Received: 27 April 2020 | Revised: 28 May 2020 | Accepted: 8 June 2020
DOI: 10.1002/rth2.12406

REVIEW ARTICLE

Antiviral anticoagulation

Edward L. G. Pryzdial PhD[1,2] | **Michael R. Sutherland PhD**[1,2] | **Bryan H. Lin PhD**[1,2] | **Marc Horwitz PhD**[3]

[1]Center for Innovation, Canadian Blood Services, Vancouver, BC, Canada

[2]Centre for Blood Research and Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

[3]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

Correspondence
Edward L. G. Pryzdial, Centre for Blood Research, University of British Columbia, 2350 Health Sciences Mall, Vancouver. BC V6T 1Z3, Canada.
Email: ed.pryzdial@blood.ca

Funding information
Centre for Blood Research, Grant/Award Number: Graduate Award Program Grant; Heart and Stroke Foundation of Canada, Grant/Award Number: G-19-0026524 ; Canadian Institutes of Health Research, Grant/Award Number: 273985

Handling Editor: Yotis Senis

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel envelope virus that causes coronavirus disease 2019 (COVID-19). Hallmarks of COVID-19 are a puzzling form of thrombophilia that has elevated D-dimer but only modest effects on other parameters of coagulopathy. This is combined with severe inflammation, often leading to acute respiratory distress and possible lethality. Coagulopathy and inflammation are interconnected by the transmembrane receptor, tissue factor (TF), which initiates blood clotting as a cofactor for factor VIIa (FVIIa)-mediated factor Xa (FXa) generation. TF also functions from within the nascent TF/FVIIa/FXa complex to trigger profound changes via protease-activated receptors (PARs) in many cell types, including SARS-CoV-2–trophic cells. Therefore, aberrant expression of TF may be the underlying basis of COVID-19 symptoms. Evidence suggests a correlation between infection with many virus types and development of clotting-related symptoms, ranging from heart disease to bleeding, depending on the virus. Since numerous cell types express TF and can act as sites for virus replication, a model envelope virus, herpes simplex virus type 1 (HSV1), has been used to investigate the uptake of TF into the envelope. Indeed, HSV1 and other viruses harbor surface TF antigen, which retains clotting and PAR signaling function. Strikingly, envelope TF is essential for HSV1 infection in mice, and the FXa-directed oral anticoagulant apixaban had remarkable antiviral efficacy. SARS-CoV-2 replicates in TF-bearing epithelial and endothelial cells and may stimulate and integrate host cell TF, like HSV1 and other known coagulopathic viruses. Combined with this possibility, the features of COVID-19 suggest that it is a TFopathy, and the TF/FVIIa/FXa complex is a feasible therapeutic target.

KEYWORDS
coagulation, COVID-19, herpes simplex virus, inflammation, protease-activated receptor, tissue factor

Essentials

- Severe acute respiratory syndrome coronavirus 2 is an envelope virus that causes coagulopathy and acute inflammation in coronavirus disease 2019.
- Model coagulopathic viruses have envelope tissue factor (TF) required for infection in mice.
- TF is a key membrane cofactor linking clotting factor Xa (FXa) production and inflammation.
- TF/FXa-specific anticoagulants are antiviral and likely broadly relevant to envelope viruses.

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1,2] is a novel envelope[3] virus that causes life-threatening thrombotic coagulopathy[4-10] and inflammation,[1,11,12] the hallmarks of coronavirus disease 2019 (COVID-19).[2] SARS-CoV-2 is highly virulent and despite the introduction of social distancing, has infected 5 million people on all inhabitable continents in approximately 6 months.[13] The overall mortality rate of confirmed infections is > 1.5%, although this is likely an overestimate since it is known that there is a dangerously significant number of unaccounted asymptomatic carriers[14] and mass screening is not yet practical. Medical scientists from all pillars of investigation have united from around the globe toward developing therapeutics that will mitigate the morbidity and mortality of COVID-19 and stop the virus replication cycle. Here, we draw attention to the fact that SARS-CoV-2 is an extreme example within a broad spectrum of coagulopathic envelope viruses. The pathology manifested is specific to the virus but may be explained by a unifying constituent, tissue factor (TF), the physiological initiator of coagulation and potent cell-modulating cofactor. Thus, therapeutics

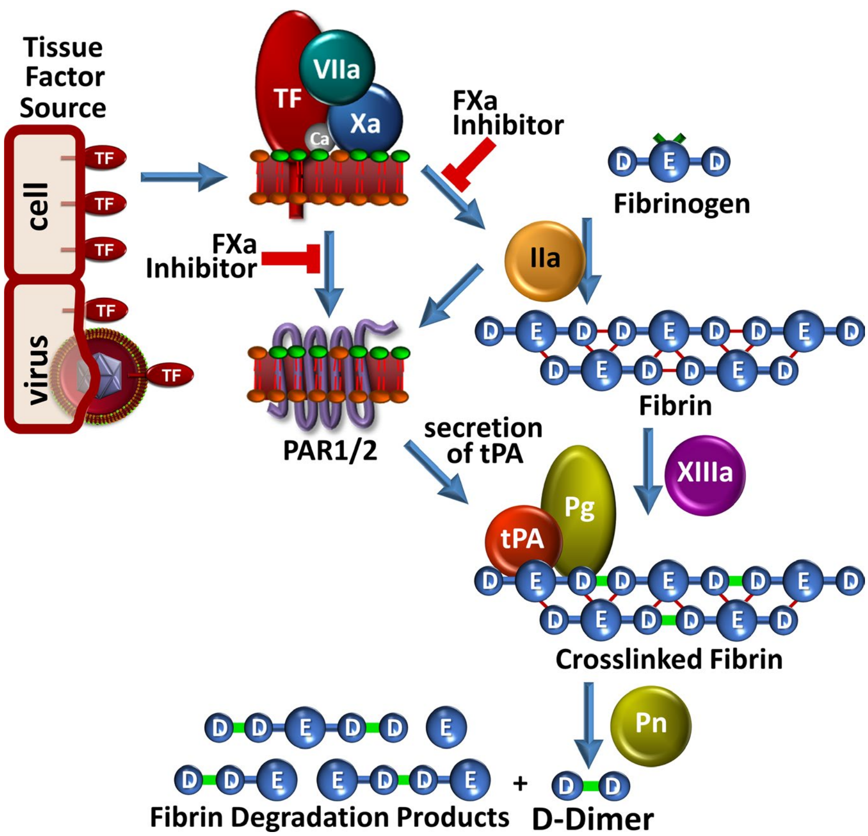

FIGURE 1 Tissue factor (TF) in viral D-dimer production. TF activity localized on the stimulated cell or on the envelope virus surface combines with the protease factor VII (FVIIa) to accelerate factor Xa (FXa) generation in the presence of anionic phospholipid (green polar head groups) and calcium. Release of FXa from the nascent TF/FVIIa/FXa complex facilitates thrombin production (factor IIa [FIIa]). Thrombin is the pivotal effector of fibrin clot formation by proteolytic excision of fibrinopeptides (green) from fibrinogen triggering noncovalent (red lines) polymerization of soluble fibrin. Thrombin also activates the transglutaminase factor XIII (FXIII), which crosslink-stabilizes the interfibrin associations (green bars). Both the TF/FVIIa/FXa complex and thrombin are potent protease-activated receptor 2 (PAR2) agonists, which may induce the release of tissue-type plasminogen activator (t-PA) from cells to enhance plasminogen (Pg) to plasmin (Pn) activation, resulting in D-dimer and fibrin degradation product formation. Thus, inhibition of FXa with small molecule inhibitors (eg apixaban) may attenuate both signaling and procoagulant branches of TF function toward D-dimer formation

targeting TF are prime candidates to consider for SARS-CoV-2 intervention.

2 | COVID-19–INDUCED COAGULOPATHY

SARS-CoV-2 infection is associated with coagulopathy.[15] The compelling clinical laboratory evidence is that COVID-19 results in elevated D-dimer,[11,16-29] which is a dogmatic metric of hypercoagulation. D-dimer is a fragment of factor XIIIa (FXIIIa)-crosslinked fibrin and is produced when tissue-type plasminogen activator (t-PA) converts plasminogen to the activated fibrinolytic protease plasmin in response to thrombin-driven clot accumulation (Figure 1). Therefore, D-dimer may also suggest hyperfibrinolysis. Supporting this possibility in COVID-19, elevated plasminogen has been reported as a risk factor.[25] Thrombin is also known to stimulate the secretion of t-PA, priming the local milieu for a fibrinolytic response.[30-32] When stratified according to severity of disease or need for mechanical ventilation, D-dimer is found to be a predictor of COVID-19 disease progression.[28] There is a clear trend showing progressive elevation of D-dimer from time of COVID-19 identification in nonsurvivors, whereas levels remain normal in survivors.[27,28] A similar trend was seen for other fibrin degradation products.[27,33] Combined, these data suggest that thrombin generation may be enhanced as virus replication persists and amplifies pathology.

Clinical laboratory results further linking COVID-19 and a hypercoagulable state is suggested by prothrombin time (PT) measurements, which are prolonged in nonsurvivors compared to survivors[27,28] and indicate depletion of clotting factors. However, this is a subtle effect of approximately 2 seconds that is relatively small. Conversion to International Normalized Ratio may conceal this relatively moderate effect.[23]

Additional evidence of coagulopathy is provided by a meta-analysis of 9 studies reporting data on platelet counts from 1779 patients with COVID-19, of which 399 were severe.[34] The weighted mean difference in this report revealed an ~ 15% drop in platelet number, which is reduced another ~ 10% for nonsurvivors. While numerous factors may contribute to a reduced platelet count in virus infection,[35] thrombocytopenia is usually attributed to enhanced thrombin production with consequent platelet activation and subsequent senescence.

Fibrinogen, clinically evaluated in the diagnosis of coagulopathy, is reported to increase in patients during severe disease compared to mild COVID-19[27,36-38] and may be the result of an acute-phase response. Interestingly, an exception to this trend was observed at late hospitalization when 2 severely diseased patients became hypofibrinogenemic.[27] This late-stage observation is consistent with the parameters of conventional disseminated intravascular coagulation (DIC).[39]

Together, these observations make a compelling argument for SARS-CoV-2–induced coagulopathy. Although elevated D-dimer alludes to DIC, COVID-19 does not satisfy the other prominent characteristics of overt thrombin generation consistent within the ISTH definition[39]; COVID-19 does not have prolonged PT of > 3 seconds, platelet count dropping to < 100×10^9/L or fibrinogen dropping to < 1 gm/L. It follows that COVID-19 coagulopathy does not lead to a hemorrhagic condition but rather to a prothrombotic state. To substantiate this, there is overwhelming evidence for prevalent pulmonary embolism, thrombotic microangiopathy, and arterial thrombosis.[4-10] Whether the virus causes these events or patient predisposition to hypercoagulation favors infection, or both, is unknown.

