A broad-spectrum molecular basis for hemostatic disease in transfusion-transmissible enveloped viruses: HIV and dengue virus

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Many viruses including human immunodeficiency virus (HIV), dengue virus (DENV), and SARS-CoV-2 have an outer membrane structure comprised of host derived lipids and proteins and virus-encoded proteins, known as the envelope.

During the formation of the envelope, the virus can acquire critical co-factors 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.

Tissue factor is present on the surface of enveloped viruses 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
- Propagate virus
- Purify and concentrate virus
- Immunogold electron microscopy: Confirm presence of TF antigen on the virus
- FXa generation assay: Assess virus-bound TF cofactor activity
- Plasma clotting assay: Assess virus-bound TF procoagulant activity

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). Patient derived HIV (B) and cultured DENV (D) co-labelled for TF and a viral envelope protein (gp120 and E protein, respectively). Cultured HIV (A) singly labelled for TF. Scale bar represents 100nm.

Fig 2. DENV 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 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 Ca2+. 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. DENV Induces plasma clotting. (A) Increasing the concentration of DENV in plasma results in a faster clotting time. (B) An anti-TF antibody or NAPc2 shows that the induction of clotting is due to TF activity, and the induction of clotting is dependent on the presence of DENV. These results suggest 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)

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 shows that the FXa generation is due to TF activity. The reaction is dependent on the presence of HIV, FX, FVIIa, and Ca2+. 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.