Coagulation Initiated by Tissue Factor on a Coronavirus

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INTRODUCTION

- Human coronavirus infection can precede a procoagulant state, affecting numerous organ systems
- Enveloped viruses can acquire host proteins as they egress from the cell (Fig. 1A)

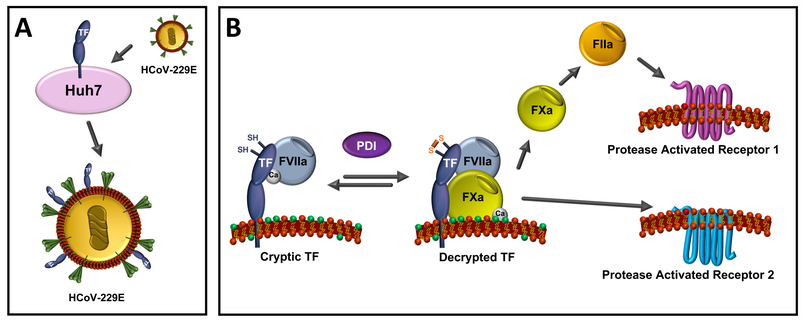


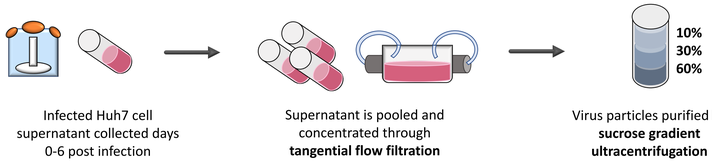
Figure 1. A) Huh7 cell infection. **B).** Protease Activated Receptor signaling by tissue factor (TF) and clotting proteases.

- TF acts as a cofactor for FVIIa, promoting the conversion of FX into FXa
- TF can participate in cell signaling directly through protease activated receptor 2 activation by TF/FVIIa/FXa complex or protease activated receptor 1 activation by downstream FIIa (Fig. 1B)

We hypothesize that any enveloped virus can acquire TF if replicated in a TF-bearing cell, providing a novel broad-spectrum antiviral target.

AIMS AND METHODS

Generating a purified HCoV-229E preparation.

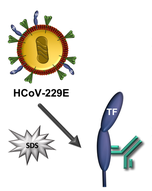


Infected Huh7 cell supernatant collected days 0-6 post infection

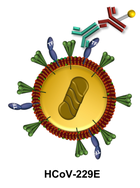
Supernatant is pooled and concentrated through **tangential flow filtration**

Virus particles purified by **sucrose gradient ultracentrifugation**

1: Characterize TF antigen on HCoV-229E through immunoblot and electron microscopy.

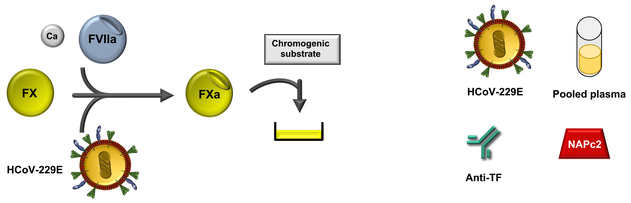


- Virus lysate generated through by adding sodium dodecyl sulfate (SDS)
- 10ug/ul total [protein] loaded



- HCoV-229E was labelled with α-TF monoclonal antibody 9B4 (primary) and goat α-mouse anti conjugated to 15nm gold bead (secondary)
- Methylamine tungstate was used as a negative stain

2 + 3: Functional assays to characterize HCoV-229E TF activity.



1: DETECTION OF TF ANTIGEN

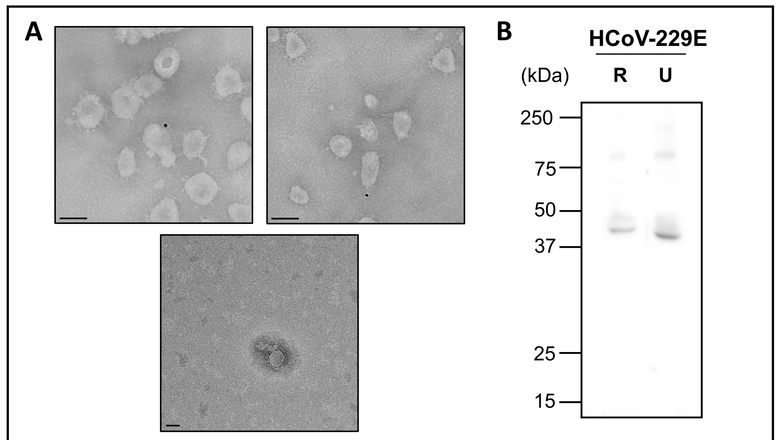


Figure 2. A) Immunogold electron microscopy of purified HCoV-229E, labeled with α-TF monoclonal antibody 9B4 and goat α-mouse anti conjugated to 15nm gold bead. Scale bar represents 100nm. **B)** HCoV-229E lysate samples, both reduced with dithiothreitol (R) and unreduced (U), were probed with α-TF monoclonal antibody 9B4.