3 | COVID-19–DEPENDENT INFLAMMATION

Severe pneumonia and the associated respiratory distress, originally attributed as the leading cause of death in COVID-19,[1,11] is now known to involve pulmonary embolism.[4-10] The severity of the hallmark pulmonary inflammation correlates to lymphocyte subgroups[40] and glassy alveolar opacities have been documented in computed tomography images.[6] When uncontrolled, the prolonged inflammatory imbalance ultimately leads to multiple organ failure. This progression may be influenced by the broad tissue distribution of the virus's primary host cell docking site, the angiotensin-converting enzyme 2 (ACE2) receptor,[41,42] found in the lungs, kidneys, brain, gastrointestinal tract, and cardiovascular system.[43,44]

The severity of COVID-19 presentation and disease progression range widely for unknown reasons, and thus treatment options vary. However, prophylactic anticoagulation is the accepted standard. General predictors of poor outcome were identified quite early in the SARS-CoV-2 pandemic as advanced age and male sex,[45,46] while comorbidities include, diabetes,[16,28] hypertension, cardiovascular disease,[12] and obesity.[47] These underlying pathologies are all characterized by chronic inflammation, presenting clinically as elevated levels of acute-phase reactants, most notably C-reactive protein.[11,16,20,21,29] Secretion of high levels of circulating proinflammatory cytokines, interleukin (IL)-6, IL-1, interferon-γ, and tumor necrosis factor have also been documented and attributed to an immune-surveillance response.[11,16,28] While most virus infections are opportunistic and enhanced by immunosuppression, elevation of COVID-19 or other coronavirus diseases in immunosuppressed transplant recipients is atypical and does not increase.[48] Similarly, in a transgenic mouse model deficient in the innate immune response to pathogens that promotes inflammation and neutralization, complement component C3 was shown to facilitate respiratory dysfunction and cytokine increase upon infection by other coronavirus family members[49] compared to wild-type controls. Combined, these reports indicate that both symptoms and virus replication may be amplified by the innate immune response.

4 | TF CONNECTS COAGULATION AND INFLAMMATION

It is not surprising that evidence is accumulating to show the etiology of COVID-19 pneumonia is both coagulopathic (ie, elevated

D-dimer) and inflammatory (ie, elevated IL-6), since the 2 pathways are intimately connected. The molecular bridge between hemostasis and the innate inflammatory response is the coagulation trigger, TF.[50] TF has been unequivocally identified as a mechanistic pathophysiological mediator in numerous mouse models of disease and clinical correlations have been made; examples include cancer,[51,52] sickle cell disease,[53,54] obesity and diabetes,[55-57] rheumatoid arthritis,[58,59] and cardiovascular disease.[60,61] Thus, TF is a probable effector of the progression and severity of thrombosis and inflammation seen in COVID-19.

TF is a transmembrane receptor essential for mammalian life.[62-64] It is pivotal in the blood clotting mechanism and best understood as the extrinsic tenase cofactor,[65] functioning to accelerate factor VIIa (FVIIa)-dependent proteolytic activation of factor X (FX) to FXa in the presence of an anionic phospholipid (aPL)-containing membrane and calcium (Figure 1). However, TF also participates to accelerate FVIIa activity toward the initial activation of factor IX (FIX) to FIXa and FVIII to FVIIIa, and autoactivation of FVII.[66-70] The coagulopathic consequence of enhanced clotting factor activation is that downstream thrombin acts as its own feedback amplifier for subsequent clot formation. Thus, enhanced TF activity may be extrapolated using clinical laboratory values of D-dimer elevation as a surrogate marker.

Of equal or greater importance to the clotting function of TF is its critical role as a cell-signaling cofactor from within the TF/VIIa cofactor/protease and nascent TF/FVIIa/FXa cofactor/protease/product complexes.[71] These facilitate cell signaling via protease activated receptors (PARs) (Figure 1). PAR extracellular domains are cleaved by the TF-enhanced protease, and the new N-terminus acts as a tethered ligand that sends a transmembrane signal transduced by G-protein– and β-arrestin–coupled intracellular pathways.[72] These stimulate fundamental biochemical pathways such as kinase cycles, gene transcription, and protein synthesis.[73,74] The biological result may be profound, ranging from effects on storage granule release (eg, cytokines) to cell trafficking, which likely impacts COVID-19–dependent pulmonary inflammation.

The stimulatory effects of the TF-protease complexes are predominantly conferred through PAR1 and PAR2, although indirect effects on PAR3 and PAR4 also occur through mobilization of effector proteases. TF is prevalent throughout the body and is constitutively expressed by fibroblasts, pericytes, smooth muscle cells, epithelial cells, astrocytes, and cardiomyocytes, and inducibility expressed on endothelial and monocyte lineage cells.[64] Similarly, PARs have an extremely broad cellular and tissue distribution that includes key contributors in COVID-19 progression: vascular endothelium, platelets, leukocytes, smooth muscle cells, and airway epithelium.[75] Thus, the TF-PAR pathway is positioned at crucial interfaces where a multitude of relevant physiological and pathological processes occur.[71,76,77]

TF/FVIIa exclusively cleaves and activates PAR2 with relatively low affinity; however, the cofactor signaling effects of TF are greatly enhanced after thiol oxidation and in the presence of nascent FXa.[71] Within the ternary TF/FVIIa/FXa complex, FXa becomes the proteolytic subunit. TF-mediated signaling is enhanced by additional cell-specific receptors. On the endothelial cell vascular lining and alveolar epithelial lining,[78] a major site of SARS-CoV-2 infection, the endothelial protein C receptor–TF/FVIIa/FXa complex cleaves and activates PAR2 and PAR1.[79,80] Consequently, the effective concentration of FVIIa is reduced by more than 10-fold.[79,80] Thrombin is also an efficient activator of PAR1 and does not require an accessory cofactor because PAR1 has a high-affinity binding site.[81] The combined effects of cell surface–localized hemostatic proteases in the vicinity of PARs creates a potent trigger for inflammation and other pathophysiological consequences. To stimulate discussion in a novel area of clinical intervention strategies to alleviate COVID-19, here anticoagulation of the TF-PAR axis is proposed as having an additional antiviral therapeutic value.

5 | TF AND VIRUSES

Different viruses manifest diverse illnesses because of the unique proteins encoded by their genome and the cell and organ tropism dictated by those proteins. As an example, the SARS-CoV-2 envelope surface "spike" protein facilitates fundamental docking with the cell surface receptor ACE2. However, contrary to the dogma that each virus encodes unique proteins and must therefore give rise to unique pathology, numerous virus types have in common the modulation of the blood clotting system with correlations to hemostatic pathology. The symptoms range widely depending on the virus type and are driven by complicated virus-host mechanisms, involving hemostatic proteins (clotting, anticoagulant, and fibrinolytic),[82-88] platelets,[35] endothelial cells,[89,90] leukocytes,[91] and complement proteins.[92] In some cases, clotting protein activation may lead to thrombosis, such as for HIV.[93,94] For other viruses, clotting factors may become depleted due to extensive activation and clearance, which contributes to the bleeding seen during infection of hemorrhagic viruses like dengue virus.[83,95-98] To highlight the prevalence and importance of the virus-hemostasis association, in addition to the reports accumulating for SARS-CoV-2, other important and highly prevalent virus examples include the hepatitis C virus (HCV),[88,99,100] influenza virus,[101,102] Ebola virus,[89,103,104] Zika virus,[105] genital herpes (herpes simplex virus type 2 [HSV2]),[106,107] cytomegalovirus (CMV),[108,109] and the cold sore virus (herpes simplex virus type 2 [HSV1]).[110-113] We propose that a mutual molecular basis explains this diverse and extensive list.

Each of the viruses above and many more have an envelope as a common structural feature, which is a surrounding phospholipid bilayer acquired from infected host cellular membranes. Within the envelope are membrane-associated proteins. Some of these envelope proteins are encoded by the virus genome, like the SARS-CoV-2 spike protein.[114,115] However, many other proteins are associated with the envelope but are encoded by the host and derived from the cell where the virus replicates and acquires the envelope. While much is known about the roles of virus-encoded envelope proteins and their roles in the infection mechanism, the functions of host-encoded proteins on the virus surface have been given relatively little consideration in the prevailing paradigm.[116] Many cells known to bear TF are permissive to infection by clinically important enveloped viruses, including SARS-CoV-2,[117] HSV1,[118] Ebola,[119] influenza,[120] HIV,[121] dengue,[122] Zika virus,[123] HCV,[124] and others. It is reasonable to speculate that the surface of these and other viruses display TF, which may account for hemostatic and inflammatory symptoms associated with their infection. Therefore, the TF-initiated mechanisms may serve as a broad-specificity target to alleviate viral pathology, such as in COVID-19.

6 | TF ON THE VIRUS ENVELOPE

To investigate TF as a general surface constituent of envelope viruses, we have studied HSV1 as a model virus. Over two-thirds of the world's population is infected by HSV1, which is the leading cause of infectious blindness,[125] sporadic encephalitis,[126] and genital herpes[127,128] and is associated with intestinal dysregulation.[129] Although known as the cold sore virus and typically not life threatening, there are numerous correlations between HSV1 and other members of the herpesvirus family to cardiovascular disease,[130,131] suggesting links to TF: (i) HSV1 seropositivity is associated with a 2-fold increase in myocardial infarction incidence and death due to coronary heart disease[113]; (ii) fibrin deposits in the microvasculature are linked to HSV1 infection[132,133]; (iii) DIC in neonates may occur during severe HSV1 infection[134]; (iv) HSV2 is linked to ischemic and hemorrhagic stroke due to DIC[107,135]; (v) a history of CMV infection is linked to subclinical and clinical arterial thickening[136-138]; (vi) CMV is strongly correlated to accelerated atherosclerosis in immunosuppressed organ transplant recipients[139-142]; and (vii) CMV infection is a strong risk factor for restenosis after angioplasty.[143,144] When paired with other known cardiovascular risk factors, viral correlation to vascular disease is strong.[110-113] A clear cause-and-effect relationship has been established in several animal models, which confirm that herpesviruses accelerate thrombosis and atherosclerosis.[145-147] Indeed, HSV1 and CMV are known to induce TF activity on vascular endothelial cells,[148,149] which support infection and from which the replicative viruses derive their envelope.[74]

Electron microscopy can definitively identify the presence of a macromolecular structure associated with the surface of a virus. Using HSV1 propagated in TF-expressing cultured cells and purified by sucrose gradient differential ultracentrifugation,[74] multiple-sized electron-dense gold beads were used to simultaneously distinguish 3 constituents on a single HSV1.[150] Figure 2 shows several transmission electron micrograph examples of particles that have the diameter and morphology of HSV1, which are triple labeled. Each identically stained representative example clearly demonstrates that multiple TF molecules exist on the HSV1 surface. Since the HSV1 genome does not encode TF, the source must be of host cell in origin and implies any envelope virus may assimilate TF.

