who had disodium ethylenediaminetetraacetate (EDTA) anticoagulated plasma samples available, obtained about 7 days after admission. We used commercially available fluorometric ACE2 assays (Abcam), and a SpectraMax® M5 microplate reader (Molecular Devices), measuring Relative Fluorescent Unit (RFU, Ex/Em = 320/420 nm) in a kinetic mode every 20 min at 37℃. ACE2 inhibitor provided in the assay kit was used for additional controls. In some control experiments, we added Zn^{2+} to plasma, or conducted serial dilutions to decrease EDTA concentration. To deplete Igs, we passed plasma samples through a 0.45 μm filter to remove large debris, then passed the material through 100kDa cut-off ultrafiltration membrane (Pierce™) columns, and finally used protein A/G Magnetic Beads (Life Technologies) to specifically deplete Ig, removing >99.99% of Ig as assessed with a human IgG ELISA Kit (Abcam).

RESULTS



Inhibition of exogenously added recombinant ACE2 substrate cleavage activity in undiluted EDTA-anticoagulated pooled plasma samples with and without added $ZnCl_2$. ACE2 is a Zn^{2+} metalloprotease. No ACE2 activity was detectable when exogenous ACE2 was added to plasma, unless Zn^{2+} was added, or unless the plasma was diluted. Adding an ACE2 inhibitor blocked detection of the ACE2 activity. No native ACE2 activity should be detectable in EDTA-anticoagulated plasma. Any ACE2 substrate cleavage activity observed in the EDTA-anticoagulated plasma should be due to something other than ACE2.

Some COVID-19 patients have an activity in plasma that cleaves ACE2 substrate that is associated with Ig: ACE-2-like abzymes.



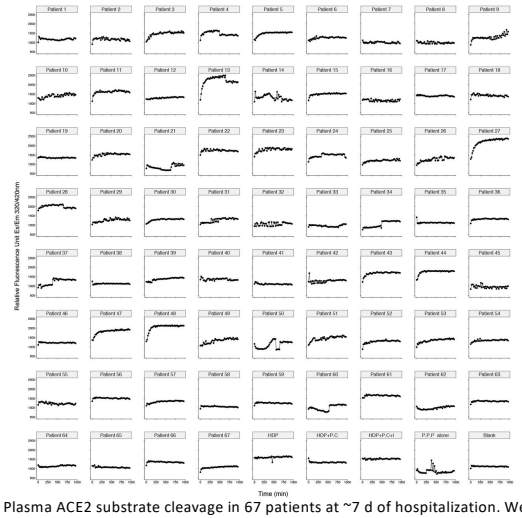
Hypothesized Anti-RBD Antibodies with Catalytic Activity: Anti-RBD Abzymes

Schematic diagram illustrating the hypothesis. The SARS-COV-2 Spike Protein Receptor Binding Domain (RBD) binds ACE2 on the surface of a potential host cells. The RBD has a partial negative image of ACE2. Some antibodies against the RBD may then have a conformation enough like ACE2 to have proteolytic activity similar to ACE2. Because the negative image of RBD present antibodies is imperfect, some antibodies with proteolytic activity may have an altered substrate specificity, potentially affecting other proteolytic regulatory cascades.

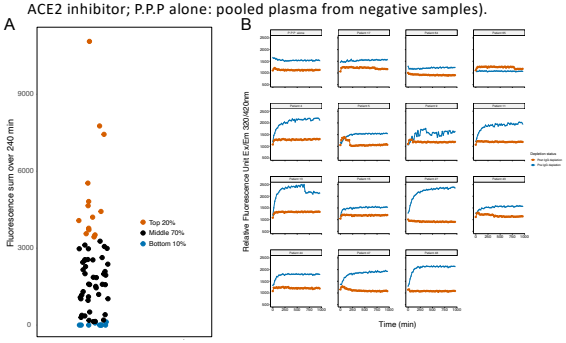
ADDITIONAL KEY INFORMATION

Author Contact Information: zeichner@virginia.edu

Acknowledgements: We thank the patients whose samples we studied and and the research support staff who enrolled the study participants (led by Linda Bailes RN, Allison Raymond RN and Sarah Struchen RN). We also thank the University of Virginia Biorepository and Tissue Research Facility for processing the samples used in the study and for providing specimens (Technical Director, Patcharin Pramoonjago PhD). The sample collection protocol was approved by the UVA IRB (HSR #200110), and approval was obtained to work on the specimens (HSR #HSR200362). Informed consent was obtained from the study participants.



Plasma ACE2 substrate cleavage in 67 patients at ~7 d of hospitalization. We selected 11 patients within the top 20% of RFU values with sufficient sample for the Ig depletion protocol for further study (HDP: purchased pooled donor plasma; HDP+P.C: HDP plus recombinant human ACE2; HDP+P.C+I: HDP+P.C.+ ACE2 inhibitor; P.P.P alone: pooled plasma from negative samples).



A. RFU scatter plot. B. ACE2 substrate cleavage before and after Ig depletion. Also shown: pooled patient plasma (PPP) controls and samples from three arbitrary patients within the bottom 10% of RFU. For the 11 patients with high ACE2 substrate cleavage, cleavage was depleted in an Ig-associated manner.

CONCLUSIONS

- **Some COVID-19 patients have a plasma catalytic activity co-depleting with immunoglobulin that can cleave ACE2 substrate.**
- **This suggests that some COVID-19 patients may produce antibodies with catalytic activity – abzymes.**
- **Many physiologic processes are regulated via proteolytic pathways. If COVID-19 patients make abzymes, the abzymes could contribute to COVID-19 pathogenesis.**