- TF is labeled on purified HCoV-229E, identifiable by the distinct spike "corona"
- A distinct TF band is observed at approximately 47kDa by immunoblot analysis

TF antigen is observed on virus particles in the purified HCoV-229E preparation.

2: DOES VIRAL TF HAVE CO-FACTOR ACTIVITY?

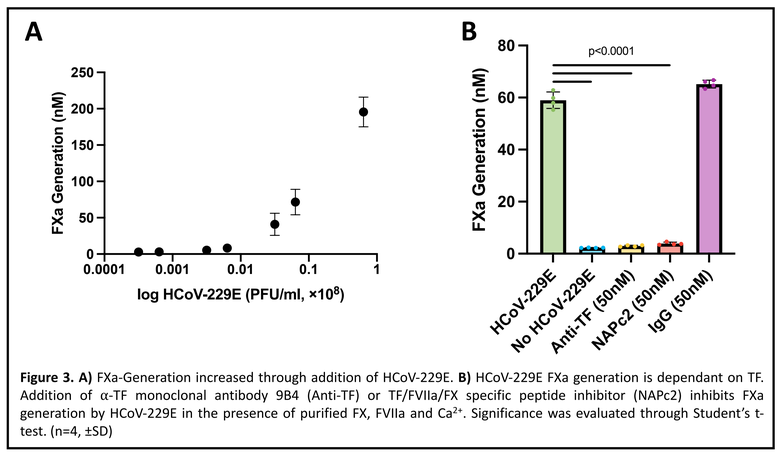


Figure 3. A) FXa-Generation increased through addition of HCoV-229E. **B)** HCoV-229E FXa generation is dependant on TF. Addition of α-TF monoclonal antibody 9B4 (Anti-TF) or TF/FVIIa/FX specific peptide inhibitor (NAPc2) inhibits FXa generation by HCoV-229E in the presence of purified FX, FVIIa and Ca^{2+}. Significance was evaluated through Student's t-test. (n=4, ±SD)

- Fig. 3A: Purified HCoV-229E is sufficient to generate procoagulant FXa species
- Fig. 3B: HCoV-229E FXa generation is TF-dependant and can be reduced by two different types of TF-specific inhibitors

HCoV-229E TF has the expected cofactor activity, allowing for the production of FXa from purified FX. FXa generated by HCoV-229E may facilitate procoagulant activity and cell signaling functions.

3: DOES VIRAL FORM A CLOT?

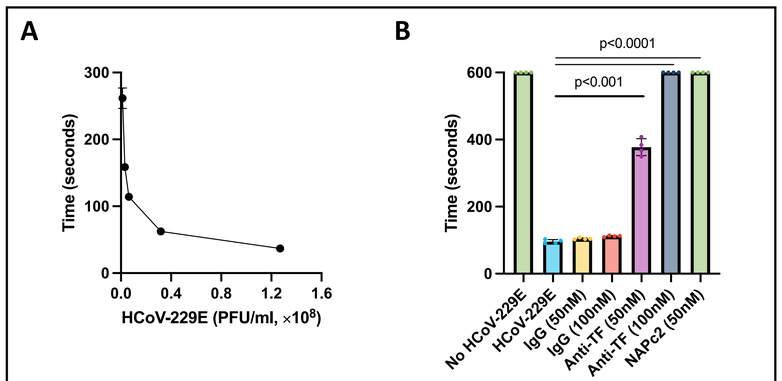


Figure 4. A) Addition of HCoV-299E shortens plasma clotting time in a concentration dependant manner. **B)** Pooled plasma and purified HCoV-229E treated with α-TF monoclonal 9B4 (Anti-TF), TF/FVIIa/FX specific peptide inhibitor (NAPc2) and IgG isotype control with a clotting time endpoint of 600 seconds. Significance was evaluated through Student's t-test. (n=4, ±SD)

- Fig. 4B: Clot formation is dependant on HCoV-229E addition
- Fig. 4B: HCoV-229E clotting activity is successfully inhibited through addition of 100nM Anti-TF and 50nM NAPc2

HCoV-229E has TF-dependent clotting activity. This further supports chromogenic data that TF on virus surface acts to generate FXa and subsequently FIIa prior to fibrin clot formation.

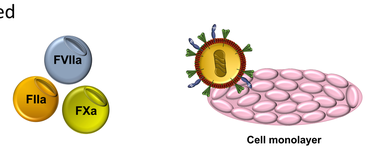
CONCLUSIONS

Summary:

- TF antigen is identifiable on purified HCoV-229E preparation
- TF antigen has expected FXa-generating co-factor activity, inhibited by TF-specific antibody and peptide
- The TF-dependent clotting activity of HCoV-229E shortens plasma clotting time in a concentration dependent manner

Future directions and implications:

- Cell infectivity assays will be conducted to assess the role of TF-generated proteases during *in vitro* infection
- Change in plaque formation following addition of purified coagulation proteases will be calculated



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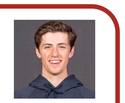

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