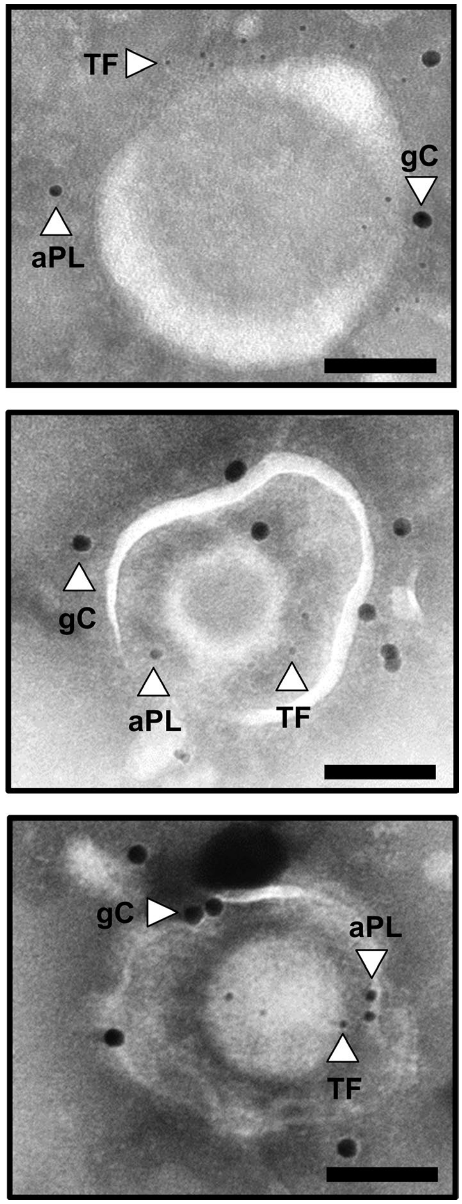

FIGURE 2 Coagulation initiators tissue factor (TF) and anionic phospholipid (aPL) are available on the herpes simplex virus type 1 (HSV1) surface [150]. Representative triple-labeled immunogold electron micrographs simultaneously identifying the HSV1 marker, glycoprotein C (gC; 15 nm gold bead), aPL (10 nm bead), and TF (6 nm gold bead). (Scale bars = 100 nm. n = 3)

Only when grown in TF-bearing cell types do purified HSV1 preparations initiate plasma clotting[106,150] and FVIIa-dependent FX activation in experimental systems using purified proteins.[106,150-152] Demonstrating specificity, these activities are inhibited by direct antagonists of TF function.[150,151] These data imply the availability of envelope aPL for macromolecular assembly of the viral TF-FVIIa-FX complex (Figure 1). Consistent with these observations, the aPL-binding protein, annexin A5, labeled with a medium-diameter gold bead, binds to TF-positive HSV1 indicating a calcium-dependent interaction with the virus (Figure 2). These data show the 2 essential cellular initiators of

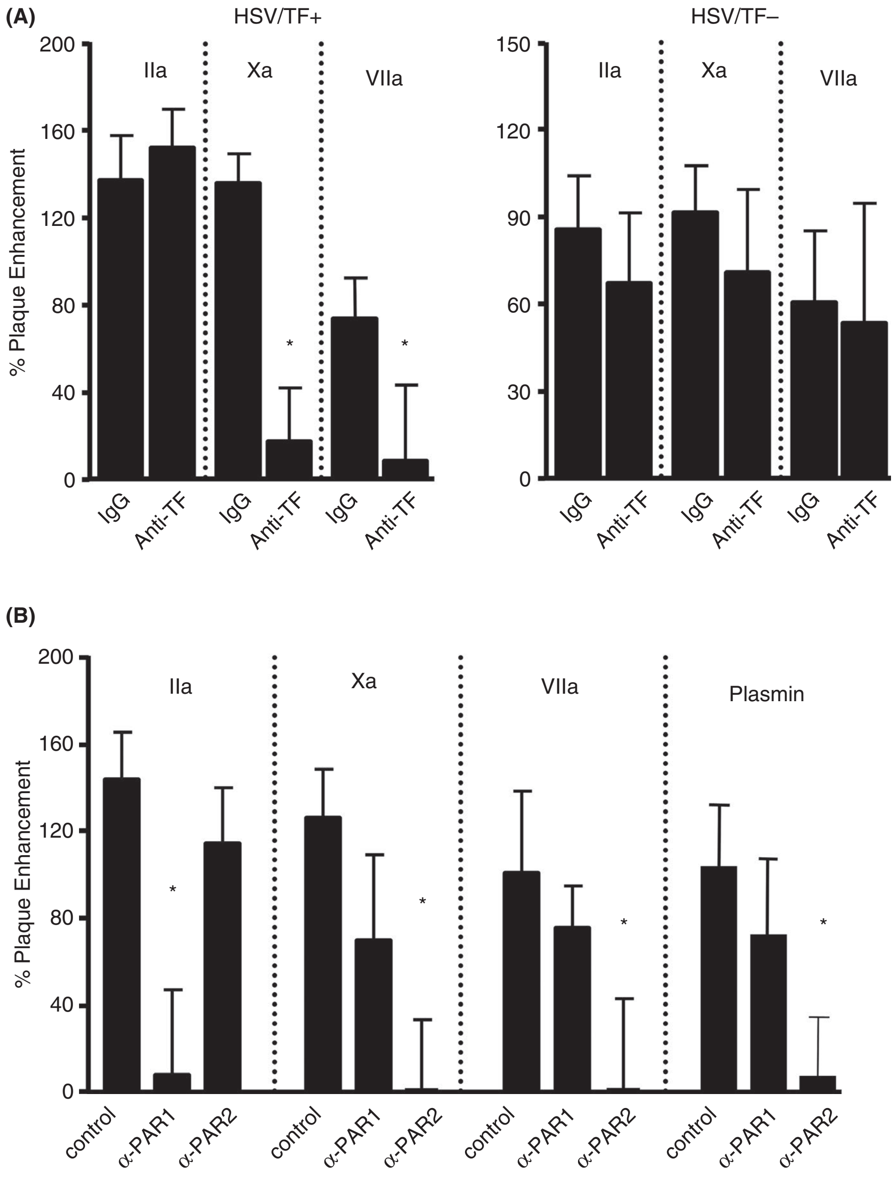

FIGURE 3 Viral tissue factor (TF) and hemostatic proteases enhance infection via protease-activated receptors (PARs) in vitro.[73,74] (A) Human umbilical vein endothelial cells (HUVECs) were incubated with a constant amount of TF+ (left panel) or TF– (right panel) herpes simplex virus type 1 (HSV1; 4.5×10^5 vp/mL) and thrombin (IIa; 10nM), factor Xa (FXa; 1nM), or factor VIIa (FVIIa; 2.5 nM) with mouse IgG (55 nM) plus enzyme (IgG) or enzyme plus anti-TF (55nM). The data were corrected for the amount of infection detected without added protease (n = 4; data are presented as mean ± SEM). *$P \le .05$ compared with mouse IgG plus enzyme. (B) HUVECs were incubated with TF + HSV1 (4.5×10^5 vp/mL) and thrombin (IIa; 10nM), FXa (1nM), FVIIa (2.5 nM) or plasmin (50 nM) with control mouse IgG (control; 50 M) plus enzyme, anti- PAR1 (α-PAR1; 150 nM) plus enzyme, or anti-PAR2 (α-PAR2; 50 nM) plus enzyme. The data were corrected for the amount of infection without added protease in the presence of control IgG (n = 4; data are presented as mean ± SEM). *$P \le .05$ compared with control IgG plus enzyme

coagulation, TF and aPL, are trafficked to the virus during envelope formation.

Confirming the identity of the particle as HSV1, the largest gold bead shown in Figure 2 denotes HSV1 encoded glycoprotein C (gC). gC is a multifunctional contributor to virus infection known to participate in virus attachment to the cell through association with heparan sulfate proteoglycan and in the evasion of host defense by complement.[153,154] When expressed on the surface of infected cells, gC has been shown to be involved in FX activation and binding,[148,155,156] which is another reason it was selected as a marker to confirm virus identity. We have reported that purified gC and gC on the virus surface mimics the cofactor function of TF toward FVIIa-enhanced FXa generation.[150,151] Like TF, it binds directly to both FVIIa and FX forming a cofactor-protease-substrate complex.[150] A further similarity to TF is that FX stabilizes gC-FVIIa cofactor-protease assembly, which for TF would rigorously localize the hemostatic response to sites of aPL accessibility. This similarly applies to the virus surface and would initiate symptomatic consequences with severity dependent on accessory constituents on the envelope and the cells that are affected.

7 | TF ENHANCES VIRUS INFECTION IN VITRO

The mimicry of TF by gC implies an advantage to the virus when hemostatic proteases are activated at the site of virus-cell docking. Pretreatment of endothelial cell monolayers with nanomolar concentrations of proteases known to trigger PARs, including FVIIa, FXa, thrombin, and plasmin, enhanced viral plaque formation by up to an order of magnitude when in combination,[73,74] as did in situ FX zymogen activation.[74] To discriminate between viral and cellular effects of TF in the infection cycle, a novel panel of HSV1 was created using a TF-inducible human A7 melanoma cell line[157] and combining this with engineered HSV1 deficient in gC production.[74] Thus, HSV1/TF–/gC–, HSV1/TF+/gC–, HSV1/TF–/gC + and HSV1/TF+/

