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Antiviral anticoagulation

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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
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel envelope virus that causes life-threatening thrombotic coagulopathy and inflammation, the hallmarks of coronavirus disease 2019 (COVID-19). 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. 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 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...
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. The compelling clinical laboratory evidence is that COVID-19 results in elevated D-dimer, 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. Thrombin is also known to stimulate the secretion of t-PA, priming the local milieu for a fibrinolytic response. 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. 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. A similar trend was seen for other fibrin degradation products. 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 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.

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. 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, 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 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. This late-stage observation is consistent with the parameters of conventional disseminated intravascular coagulation (DIC). 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; COVID-19 does not have prolonged PT of >3 seconds, platelet count dropping to 100 × 10⁹/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. 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, is now known to involve pulmonary embolism. The severity of the hallmark pulmonary inflammation correlates to lymphocyte subgroups and glassy alveolar opacities have been documented in computed tomography images. 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, found in the lungs, kidneys, brain, gastrointestinal tract, and cardiovascular system.

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, while comorbidities include, diabetes, hypertension, cardiovascular disease, and obesity. These underlying pathologies are all characterized by chronic inflammation, presenting clinically as elevated levels of acute-phase reactants, most notably C-reactive protein. 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. 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. 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 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 (e.g., 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 (e.g., 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 inducibly 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,89 endothelial cells,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),58,99,100 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.\textsuperscript{116} Many cells known to bear TF are permissive to infection by clinically important enveloped viruses, including SARS-CoV-2,\textsuperscript{117} HSV1,\textsuperscript{118} Ebola,\textsuperscript{119} influenza,\textsuperscript{120} HIV,\textsuperscript{121} dengue,\textsuperscript{122} Zika virus,\textsuperscript{123} HCV,\textsuperscript{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,\textsuperscript{125} sporadic encephalitis,\textsuperscript{126} and genital herpes\textsuperscript{127,128} and is associated with intestinal dysregulation.\textsuperscript{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,\textsuperscript{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\textsuperscript{113}; (ii) fibrin deposits in the microvasculature are linked to HSV1 infection\textsuperscript{132,133}; (iii) DIC in neonates may occur during severe HSV1 infection\textsuperscript{134}; (iv) HSV2 is linked to ischemic and hemorrhagic stroke due to DIC\textsuperscript{107,135}; (v) a history of CMV infection is linked to subclinical and clinical arterial thickening\textsuperscript{136-138}; (vi) CMV is strongly correlated to accelerated atherosclerosis in immunosuppressed organ transplant recipients\textsuperscript{139-142}; and (vii) CMV infection is a strong risk factor for restenosis after angioplasty.\textsuperscript{143,144} When paired with other known cardiovascular risk factors, viral correlation to vascular disease is strong.\textsuperscript{110-113} A clear cause-and-effect relationship has been established in several animal models, which confirm that herpesviruses accelerate thrombosis and atherosclerosis.\textsuperscript{145-147} Indeed, HSV1 and CMV are known to induce TF activity on vascular endothelial cells,\textsuperscript{148,149} which support infection and from which the replicative viruses derive their envelope.\textsuperscript{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,\textsuperscript{74} multiple-sized electron-dense gold beads were used to simultaneously distinguish 3 constituents on a single HSV1.\textsuperscript{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](https://example.com/figure2.png)

FIGURE 2 Coagulation initiators tissue factor (TF) and anionic phospholipid (aPL) are available on the herpes simplex virus type 1 (HSV1) surface.\textsuperscript{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\textsuperscript{106,150} and FVIIa-dependent FX activation in experimental systems using purified proteins.\textsuperscript{106,150-152} Demonstrating specificity, these activities are inhibited by direct antagonists of TF function.\textsuperscript{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
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. When expressed on the surface of infected cells, gC has been shown to be involved in FX activation and binding, 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 mimic the cofactor function of TF toward FVIIa-enhanced FXa generation. Like TF, it binds directly to both FVIIa and FX forming a cofactor-protease-substrate complex. 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, as did in situ FX zymogen activation. 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 and combining this with engineered HSV1 deficient in gC production. Thus, HSV1/TF−/gC−, HSV1/TF+/gC−, HSV1/TF−/gC + and HSV1/TF+/...
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. 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). 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. 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, implying the involvement of additional virus surface constitutents. 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±. 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/FXa 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,124 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.164 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 × 10⁵ plaque-forming units (PFUs) of tissue factor (TF)-competent (TF+) 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.

- Hirudin - + - - -
- NAPc2 - - + - -
- Apixaban - - + + -
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. 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, thrombolysis with recombinant t-PA has been used to treat patients with respiratory distress syndrome. 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. 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, influenza H5N1, and chikungunya viruses. For HIV, IL-6 has been identified as a stronger predictor of the severity of clinical events than D-dimer. 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. These authors furthermore used another FXa-specific DOAC, rivaroxaban, to avert symptoms in their sickle cell model. 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 and must be approached cautiously. The important role of PARs has been summarized in a comprehensive review. 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 or improve outcome to the animal in influenza or HSV1 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 or increase influenza symptoms or increase HSV1 titer. An interesting consistency between groups is the preparation of virus inoculum in cells known to express TF or not, which may indicate biases in signaling mechanisms during infection.

For infection models of HSV1, in vitro and in vivo experiments both showed that PAR1 participates to enhance infection. Like the PAR1 results, HSV1 infection of cells in culture was also increased by PAR2. However, in a mouse KO model of infection, the presence of PAR2 attenuated the infection of most organs evaluated by HSV1, 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. 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. 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, 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 and anti-inflammatory 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.

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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.

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