Tissue Factor as the molecular basis for hemostatic disease in enveloped virus infection: HIV and dengue virus

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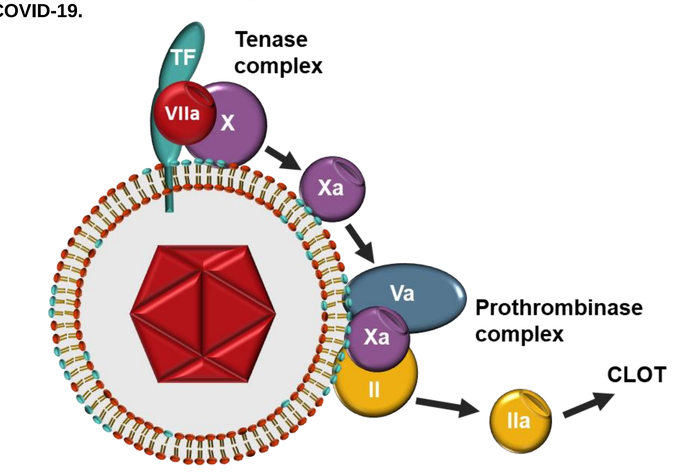
BACKGROUND

- Many viruses, including human immunodeficiency virus (HIV) and dengue virus (DENV) have an outer membrane structure, the envelope, comprised of host derived lipids and proteins, and virus-encoded-proteins.
- During the formation of the envelope, the virus can acquire critical cofactors of coagulation including the transmembrane protein tissue factor (TF), bypassing normal regulation.
- Most enveloped virus infections have been reported to affect hemostasis. As examples, HIV infection is strongly correlated to early onset thrombosis, DENV infection leads to hemorrhage and SARS-CoV-2 causes thrombotic complications in COVID-19.



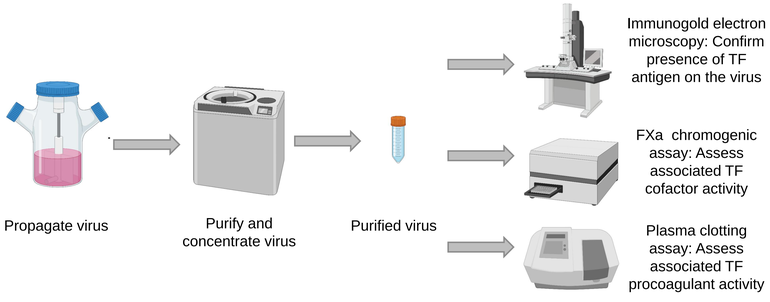
Hypothesis

Tissue factor is present on the surface of HIV and DENV and retains its procoagulant activity.

Goals

- Identify TF on the surface of HIV and DENV
- Demonstrate TF-mediated FXa generation by HIV and DENV
- Show purified HIV and DENV induce clotting via TF

Workflow



RESULTS

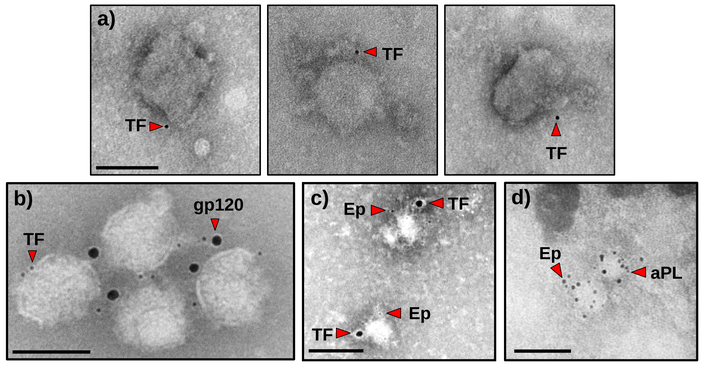


Fig 1. Demonstration of TF on DENV and HIV. Immunogold electron microscopy showing purified (a) and patient-derived (b) HIV, and cultured DENV (c, d). Cultured HIV (a) was singly labelled for TF. Patient-derived HIV (b) and cultured DENV (c, d) were co-labelled for TF and a virus-encoded envelope protein (gp120 and E protein, respectively). DENV was also labeled for anionic phospholipid by biotin-annexin V. Scale bar represents 100nm. Isotype controls or biotin-annexin V in the absence of calcium were completely blank of gold beads (not shown).