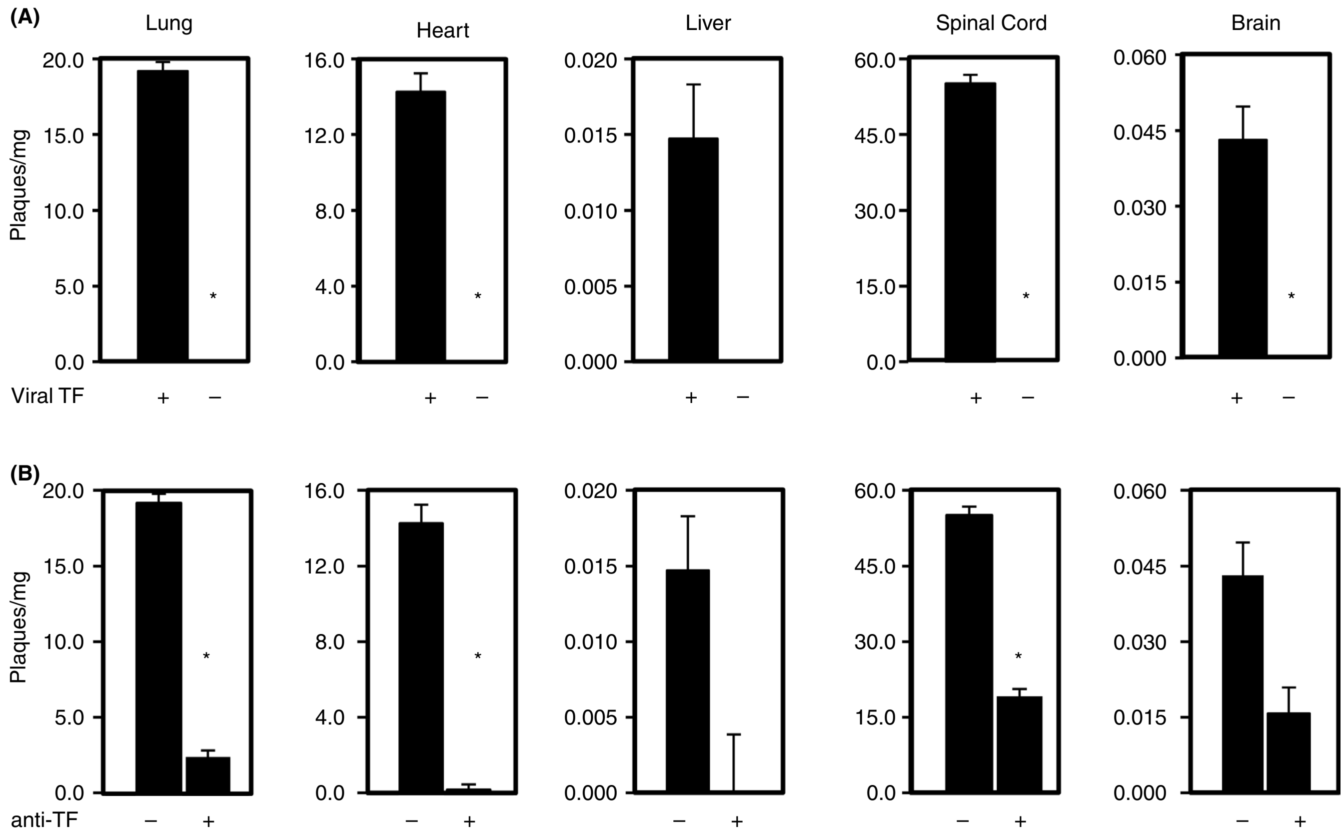

FIGURE 4 Tissue factor (TF) on herpes simplex virus type 1 (HSV1) enhances infectious virus production in mice.[158] (A) Eight-week-old female BALB/c mice were inoculated intravenously with 5 × 10^5 plaque-forming units (PFUs) of either TF-competent (TF+; n = 24) or TF-deficient (TF−; n = 13) herpes simplex virus type 1 (HSV1) via the tail vein. Three days after infection, the mice were processed and the amounts of infectious virus (plaque-forming units/mg) were determined. (B) Additional experiments were conducted after preimmunization of mice with mouse IgG or a mixture of three anti-TF IgG1 monoclonal antibodies (5G9, 9C3, and 6B4; 0.33 mg each per mouse; n = 10), 4 h prior to injection of the virus. In all panels, data are expressed as mean ± SEM. As determined with Student's *t* test, *$P \le .05$ when compared with the TF + virus alone

gC + derive their envelope from the same cell background, with TF and gC being the only known membrane protein differences.

Under conditions that facilitated FVIIa-dependent in situ FX activation during inoculation of cultured endothelial cell monolayers, TF on the virus enhanced infection as measured by standard plaque assays.[74] Using purified proteases, the enhancement due to envelope TF required FXa and FVIIa and was inhibitable by an anti-TF antibody (Figure 3A). The anti-TF antibody had no impact on thrombin-mediated enhancement of virus infection, since its cell signaling is not directly affected by TF. Further dissecting the cell surface hemostatic mechanism exploited by the virus, antibodies that specifically inhibited the stimulation of either PAR1 or PAR2 were used. The enhancement of HSV1/TF + infection due to pretreating endothelial cells with FXa, FVIIa, or plasmin was only inhibitable by anti-PAR2, whereas pretreatment with thrombin was inhibitable by anti-PAR1 (Figure 3B).[73,74] These observations reveal that TF and PARs are antiviral therapeutic targets.

The parallels between the cofactor effects of TF and gC on FX activation by FVIIa suggest that gC may be similar in PAR2-mediated infection. Comparing HSV1/TF−/gC + to HSV1/TF−/gC− demonstrated a novel binary gC/FXa combination that increased PAR2-mediated infection.[74] Interestingly, unlike purified protein experiments where gC and TF function independently in FX activation by FVIIa, TF was required for gC to appreciably enhance FXa generation on the virus envelope,[150] implying the involvement of additional virus surface constituents. The combined findings of our in vitro assay designed to study the early events of virus infection that influence the first hour of cell infection, support a model where coagulation pathway protease activation initiated by envelope TF and gC engage PAR2 and PAR1 to enhance virus replication.

8 | TF ENHANCES VIRUS INFECTION IN VIVO

The effects of envelope TF on infection have been investigated in mice using HSV1/TF±.[158] These experiments were designed to represent a model of viremia with general applicability to any envelope virus, therefore mice were inoculated via the tail vein. Unlike our well-defined in vitro model of infectivity, the pathophysiological effects of envelope TF accumulate over 3 days in vivo prior to harvesting organs for analysis. These effects could include those on the

TF-triggered innate immune response, which was not present in the in vitro model.

Substantiating the in vitro assays, a remarkable all-or-nothing difference was seen in the infectability of all 5 organs that were investigated depending on the availability of envelope TF (Figure 4A).[158] Viral plaques were undetectable in samples from mice that were inoculated with HSV1/TF–, although HSV1/TF + and HSV1/TF– had equivalent in vitro infectivity measured by traditional viral plaque assays where no clotting factor proteases are available. This in vivo model provides an ideal platform to unambiguously determine if therapeutic modulation of envelope TF affects infection. Using anti-TF antibodies that specifically recognize human TF, only the TF on the purified HSV1/TF + envelope was engaged and not the TF endogenous to the mouse. Like a TF deficiency on the virus, Figure 4B shows that therapeutically reducing viral TF activity in vivo significantly attenuates replication in each of 5 organs analyzed.

Further studies were conducted to exploit the viral TF pathway as an antiviral prophylactic target. The effects of highly specific small molecule inhibitors were evaluated in vivo. Figure 5 shows that when administered at the time of inoculation, a thrombin inhibitor (hirudin),[159,160] a TF/FVIIa/FX(a) complex inhibitor (nematode anticoagulant protein c2 [NAPc2]),[161] or a FXa-specific oral anticoagulant (apixaban),[162] each have potent antiviral activity. Of these, apixaban is a well-tolerated anticoagulant currently prescribed for several thrombotic conditions.[162] Like all anticoagulants, apixaban must be considered as a risk to bleeding. In the current mouse antiviral experiments, apixaban was used at a dose that is similar to those previously reported (1.0 mg/kg) that facilitated anticoagulation in mice[163,164] and, like these studies, had no evidence of bleeding. While this is nearly twice the therapeutic dose in humans, it facilitated complete inhibition of virus infection, implying a much lower antiviral dose will also be efficacious but this remains untested. Consistent with in vitro PAR studies,[74] these in vivo data show that directly anticoagulating the nascent TF/FVIIa/FXa complex or the proteases subsequently generated in the TF pathway, FXa and thrombin, is antiviral.

While the specific involvement of TF in coagulopathy induced by SARS-CoV-2 or other viruses has not yet been widely studied, enhanced TF activity has been associated with the primary complication of COVID-19, acute respiratory distress syndrome (ARDS).[165] ARDS typifies severe influenza virus infection, and this correlates to patient microvesicle-associated TF.[166] TF is known to play a role in Ebola virus–induced coagulopathy,[89,167] where NAPc2 reduced symptoms and increased survival of infected rhesus macaques. Of note, NAPc2 treatment also reduced virus load.[104] Combined with HSV1 results (Figures 4 and 5), TF is emerging as a key effector of viral pathophysiology and replication cycle.

Like severe COVID-19, D-dimer is elevated in Ebola virus infection.[103] In surviving Ebola-infected animals, treatment with NAPc2 reduced D-dimer. Clinical studies to establish the corollary parameter would also be of great value. Is D-dimer a prognostic indicator of recovery from SARS-CoV-2 infection? Following the finding that the use of predominantly low-molecular-weight heparin (LMWH) gave improved survival in COVID-19 patients stratified for high D-dimer and sepsis-induced coagulopathy score,[23] the ISTH established management guidelines that involves LMWH treatment.[168] Viewed predominantly as an anticoagulant, LMWH and larger polymeric forms of heparin have multiple therapeutic effects that may impact COVID-19 treatment, not the least of which is well-established anti-inflammatory benefit.[169,170] Heparin is also known to compete against initial weak virus-cell heparan sulfate proteoglycan interactions, such as for dengue virus.[171]

Whether anticoagulant and anti-inflammatory effects are provided by LMWH treatment of COVID-19 in addition to virus

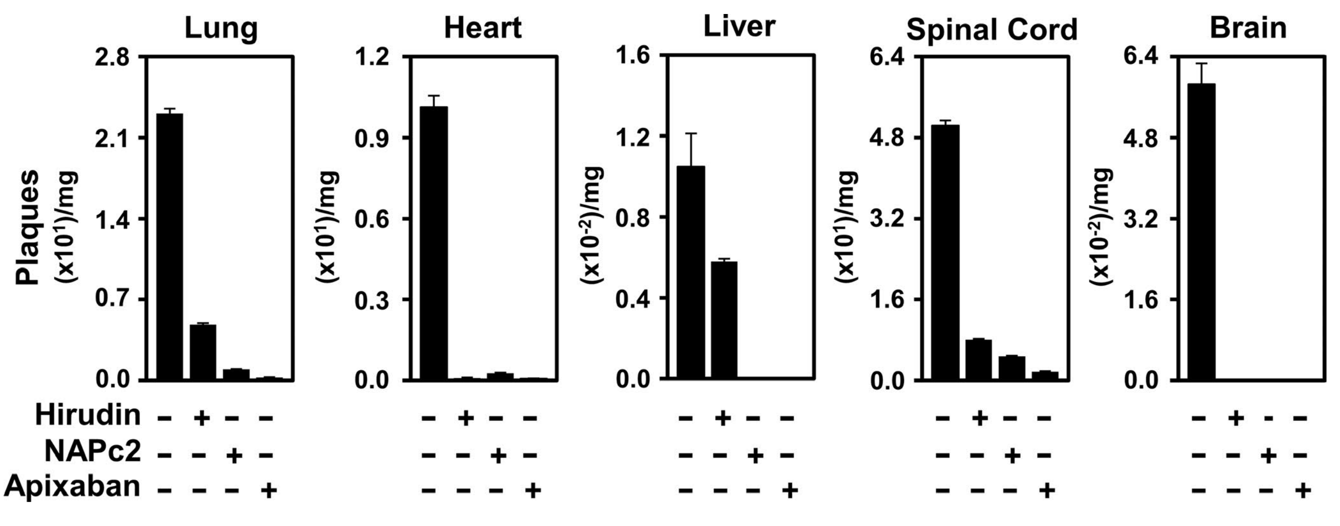

FIGURE 5 Infection of BALB/c mice is inhibited by anticoagulation.[158] Eight-week-old female BALB/c mice were inoculated intravenously with 5 × 10^5 plaque-forming units (PFUs) of tissue factor (TF)-competent (TF+; n = 24) HSV1 alone or simultaneously with hirudin (1 mg/kg, n = 9), nematode anticoagulant protein c2 (NAPc2) (1 mg/kg, n = 18), or apixaban (1 mg/kg, n = 18), via the tail vein. In each case, 3 days after infection, the mice were processed, and the amount of infectious virus (PFUs/mg) was determined in each organ. In all panels, data are expressed as mean ± SEM. As determined with Student's *t* test, *P* ≤ .05 as compared with the TF + virus alone for all data points except liver treated with hirudin

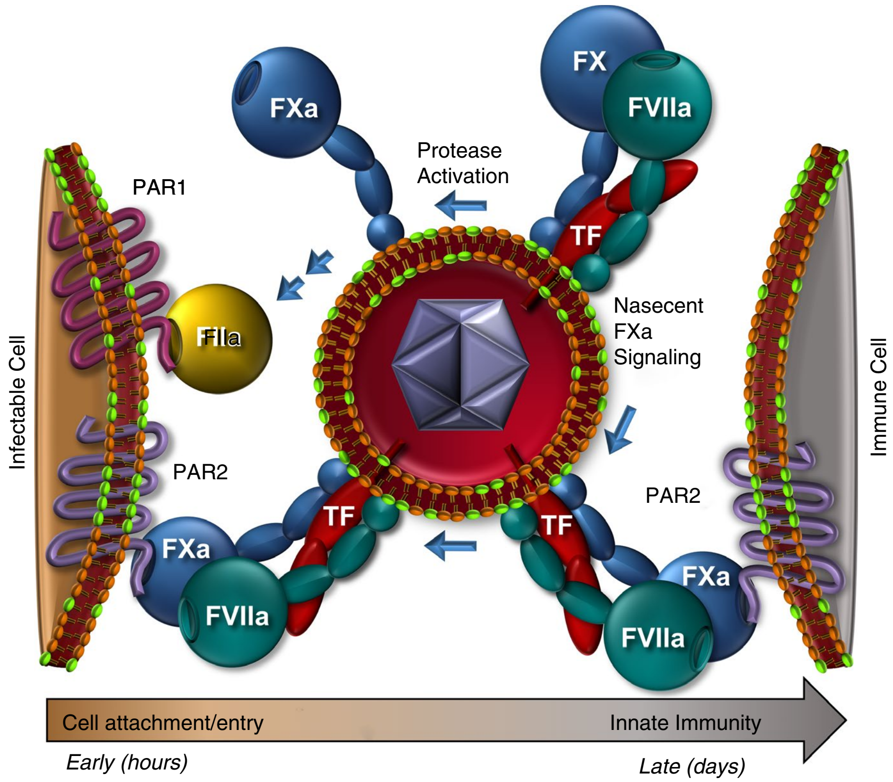

FIGURE 6 Viral tissue factor (TF) in infection. A model envelope virus is depicted showing a phospholipid bilayer. Several pools of TF may be resent during virus infection including, cellular, viral or associated with extracellular vesicles. Based on studies with herpes simplex virus type 1 (HSV1), TF is embedded in the envelope and assembled with factor VIIa (FVIIa). The known domain organization of proteins is depicted including an active site on respective protease domains (cleft). The TF/FVIIa tenase activates factor X (FX) to FXa bound to viral anionic phospholipid polar headgroups (green). The nascent FXa may either remain bound and engage in signaling through protease-activated receptor 2 (PAR2) or dissociate and participate in downstream thrombin (factor IIa [FIIa]) generation. When early events of infection were monitored in the absence of the immune system in vitro, both PAR2 via TF/FVIIa/FXa or protease-activated receptor 1 (PAR1) via thrombin-enhanced infection. In mice, the absence of envelope TF prevented infection of HSV1 in all organs evaluated. In these in vivo experiments, PAR1 continued to enhance HSV1/TF + virus infection. Highlighting a switch in the role of PAR2 function compared to only evaluating the early stages of infection in vitro (eg, cell attachment and entry), PAR2 reduced virus infection in vivo, presumably through innate immune cell recruitment

receptor-mediated effects is unknown. However, based on the finding that hindering the TF/FVIIa/FXa signaling mechanism will curtail virus infection, it may be possible to attenuate thrombosis and virus replication with a single anticoagulant. LMWH affects coagulation indirectly predominantly by accelerating antithrombin-mediated inhibition of FXa inhibition, and this is precluded when FXa and other hemostatic proteases are in complex with other macromolecules.[172-174] Therefore, FXa-specific small direct oral anticoagulants (DOACs), such as apixaban, that are not susceptible to the steric limitations of antithrombin would be preferable as potential dual-purpose antiviral-anticoagulant agents. Numerous patient factors must be considered, such as the heterogeneity in patient presentation and risk factors, and oral versus intravenous mode of drug delivery. However, simultaneously mitigating thromboinflammation and the underlying basis, persistent virus replication, will reduce the duration of morbidity and mitigate tissue damage.

To address the high prothrombotic rates that are being reported for COVID-19,[4-10] thrombolysis with recombinant t-PA has been used to treat patients with respiratory distress syndrome.[175] In this case report, 3 patients initially showed symptomatic improvement, with 1 surviving. However, the downstream enzyme produced by t-PA, plasmin, has been predicted to proteolytically prepare the SARS-CoV-2 spike-protein for entry into ACE2-containing cells.[25] Thus, the demise of the other patients treated with thrombolytic agent may be due to a surge in viral pathogenicity.

While not typically measured unless symptomatically indicated, like SARS-CoV-2 D-dimer is elevated in other virus infections, such as HIV,[176,177] influenza H5N1,[178] and chikungunya[179] viruses. For HIV, IL-6 has been identified as a stronger predictor of the severity of clinical events than D-dimer.[180] Drawing on recent evidence from another inflammatory pathology, sickle cell disease, a mouse model has shown that endothelial cell–specific deletion of TF and separate deletion of PAR1, attenuated IL-6 production and averted inflammation and symptoms.[181,182] These authors furthermore used another FXa-specific DOAC, rivaroxaban, to avert symptoms in their sickle cell model.[182] These examples combined with our direct observations that reduction of TF activity in mice decreases infection suggest that use of a FXa-specific DOAC may also attenuate IL-6 driven inflammation.

9 | PROTEASE-ACTIVATED RECEPTOR INHIBITORS IN VIRUS INFECTION

For therapeutic intervention of the TF pathway as an antiviral strategy, careful consideration must be taken because of the risk of bleeding. However, TF-dependent cell signaling by hemostatic proteases via PARs is also a fundamental aspect of the innate immune system and inflammation. Thus, inhibition by DOACs or other anticoagulants could have complex and unpredictable consequences[183] and must be approached cautiously. The important role of PARs has been summarized in a comprehensive review.[87] The general model that is emerging from several labs is that PAR2-mediated stimulation initiates an intricate mechanism. This enables a network of proteases, including thrombin, that additionally engage PAR1, PAR3, and PAR4. Inflammatory cells are consequently recruited. However, the results from PAR knockout (KO) animals are somewhat conflicting since inflammation may both resolve the virus and have a deleterious impact on tissues.

As examples of envelope viruses that may carry host cell-derived TF on their surface, influenza and HSV1 have been studied in PAR2 or PAR1 KO mice. Because of distinct sets of parameters that are measured, it is difficult to unambiguously conclude whether virus replication, immune clearance, or pathological inflammation are affected exclusively or in combination. In experiments conducted using PAR2 KO mice, PAR2 was found to exacerbate influenza infection severity[184] or improve outcome to the animal in influenza[185] or HSV1[158] infection. Similarly, there is reported discrepancy in the infection of PAR1 KO mice, where PAR1 was concluded to either contribute to reducing influenza virus load,[186] or increase influenza symptoms[187] or increase HSV1 titer.[158] An interesting consistency between groups is the preparation of virus inoculum in cells known to express TF[158,185,187] or not,[184,186] which may indicate biases in signaling mechanisms during infection.

For infection models of HSV1, in vitro[74] and in vivo[158] experiments both showed that PAR1 participates to enhance infection. Like the PAR1 results, HSV1 infection of cells in culture was also increased by PAR2.[74] However, in a mouse KO model of infection, the presence of PAR2 attenuated the infection of most organs evaluated by HSV1,[158] which contradicts the in vitro experiments. This inconsistency likely involves effects of immune surveillance, which plays a role only in the in vivo model and timing is also a probable factor (Figure 6). It is reasonable to speculate that the earliest stage of virus infection, consisting of virus-cell attachment and entry, is enhanced by PAR1 and PAR2. These events are reported by the in vitro virus plaque formation assays that consist of a 1-hour inoculation period [74]. During the 3-day duration of the in vivo experiments, the multifunctional effects of PARs are enabled. While the roles localized to the site of virus-cell docking may still be progressing, these are overwhelmed by opposing roles of PAR2 in the immune and inflammatory responses,[71,76] which impair propagation of the virus. These latter effects may involve PAR2 signaling that is distal from the initial role played by envelope TF. Thus, the timing that anticoagulant therapy is delivered may impact its concomitant anticoagulant, antiviral, and anti-inflammatory properties.

10 | CONCLUSION

Envelope TF may be a virulence effector and the long-sought common denominator linking numerous prevalent envelope viruses. The monumental question is how to singularly exploit TF as an antiviral target and to diminish inflammation when its roles in physiology are vast? FXa-specific DOACs have been reported as having both antiviral[158] and anti-inflammatory[182] effects when administered early in mouse models of disease. This may be similar for infection by SARS-CoV-2 and other viruses that can propagate in TF-bearing cells. If administered early in the infection cycle, DOACs may have antiviral, anti-inflammatory, and their intended anticoagulant benefit, whereas later-stage infection may predominantly alleviate symptomatic thrombotic and inflammatory disease. Both early and late stages of infection involve TF, and both are prime targets for combinations of anticoagulation, anti-PAR, and anti-inflammatory innovations.

ACKNOWLEDGMENTS

We are indebted to Scott Meixner (Centre for Innovation, Canadian Blood Services, and Centre for Blood Research, University of British Columbia) for invaluable editorial discussion. This work was supported by the Canadian Institutes of Health Research grant 273985 (EP) and Heart and Stroke Foundation of Canada G-19-0026524 (EP). BL was supported by a Centre for Blood Research Graduate Award Program Grant.

RELATIONSHIP DISCLOSURE

The authors declare nothing to report.

AUTHOR CONTRIBUTIONS

EP wrote the manuscript; MS analyzed data, prepared figures, and edited the manuscript; BL prepared figures, and edited the manuscript. MH edited the manuscript.

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506.
2. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5:536–44.
3. Schoeman D, Fielding BC. Coronavirus envelope protein: current knowledge. Virol J. 2019;16:69.
4. Danzi GB, Loffi M, Galeazzi G, Gherbesi E. Acute pulmonary embolism and COVID-19 pneumonia: a random association? Eur Heart J. 2020;41(19):1858.