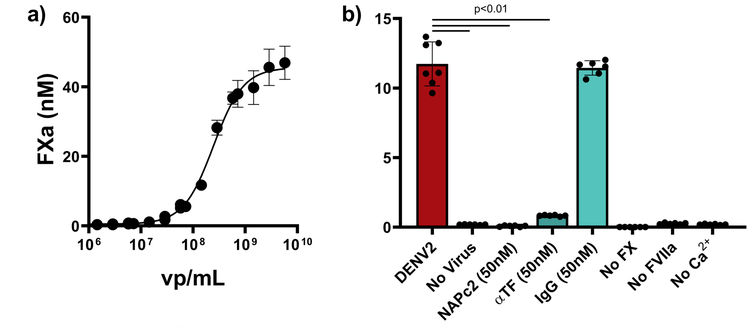


Fig 2. DENV2 mediates FXa generation in a TF-like manner. (A) The amount of FXa generated is dependent on the amount of virus particles (vp) in the reaction. **(B)** An anti-TF antibody or peptide inhibitor of the TF/FVIIa/FX complex (NAPc2) shows that the FXa generation is due to TF activity. The reaction is dependent on the presence of DENV, FX, FVIIa, and Ca^{2+}. Taken together, this suggests that TF activity on the virus is responsible for the generation of FXa. Significance was evaluated by use of Student's t-test. (n=6; ±SD)

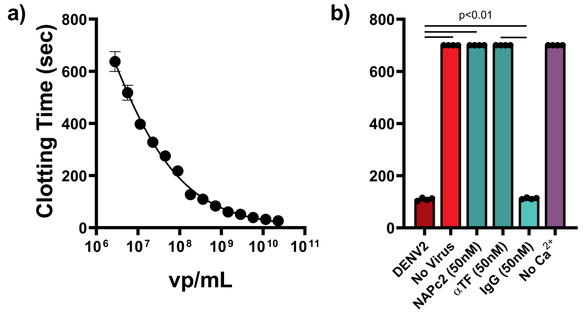


Fig 3. DENV2 Induces plasma clotting. (A) Increasing the concentration of DENV in plasma results in a faster clotting time. **(B)** An inhibitory anti-TF antibody or NAPc2 showed that the induction of clotting involved TF activity associated with DENV. Significance was evaluated by use of Student's t-test. (n=4; ±SD)

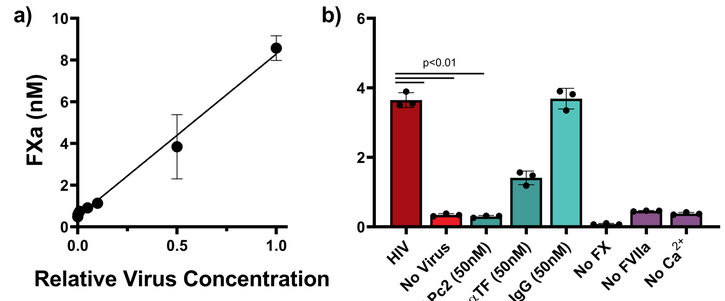


Fig 4. HIV mediates FXa generation in a TF-like manner. (A) The amount of FXa generated is dependent on the amount of virus in the reaction. **(B)** An anti-TF antibody or NAPc2 showed that FXa generation is due to TF activity. The reaction is dependent on the presence of HIV, FX, FVIIa, and Ca^{2+}. These results suggest that TF activity on the virus is responsible for the generation of FXa. Significance was evaluated by use of Student's t-test. (n=3; ±SD)

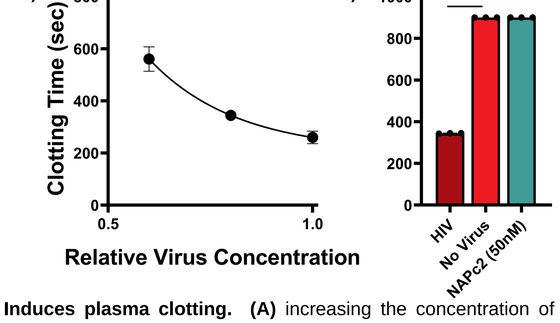


Fig 5. HIV Induces plasma clotting. (A) increasing the concentration of HIV in plasma results in a faster clotting time. **(B)** Use of NAPc2 shows that the induction of clotting is due to TF activity, and the induction of clotting is dependent on the presence of HIV. Taken together, this suggests that TF activity on the virus is responsible for the initiation of plasma clotting. Significance was evaluated by use of Student's t-test. (n=4; ±SD)

CONCLUSIONS

- TF is on the envelope of patient-derived HIV, and cultured HIV and DENV
- HIV and DENV have TF activity, explaining viral pathology

SIGNIFICANCE

- These data support a mechanism that unifies virus-induced coagulopathy to envelope TF, suggesting TF as a target for the development of a broad-spectrum antiviral.

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