5. Luo W, Yu H, Guo J, Li X, Sun Y, Li J, Liu L. Clinical pathology of critical patient with novel coronavirus pneumonia (COVID-19). Pre-prints. 2020. 2020020407.
6. Xu X, Yu C, Qu J, Zhang L, Jiang S, Huang D, et al. Imaging and clinical features of patients with 2019 novel coronavirus SARS-CoV-2. Eur J Nucl Med Mol Imaging. 2020;47:1275–80.
7. Fox SE, Almatbekov A, Harbert JL, Li G, Brown Q, Vander Heide RS. Pulmonary and cardiac pathology in Covid-19: The first autopsy series from New Orleans. medRxiv preprint. 2020. https://doi.org/10.1101/2020.04.06.20050575
8. Wang T, Chen R, Liu C, Liang W, Guan W, Tang R, et al. Attention should be paid to venous thromboembolism prophylaxis in the management of COVID-19. Lancet Haematol. 2020;7(5):e362.
9. Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145–7.
10. Middeldorp S, Coppens M, van Haaps TF, Foppen M, Vlaar AP, Muller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020. https://doi.org/10.1111/jth.14888
11. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunologic features in severe and moderate Coronavirus Disease 2019. J Clin Invest. 2020;130(5):2620–9.
12. Madjid M, Safavi-Naeini P, Solomon SD, Vardeny OPotential effects of coronaviruses on the cardiovascular system. A review. JAMA Cardiol. 2020. https://doi.org/10.1001/jamacardio.2020.1286
13. Johns Hopkins University Coronavirus Resource Center 2020. [Accessed 2020 May 28] Available from https://coronavirus.jhu.edu
14. Lai CC, Liu YH, Wang CY, Wang YH, Hsueh SC, Yen MY, et al. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths. J Microbiol Immunol Infect. 2020;53(3):404–412.
15. Levi M, Hunt BJ. Thrombosis, Thromboprophylaxis & Coagulopathy in COVID- 19 Infections. *International Society of Thrombosis and Haemostasis Webinar.* 2020; [Accessed 2020 April 9] Available from https://academy.isth.org/isth/2020/covid-19/291581/marcel.levi.26.beverley.jane.hunt.thrombosis.thromboprophylaxis.26.coagulopathy.html?f=menu%3D8%2Abrowseby%3D8%2Asortby%3D2%2Alabel%3D19794
16. Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab Res Rev. 2020;80:e3319.
17. Li X, Wang L, Yan S, Yang F, Xiang L, Zhu J, et al. Clinical characteristics of 25 death cases with COVID-19: a retrospective review of medical records in a single medical center, Wuhan, China. Int J Infect Dis. 2020;3:3.
18. Zhang JJ, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy. 2020;19:19.
19. Yin S, Huang M, Li D, Tang N. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. J Thromb Thrombolysis. 2020;3:3.
20. Mi B, Chen L, Xiong Y, Xue H, Zhou W, Liu G. Characteristics and Early Prognosis of COVID-19 Infection in Fracture Patients. J Bone Joint Surg Am. 2020;2020:1.
21. Wan S, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J Med Virol. 2020;21:21.
22. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. Lancet Infect Dis. 2020;25:25.
23. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost. 2020;27:27.
24. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China . JAMA Int Med. 2020;13:13.
25. Ji HL, Zhao R, Matalon S, Matthay MA. Elevated plasmin(ogen) as a common risk factor for COVID-19 susceptibility. Physiol Rev. 2020;100:1065–75.
26. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ. 2020;368:m1091.
27. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18:844–7.
28. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054–62.
29. Zhang G, Zhang J, Wang B, Zhu X, Wang Q, Qiu S. Analysis of clinical characteristics and laboratory findings of 95 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a retrospective analysis. Respir Res. 2020;21:74.
30. Hanss M, Collen D. Secretion of tissue-type plasminogen activator and plasminogen activator inhibitor by cultured human endothelial cells: modulation by thrombin, endotoxin, and histamine. J Lab Clin Med. 1987;109:97–104.
31. Emeis JJ. Regulation of the acute release of tissue-type plasminogen activator from the endothelium by coagulation activation products. Ann N Y Acad Sci. 1992;667:249–58.
32. Oliver JJ, Webb DJ, Newby DE. Stimulated tissue plasminogen activator release as a marker of endothelial function in humans. Arterioscler Thromb Vasc Biol. 2005;25:2470–9.
33. Han H, Yang L, Liu R, Liu F, Wu KL, Li J, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med. 2020;58:1116–20.
34. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. Clin Chim Acta. 2020;506:145–8.
35. Pryzdial ELG, Lin BH, Sutherland MR. Virus-platelet associations. In: Gresele P, Lopez JA, Klieman NS, Page CP, editors. Platelets in thrombotic and non-thrombotic disorders, 2nd edn. New York: Springer; 2017. p. 1085–102.
36. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol. 2020;17:17.
37. Zhang Y, Cao W, Xiao M, Li YJ, Yang Y, Zhao J, et al. Clinical and coagulation characteristics of 7 patients with critical COVID-2019 pneumonia and acro-ischemia. Chin J Hematol. 2020;41:e006.
38. Li CX, Wu B, Luo F, Zhang N. Clinical study and CT findings of a familial cluster of pneumonia with coronavirus disease 2019 (COVID-19). J Sichuan Univ Med Sci Ed. 2020;51:155–8.
39. Taylor FB, Toh CH, Hoots WH, Wada H, Levi M. Towards a definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. ISTH Scientific and Standardization Committee Communications. 2001;86(5):1327–30.
40. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. Br J Haematol. 2020;189(3):428–37.
41. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–3.
42. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and

TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271–80.
43. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin 1–7 axis of the renin-angiotensin system in heart failure. Circ Res. 2016;118:1313–26.
44. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem. 2000;275:33238–43.
45. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy Region, Italy. JAMA. 2020;323(16):1574.
46. Xu K, Chen Y, Yuan J, Yi P, Ding C, Wu W, et al. Factors associated with prolonged viral RNA shedding in patients with COVID-19. Clin Infect Dis. 2020. https://doi.org/10.1093/cid/ciaa351
47. Cai Q, Chen F, Wang T, Luo F, Liu X, Wu Q, et al. Obesity and COVID-19 severity in a designated hospital in Shenzhen, China. Diabetes Care. 2020;43(7):1392–8.
48. D'Antiga L. Coronaviruses and immunosuppressed patients. The facts during the third epidemic. Liver Transpl. 2020;26(6):832–4.
49. Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR, et al. Complement activation contributes to severe acute respiratory syndrome coronavirus pathogenesis. MBio. 2018;9:5.
50. Foley JH, Conway EM. Cross talk pathways between coagulation and inflammation. Circ Res. 2016;118:1392–408.
51. Versteeg HH, Schaffner F, Kerver M, Petersen HH, Ahamed J, Felding-Habermann B, et al. Inhibition of tissue factor signaling suppresses tumor growth. Blood. 2008;111:190–9.
52. Forster Y, Meye A, Albrecht S, Schwenzer B. Tissue factor and tumor: Clinical and laboratory aspects. Clin Chim Acta. 2006;364:12–21.
53. Chantrathammachart P, Mackman N, Sparkenbaugh E, Wang JG, Parise LV, Kirchhofer D, et al. Tissue factor promotes activation of coagulation and inflammation in a mouse model of sickle cell disease. Blood. 2012;120:636–46.
54. Shet AS, Aras O, Gupta K, Hass MJ, Rausch DJ, Saba N, et al. Sickle blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. Blood. 2003;102:2678–83.
55. Badeanlou L, Furlan-Freguia C, Yang G, Ruf W, Samad F. Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. Nat Med. 2011;17:1490–7.
56. Soma P, Swanepoel AC, Bester J, Pretorius E. Tissue factor levels in type 2 diabetes mellitus. Inflamm Res. 2017;66:365–8.
57. Zhang C, Ou Q, Gu Y, Cheng G, Du R, Yuan L, et al. Circulating tissue factor-positive procoagulant microparticles in patients with type 1 diabetes. Diabetes Metab Syndr Obes. 2019;12:2819–28.
58. Bokarewa MI, Morrissey J, Tarkowski A. Intra-articular tissue factor/factor VII complex induces chronic arthritis. Inflamm Res. 2002;51:471–7.
59. Chen L, Lu Y, Chu Y, Xie J, Ding W, Wang F. Tissue factor expression in rheumatoid synovium: a potential role in pannus invasion of rheumatoid arthritis. Acta Histochem. 2013;115:692–7.
60. Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. Arterioscler Thromb Vasc Biol. 2018;38:709–25.
61. Tremoli E, Camera M, Toschi V, Colli S. Tissue factor in atherosclerosis. Atherosclerosis. 1999;144:273–83.
62. Rosen ED, Chan JC, Idusogie E, Clotman F, Vlasuk G, Luther T, et al. Mice lacking factor VII develop normally but suffer fatal perinatal bleeding. Nature. 1997;390:290–4.
63. Carmeliet P, Mackman N, Moons L, Luther T, Gressens P, Van Vlaenderen L, et al. Role of tissue factor in embryonic blood vessel development. Nature. 1996;383:73–5.
64. Butenas S. Tissue factor structure and function. Scientifica (Cairo). 2012;2012:964862.
65. Smith SA, Travers RJ, Morrissey JH. How it all starts: Initiation of the clotting cascade. Crit Rev Biochem Mol Biol. 2015;50:326–36.
66. Rao LV, Rapaport SI. Activation of factor VII bound to tissue factor: a key early step in the tissue factor pathway of blood coagulation. Proc Natl Acad Sci U S A. 1988;85:6687–91.
67. Krishnaswamy S, Field KA, Morrissey JH, Edgington TS, Mann KG. Activation of factor X by the extrinsic pathway. Thromb Haemostasis. 1991;65:296.
68. Kamikubo Y, Mendolicchio GL, Zampolli A, Marchese P, Rothmeier AS, Orje JN, et al. Selective factor VIII activation by the tissue factor-factor VIIa-factor Xa complex. Blood. 2017;130:1661–70.
69. Pryzdial ELG. Maestro tissue factor reaches new hEIGHT. Blood. 2017;130:1604–5.
70. Krishnaswamy S. The interaction of human factor VIIa with tissue factor. J Biol Chem. 1992;267:23696–706.
71. Zelaya H, Rothmeier A, Ruf W. Tissue Factor at the crossroad of coagulation and cell signaling. J Thromb Haemost. 2018;16(10):1941–52.
72. Hamilton JR, Trejo J. Challenges and opportunities in protease-activated receptor drug development. Annu Rev Pharmacol Toxicol. 2017;57:349–73.
73. Gershom ES, Vanden Hoek AL, Meixner SC, Sutherland MR, Pryzdial ELG. Herpesviruses enhance fibrin clot lysis. Thromb Haemost. 2012;107:760–8.
74. Sutherland MR, Ruf W, Pryzdial ELG. Tissue factor and glycoprotein C on herpes simplex virus type 1 are protease-activated receptor 2 cofactors that enhance infection. Blood. 2012;119:3638–45.
75. Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. Pharmacol Rev. 2001;53:245–82.
76. Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. Arterioscler Thromb Vasc Biol. 2018;38:709–25.
77. D'Alessandro E, Posma JJN, Spronk HMH, Ten Cate H. Tissue factor (: Factor VIIa) in the heart and vasculature: More than an envelope. Thromb Res. 2018;168:130–7.
78. Wang L, Bastarache JA, Wickersham N, Fang X, Matthay MA, Ware LB. Novel role of the human alveolar epithelium in regulating intra-alveolar coagulation. Am J Respir Cell Mol Biol. 2007;36:497–503.
79. Disse J, Petersen HH, Larsen KS, Persson E, Esmon N, Esmon CT, et al. The endothelial protein C receptor supports tissue factor ternary coagulation initiation complex signaling through protease-activated receptors. J Biol Chem. 2011;286:5756–67.
80. Disse J, Ruf W. Endothelial protein C receptor is required for tissue factor ternary complex signaling in the mouse. J Thromb Haemost. 2011;9:2516–8.
81. Rana S, Yang L, Hassanian SM, Rezaie AR. Determinants of the specificity of protease-activated receptors 1 and 2 signaling by factor Xa and thrombin. J Cell Biochem. 2012;113:977–84.
82. Chuansumrit A, Chaiyaratana W. Hemostatic derangement in dengue hemorrhagic fever. Thromb Res. 2014;133:10–6.
83. Goeijenbier M, van Wissen M, van de Weg C, Jong E, Gerdes V, Meijers J, et al. Brandjes DP, van Gorp EC. Review: Viral infections and mechanisms of thrombosis and bleeding. J Med Virol. 2012;84:1680–96.
84. Baker JV, Brummel-Ziedins K, Neuhaus J, Duprez D, Cummins N, Dalmau D, et al. HIV replication alters the composition of extrinsic pathway coagulation factors and increases thrombin generation. J Am Heart Assoc. 2013;2:e000264.
85. Chuang YC, Lin YS, Liu CC, Liu HS, Liao SH, Shi MD, et al. Factors contributing to the disturbance of coagulation and fibrinolysis in dengue virus infection. J Formos Med Assoc. 2013;112:12–7.

86. Pryzdial EL, Sutherland MR, Ruf W. The procoagulant envelope virus surface: contribution to enhanced infection. Thromb Res. 2014;133(Suppl 1):S15–S17.
87. Antoniak S, Mackman N. Multiple roles of the coagulation protease cascade during virus infection. Blood. 2014;123:2605–13.
88. Gonzalez-Reimers E, Quintero-Platt G, Martin-Gonzalez C, Perez-Hernandez O, Romero-Acevedo L, Santolaria-Fernandez F. Thrombin activation and liver inflammation in advanced hepatitis C virus infection. World J Gastroenterol. 2016;22:4427–37.
89. Ruf W. Emerging roles of tissue factor in viral hemorrhagic fever. Trends Immunol. 2004;25:461–4.
90. Mackow ER, Gorbunova EE, Gavrilovskaya IN. Endothelial cell dysfunction in viral hemorrhage and edema. Front Microbiol. 2014;5:733.
91. Bouwman JJ, Visseren FL, Bosch MC, Bouter KP, Diepersloot RJ. Procoagulant and inflammatory response of virus-infected monocytes. Eur J Clin Invest. 2002;32:759–66.
92. Agrawal P, Nawadkar R, Ojha H, Kumar J, Sahu A. Complement Evasion Strategies of Viruses: an Overview. Front Microbiol. 2017;8:1117.
93. Witz M, Lehman J, Korzets Z. Acute brachial artery thrombosis as the initial manifestation of human immunodeficiency virus infection. Am J Hematol. 2000;64:137–9.
94. Wasif M, Greenberg B. HIV and Thrombosis: a review. AIDS Patient Care and STDs. 2001;15:15–24.
95. Srichaikul T, Nimmannitya S. Haematology in dengue and dengue haemorrhagic fever. Baillieres Best Pract Res Clin Haematol. 2000;13:261–76.
96. Wills BA, Oragui EE, Stephens AC, Daramola OA, Dung NM, Loan HT, et al. Coagulation abnormalities in dengue hemorrhagic fever: serial investigations in 167 Vietnamese children with dengue shock syndrome. Clin Infect Dis. 2002;35:277–85.
97. van Gorp EC, Minnema MC, Suharti C, Mairuhu AT, Brandjes DP, ten Cate H, et al. Activation of coagulation factor XI, without detectable contact activation in dengue haemorrhagic fever. Br J Haematol. 2001;113:94–9.
98. Sundberg E, Hultdin J, Nilsson S, Ahlm C. Evidence of disseminated intravascular coagulation in a hemorrhagic fever with renal syndrome-scoring models and severe illness. PLoS One. 2011;6:e21134.
99. Ishizaka N, Ishizaka Y, Takahashi E, Tooda E-I, Hashimoto H, Nagai R, et al. Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima-media thickening. Lancet. 2002;359:133–5.
100. Boyd JT, Wangensteen KJ, Krawitt EL, Hamill RW, Kao CH, Tsai HH. Hepatitis C virus infection as a risk factor for Parkinson disease: a nationwide cohort study. Neurology. 2016;87:342.
101. Yang Y, Tang H. Aberrant coagulation causes a hyper-inflammatory response in severe influenza pneumonia. Cell Mol Immunol. 2016;13:432–42.
102. Goeijenbier M, van Gorp EC, Van den Brand JM, Stittelaar K, Bakhtiari K, Roelofs JJ, et al. Activation of coagulation and tissue fibrin deposition in experimental influenza in ferrets. BMC Microbiol. 2014;14:134.
103. Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. Lancet. 2003;362:1953–8.
104. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. J Infect Dis. 2003;188:1618–29.
105. Wu Y, Cui X, Wu N, Song R, Yang W, Zhang W, et al. A unique case of human Zika virus infection in association with severe liver injury and coagulation disorders. Sci Rep. 2017;7:11393.
106. Sutherland MR, Raynor CM, Leenknegt H, Wright JF, Pryzdial ELG. Coagulation initiated on herpesviruses. Proc Natl Acad Sci U S A. 1997;94:13510–4.
107. Forbes HJ, Benjamin L, Breuer J, Brown MM, Langan SM, Minassian C, et al. The association between human herpesvirus infections and stroke: a systematic review protocol. BMJ Open. 2017;7:e016427.
108. Sorlie PD, Nieto FJ, Adam E, Folsom AR, Shahar E, Massing M. A prospective study of cytomegalovirus, herpes simplex virus 1 and coronary heart disease. Archives Int Med. 2000;160:2027–32.
109. Youd P, Main J, Jackson E. Cytomegalovirus infection and thrombosis: a causative association? J Inf Dis. 2003;46:141–2.
110. Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Victor A, et al. Impact of infectious burden on progression of carotid atherosclerosis. Stroke. 2002;33:2581–6.
111. Epstein SE, Zhu J, Burnett MS, Zhou YF, Vecellotti G, Hajjar D. Infection and atherosclerosis: Potential roles of pathogen burden and molecular mimicry. Arterioscler Thromb Vasc Biol. 2000;20:1417–20.
112. Zhu J, Nieto FJ, Horne BD, Anderson JL, Muhlestein JB, Epstein SE. Prospective study of pathogen burden and risk of myocardial infarction or death. Circulation. 2001;103:45–51.
113. Siscovick DS, Schwartz SM, Corey L, Grayston JT, Ashley R, Wang SP, et al. Chlamydia pneumoniae, herpes simplex virus type 1, and cytomegalovirus and incident myocardial infarction and coronary heart disease death in older adults - the Cardiovascular Health Study. Circulation. 2000;102:2335–40.
114. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;581:215–20.
115. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181:281–92.
116. Lippe R. Deciphering novel host-herpesvirus interactions by virion proteomics. Front Microbiol. 2012;3:181.
117. Park JA, Sharif AS, Tschumperlin DJ, Lau L, Limbrey R, Howarth P, et al. Tissue factor-bearing exosome secretion from human mechanically stimulated bronchial epithelial cells in vitro and in vivo. J Allergy Clin Immunol. 2012;130:1375–83.
118. Carson SD, Perry GA, Pirruccello SJ. Fibroblast tissue factor: Calcium and ionophore induce shape changes, release of membrane vesicles and redistribuion of tissue factor antigen in addition to increased procoagulant activity. Blood. 1994;84:526–34.
119. Martines RB, Ng DL, Greer PW, Rollin PE, Zaki SR. Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. J Pathol. 2015;235:153–74.
120. La Gruta NL, Kedzierska K, Stambas J, Doherty PC. A question of self-preservation: immunopathology in influenza virus infection. Immunol Cell Biol. 2007;85:85–92.
121. Clayton KL, Garcia JV, Clements JE, Walker BD. HIV infection of macrophages: implications for pathogenesis and cure. Pathog Immun. 2017;2:179–92.
122. Dalrymple NA, Mackow ER. Virus interactions with endothelial cell receptors: implications for viral pathogenesis. Curr Opin Virol. 2014;7:134–40.
123. Mladinich MC, Schwedes J, Mackow ER. Zika virus persistently infects and is basolaterally released from primary human brain microvascular endothelial cells. MBio. 2017;8:1–17.
124. Revie D, Salahuddin SZ. Role of macrophages and monocytes in hepatitis C virus infections. World J Gastroenterol. 2014;20:2777–84.
125. Farooq AV, Shukla D. Herpes simplex epithelial and stromal keratitis: an epidemiologic update. Surv Ophthalmol. 2012;57:448–62.
126. Stahl JP, Mailles A, Dacheux L, Morand P. Epidemiology of viral encephalitis in 2011. Med Mal Infect. 2011;41:453–64.

127. Malkin JE. Epidemiology of genital herpes simplex virus infection in developed countries. Herpes. 2004;11(Suppl 1):2A–23A.
128. Roterman M, Langlois KA, Severini A, Totten S. Prevalence of Chlamydia trachomatis and herpes simplex virus type 2: Results from the 2009 to 2011 Canadian Health Measures Survey. Health Rep. 2013;24(4):10–15.
129. Khoury-Hanold W, Yordy B, Kong P, Kong Y, Ge W, Szigeti-Buck K, et al. Viral spread to enteric neurons links genital HSV-1 infection to toxic megacolon and lethality. Cell Host Microbe. 2016;19:788–99.
130. Nicholson AC, Hajjar DP. Herpesvirus and thrombosis: activation of coagulation on the endothelium. Clin Chim Acta. 1999;286:23–9.
131. Visser MR, Vercellotti GM. Herpes simplex virus and atherosclerosis. Eur Heart J. 1993;14(Suppl K):39–42.
132. Schaumburg-Lever G, Saffold OE, Orfanos CE, Lever WF. Herpes gestationis. Histology and ultrastructure. Arch Dermatol. 1973;107:888–92.
133. McSorley J, Shapiro L, Brownstein MH, Hsu KC. Herpes simplex and varicella-zoster: comparative histopathology of 77 cases. Int J Dermatol. 1974;13:69–75.
134. Phinney PR, Fligiel S, Bryson YJ, Porter DD. Necrotizing vasculitis in a case of disseminated neonatal herpes simplex infection.. Arch Pathol Lab Med. 1982;106:64–7.
135. Snider SB, Jacobs CS, Scripko PS, Klein JP, Lyons JL. Hemorrhagic and ischemic stroke secondary to herpes simplex virus type 2 meningitis and vasculopathy. J Neurovirol. 2014;20:419–22.
136. Nieto FJ, Adam E, Sorlie P, Farzadegan H, Melnick JL, Comstock GW, et al. Cohort study of cytomegalovirus infection as a risk factor for carotid intimal-medial thickening, a measure of subclinical restenosis. Circulation. 1996;94:922–7.
137. Chiu B, Viira E, Tucker W, Fong IW. Chlamydia pneumoniae, cytomegalovirus and herpes simplex virus in atherosclerosis of the carotid artery. Circulation. 1997;96:2144–88.
138. Pera A, Caserta S, Albanese F, Blowers P, Morrow G, Terrazzini N, et al. CD28(null) pro-atherogenic CD4 T-cells explain the link between CMV infection and an increased risk of cardiovascular death. Theranostics. 2018;8:4509–19.
139. Wu TC, Hruban RH, Ambinder RF, Pizzorno M, Cameron DE, Baumgartner WA, et al. Demonstration of cytomegalovirus nucleic acids in the coronary arteries of transplanted hearts. Am J Pathol. 1992;140:739–47.
140. Hruban RH, Wu T-C, Beschorner WE, Cameron DE, Ambinder RF, Baumgartner WA, et al. Cytomegalovirus nucleic acids in allografted hearts. Hum Pathol. 1990;21:981–3.
141. Hruban RH, Beschorner WE, Baumgartner WA, Augustine SM, Ren H, Reitz BA, et al. Accelerated atherosclerosis in heart transplant recipients is associated with a T-lymphocyte-mediated endothelialitis. Am J Pathol. 1990;137:871.
142. Foegh ML. Chronic rejection - graft arteriosclerosis. Transplant Proceedings. 1990;22:119–22.
143. Zhou YF, Leon MB, Waclawiw MA, Popma JJ, Yu ZX, Finkel T, et al. Association between prior cytomegalovirus infection and the risk of restenosis after coronary atherectomy. New Eng J Med. 1996;335:624–30.
144. Epstein SE, Speir E, Zhou YF, Guetta E, Leon M, Finkel T. The role of infection in restenosis and atherosclerosis: focus on cytomegalovirus. Lancet. 1996;348:s13–s17.
145. Fabricant CG, Fabricant J, Minick CR, Litrenta MM. Herpesvirus induced atherosclerosis in chickens. Fed Proc. 1983;42:2476–9.
146. Span AHM, Grauls G, Bosman F, Vanboven CPA, Bruggeman CA. Cytomegalovirus infection induces vascular injury in the rat. Atherosclerosis. 1992;93:41–52.
147. Virgin HW, Weck KE, DalCanto AJ, Gould JD, Latreille P, Speck SH. Novel murine gamma-herpesvirus (HV68) model of vasculitis and oncogenesis. Am Soc Virol. 1997; 16th Meeting (abstr): 178.
148. Key NS, Bach RR, Vercellotti GM, Moldow CF. Herpes simplex virus type 1 does not require productive infection to induce tissue factor in human umbilical vein endothelial cells. Lab Invest. 1993;68:645–51.
149. Vercellotti GM, Kovacs A, Key NS, Dandelet LA, Jacob HS.Human cytomegalovirus infection of endothelial cells induces tissue factor expression. Thromb Haemost. 1997; Supplement: 203 (abs).
150. Lin BH, Sutherland MR, Rosell FI, Morrissey JH, Pryzdial ELG. Coagulation factor VIIa binds to herpes simplex virus 1-encoded glycoprotein C forming a factor X-enhanced tenase complex oriented on membranes. J Thromb Haemost. 2020;18(6):1370–80.
151. Sutherland MR, Friedman HM, Pryzdial ELG. Herpes simplex virus type 1-encoded glycoprotein C mimics the coagulation initiator, tissue factor. Thromb Haemost. 2004;393:535.
152. Livingston J, Sutherland M, Friedman H, Pryzdial E. Herpes simplex virus type 1-encoded glycoprotein C contributes to direct coagulation factor X-virus binding. Biochem J. 2006;393:529–35.
153. Friedman HM. Immune evasion by herpes simplex virus type 1, strategies for virus survival. Trans Am Clin Climatol Assoc. 2003;114:103–12.
154. Tal-Singer R, Peng C, Ponce de Leon M, Abrams WR, Banfield BW, Tufaro F, et al. Interaction of herpes simplex virus glycoprotein gC with mammalian cell surface molecules. J Virol. 1995;69:4471–83.
155. Etingin OR, Silverstein RL, Friedman HM, Hajjar DP. Viral activation of the coagulation cascade: molecular interaction at the surface of infected endothelial cells. Cell. 1990;61:657–62.
156. Altieri DC, Etingin OR, Fair DS, Brunck TK, Geltosky JE, Hajjar DP, et al. Structurally homologous ligand binding of integrin Mac-1 and viral glycoprotein-C receptors. Science. 1991;254:1200–2.
157. Dorfleutner A, Hinterman E, Tarui T, Takada Y, Ruf W. Cross-talk of integrin alpha3beta1 and tissue factor in cell migration. Molec Biol Cell. 2004;15:4416–25.
158. Sutherland MR, Simon AY, Shanina I, Horwitz MS, Ruf W, Pryzdial ELG. Virus envelope tissue factor promotes infection in mice. J Thromb Haemost. 2019;17(3):482–91.
159. Fenton JW II, Villanueva GB, Ofosu FA, Maraganore JM. How hirudin inhibits thrombin. Haemostasis. 1991;21(Suppl 1):27–31.
160. Roesken F, Vollmar B, Rucker M, Seiffge D, Menger MD. In vivo analysis of antithrombotic effectiveness of recombinant hirudin on microvascular thrombus formation and recanalization. J Vasc Surg. 1998;28:498–505.
161. Vlasuk GP. Structural and functional characterization of tick anticoagulant peptide: a potent and selective inhibitor of blood coagulation factor Xa. Thromb Haemostasis. 1993;70:212–6.
162. Yeh CH, Fredenburgh JC, Weitz JI. Oral direct factor Xa inhibitors. Circ Res. 2012;111:1069–78.
163. Bushi D, Chapman J, Wohl A, Stein ES, Feingold E, Tanne D. Apixaban decreases brain thrombin activity in a male mouse model of acute ischemic stroke. J Neurosci Res. 2018;96:1406–11.
164. Wei H, Shang J, Keohane C, Wang M, Li Q, Ni W, et al. A novel approach to assess the spontaneous gastrointestinal bleeding risk of antithrombotic agents using Apc(min/+) mice. Thromb Haemost. 2014;111:1121–32.
165. Ozolina A, Sarkele M, Sabelnikovs O, Skesters A, Jaunalksne I, Serova J, et al. Activation of coagulation and fibrinolysis in acute respiratory distress syndrome: a prospective pilot study. Front Med (Lausanne). 2016;3:64.
166. Rondina MT, Tatsumi K, Bastarache JA, Mackman N. Microvesicle tissue factor activity and interleukin-8 levels are associated with mortality in patients with influenza A/H1N1 infection. Crit Care Med. 2016;44:e574–8.
167. Geibert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. J Inf Dis. 2003;188:1618–29.

168. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020;18:1023–6.
169. Young E. The anti-inflammatory effects of heparin and related compounds. Thromb Res. 2008;122:743–52.
170. Esmon CT. Targeting factor Xa and thrombin: impact on coagulation and beyond. Thromb Haemost. 2014;111:625–33.
171. Simon AY, Sutherland MR, Pryzdial ELG. Dengue virus binding and replication by platelets. Blood. 2015;126:378–85.
172. Brufatto N, Ward A, Nesheim ME. Factor Xa is highly protected from antithrombin-fondaparinux and antithrombin-enoxaparin when incorporated into the prothrombinase complex. J Thromb Haemost. 2003;1:1258–63.
173. Teitel JM, Rosenberg RD. Protection of factor-Xa from neutralization by the heparin-antithrombin complex. J Clin Invest. 1983;71:1383–91.
174. Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J. Clot-bound thrombin is protected from inhibition by heparin - antithrombin III but is susceptible to inactivation by antithrombin III - independent inhibitors. J Clin Invest. 1990;86:385–91.
175. Wang J, Hajizadeh N, Moore EE, McIntyre RC, Moore PK, Veress LA, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): a case series. J Thromb Haemost. 2020. https://doi.org/10.1111/jth.14828
176. Baker JV, Neuhaus J, Duprez D, Kuller LH, Tracy R, Belloso WH, et al. Changes in inflammatory and coagulation biomarkers: a randomized comparison of immediate versus deferred antiretroviral therapy in patients with HIV infection. J Acquir Immune Defic Syndr. 2011;56:36–43.
177. Duprez DA, Neuhaus J, Kuller LH, Tracy R, Belloso W, De Wit S, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. PLoS One. 2012;7:e44454.
178. Soepandi PZ, Burhan E, Mangunnegoro H, Nawas A, Aditama TY, Partakusuma L, et al. Clinical course of avian influenza A(H5N1) in patients at the Persahabatan Hospital, Jakarta, Indonesia, 2005–2008. Chest. 2010;138:665–73.
179. Ramacciotti E, Agati LB, Aguiar VCR, Wolosker N, Guerra JC, de Almeida RP, et al. Zika and chikungunya virus and risk for venous thromboembolism. Clin Appl Thromb Hemost. 2019;25:1076029618821184.
180. Borges AH, O'Connor JL, Phillips AN, Neaton JD, Grund B, Neuhaus J, et al. Interleukin 6 is a stronger predictor of clinical events than high-sensitivity C-reactive protein or D-dimer during HIV infection. J Infect Dis. 2016;214:408–16.
181. Sparkenbaugh EM, Chantrathammachart P, Mickelson J, van Ryn J, Hebbel RP, Monroe DM, et al. Differential contribution of FXa and thrombin to vascular inflammation in a mouse model of sickle cell disease. Blood. 2014;123:1747–56.
182. Sparkenbaugh EM, Chen C, Brzoska T, Nguyen J, Wang S, Vercellotti GM, et al. Thrombin-mediated activation of PAR-1 contributes to microvascular stasis in mouse models of sickle cell disease. Blood. 2020;135(2):1783–7.
183. Spronk HM, de Jong AM, Crijns HJ, Schotten U, Van Gelder IC, Ten Cate H. Pleiotropic effects of factor Xa and thrombin: what to expect from novel anticoagulants. Cardiovasc Res. 2014;101:344–51.
184. Nhu QM, Shirey K, Teijaro JR, Farber DL, Netzel-Arnett S, Antalis TM, et al. Novel signaling interactions between proteinase-activated receptor 2 and Toll-like receptors in vitro and in vivo. Mucosal Immunol. 2010;3:29–39.
185. Khoufache K, LeBouder F, Morello E, Laurent F, Riffault S, Andrade-Gordon P, et al. Protective role for protease-activated receptor-2 against influenza virus pathogenesis via an IFN-gamma-dependent pathway. J Immunol. 2009;182:7795–802.
186. Antoniak S, Owens AP, Baunacke M, Williams JC, Lee RD, Weithauser A, et al. PAR-1 contributes to the innate immune response during viral infection. J Clin Inv. 2013;123:1310–22.
187. Khoufache K, Berri F, Nacken W, Vogel AB, Delenne M, Camerer E, et al. PAR1 contributes to influenza A virus pathogenicity in mice. J Clin Inv. 2013;123:206–14.

How to cite this article: Pryzdial ELG, Sutherland MR, Lin BH, Horwitz M. Antiviral anticoagulation. *Res Pract Thromb Haemost*. 2020;4:774–788. https://doi.org/10.1002/rth2.12406