C1-INH and the contact system in COVID-19-associated coagulopathy

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Abstract

COVID-19 is frequently associated with a coagulopathy with severe consequences. The mechanisms leading to a pro-coagulant state in these patients is multifactorial, including tissue destruction and inflammatory mechanisms. Based on the analysis of publicly available interactomes, we propose that SARS-CoV-2 infection causes a deficiency in C1 esterase inhibitor (C1-INH), a pathogen-specific mechanism that may help explain the pro-coagulant state in COVID-19 patients.

Introduction

Severe manifestations of COVID-19 include acute respiratory distress, cardiovascular affectation, multi-organ involvement[1] and a coagulopathy[2-4] that may be compatible with disseminated intravascular coagulation (DIC)[5] accompanied with pathological observation of venous thromboembolism in the lungs and other organs[6]. Consistent with an ongoing coagulopathy, severe

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patients display increased levels of D-dimer, thrombocytopenia and moderate prothrombin time prolongation[7] which, together with high blood levels of ferritin, interleukin-6, lactate dehydrogenase and lymphopenia, are associated with a higher risk of severe illness and death[3]. Current therapeutic recommendations in such patients include the early administration of anticoagulant agents, notably low-molecular weight heparin (LMWH), which effectively improve the recovery and prevent death in many patients[8].

The causal mechanisms of the coagulopathy associated with COVID-19 are unkwnown. It has been attributed to the cytokine storm accompanying severe inflammatory syndrome[9-11], liver dysfunction[12], antiphospholipid antibodies[13], hypertension, hypoxia, stress from mechanical ventilation, limited mobility of the patients, or a combination of factors. The high incidence of thrombotic events is not unique to COVID-19 and is also a feature of other virulent zoonotic coronavirus outbreaks[14]. In support for mechanisms of coagulopathies that are not specific to a particular infectious agent, it is argued that critically ill patients with diverse infectious complications activate multiple systemic coagulation and inflammatory responses that can lead to DIC[15] and venous thromboembolism[16].

Methods

Biogrid interaction database examination. The BioGrid interaction database (biogrid.org) was examined for published interactors for SARS-CoV proteins with human proteins and for interactors for SERPING1.

Cytoscape network construction and visualization. Networks retrieved from Pfefferle *et al*.[17] and Gordon *et al*.[18] were downloaded in XML 2.5 format, imported into Cytoscape and merged into a single network. The network was visualized as a circle layout.

Sequence alignments. SARS-CoV and SARS-CoV-2 polypeptides were aligned with the MUSCLE algorithm in the Geneious Prime package. The sequences were retrieved from the NCBI GenBank, with accession numbers NC_004718.3 (SARS-CoV) and NC_045512.2 (SARS-CoV-2). Polypeptides in open reading frames were translated with the BENCHLING platform. BLOSUM90 parameters were applied for the alignments.

Results

While examining the published interactomes of SARS-CoV (Ref. 17) and SARS-CoV-2 (Ref. 18) proteins (hereafter, CoV1 and CoV2, respectively) with human proteins, we noted that C1-INH (C1 esterase inhibitor, encoded by the SERPING1 gene) is an interactor for 7 distinct CoV1 proteins and polypeptides, encoded by ORF3b, ORF7b, ORF14, nsp2ab, nsp13ab, nsp14ab and nsp8ab (Fig. 1a). These CoV1 proteins are highly similar to their orthologous CoV2 proteins (Fig. 1b, Supplemental Information). Along with interferon and innate immune signaling components, such as IRF3, TMEM173, TBK1, IKBKE, TRIM25, MAVS or DDX58, C1-INH is one of the proteins with the highest connectivity in the merged CoV1 and CoV2 interactomes (Fig. 1c), suggesting a relevant role for these interactions in the life cycle of CoV1 and, by similarity, CoV2.

Discussion

The 105-KDa glycoprotein C1-INH, a serine protease inhibitor bearing a conserved SERPIN domain, is the main inhibitor of the classical complement enzymes C1r and C1 esterase (C1s)[19]. It is also the primary inhibitor of the activated factors XII (FXIIa) and XI (FXIa) and activated plasma kallikrein (PKa)[19] and, more modestly, of plasmin, tissue-type plasminogen activator (tPA) and thrombin (Fig. 1d). C1-INH is the sole natural inhibitor of C1r and C1s, is an inhibitor of the lectin pathway of complement activation via inactivation of mannan-binding lectin-associated serine proteinase-1 and 2 (MSP1 and MSP2), and inhibits the alternative pathway of activation by binding to C3b. Thus, C1-INH is a major regulator of all three pathways of complement activation[20]. C1-INH is the most heavily glycosylated plasma protein, and bears a sialyl Lewis[x]-related moiety through which it binds to endothelial cell-surface selectins E and P, in competition with the binding of leukocytes[21], exerting, as such, an anti-inflammatory function.

Through covalent bond formation with the complement components C1s, C1r, MASP1 and MASP2 and reversible binding to C3a (Ref. 20), C1-INH attenuates

the consequences of complement activation, including the generation of pro-inflammatory anaphylatoxins, especially C5a, and the formation of a membrane attack complex (MAC) that leads cell lysis. Of note, severe acute respiratory distress syndrome in SARS was found associated with excessive activation of C3 (Ref. 22). Further, MSP2 has been found to be a target of the N protein of MERS-CoV, SARS-CoV and SARS-CoV-2 (Ref. 23), and a blocking antibody to C5a has shown benefit in patients COVID-19 with severe lung injury.

The plasma contact system is a procoagulant and proinflammatory protease cascade that occurs on the surface of endothelial cells[24]. Upon contact with surface-bound negatively charged polymers such as polyphosphate (polyP), proteoglycans or RNA, FXII is activated to catalyze the proteolytic activation of plasma kininogen (PK) to PKa which, in turn, converts high-molecular-weight kininogen (HK) to bradykinin (BK)[19] (Fig. 1d). FXII also binds to the endothelial cell surface through interactions with the urokinase receptor (uPAR) or integrins (through fibronectin-like domain), exerting signaling functions independent of its catalytic activity. BK is liganded to the constitutively expressed cell surface G-protein-coupled receptor, B2R, causing vasodilatation and increased vascular permeability. BK is degraded by carboxypeptidase N (in plasma) or carboxypeptidase M (on endothelial cells), yielding des-arg-9 bradykinin, which interacts with a second, cytokine-induced receptor, B1R, through which it may prolong the vascular response until it is fully inactivated by angiotensin converting enzyme (ACE), aminopeptidase P, or neutral endopeptidase[25]. A deficit in C1-INH, found in the rare diseases types I and II hereditary angioedema (HAE), acquired angioedema and age-related macular degeneration, results in excessive activation of FXII and unchecked production of BK, leading to angioedema[26].

Upon contact activation, FXIa sets off the coagulation cascade through sequential activation of factors X, IX and prothrombin (factor II), resulting in the polymerization of fibrin from fibrinogen. Incidentally, factor Xa cleaves the spike protein (S) of CoV1 at the S1-S2 boundary, enhancing the fusion of viral particles to cell membranes[27]. It has not yet been determined if the CoV2 spike protein, which is cleaved at the S1-S2 boundary by TMPRSS2 (Ref. 28) and furin[29] is also cleaved by FXa. At the endothelial cell surface, C1-INH regulates

the intrinsic coagulation pathway by inhibiting multiple enzymes: the pro-coagulant enzymes FXIIa, FXIa and thrombin, and the pro-fibrinolytic enzymes tPA and plasmin (Fig. 1d). As such, loss of expression or function of C1-INH would be expected to result in augmented coagulation and fibrinolysis and, indeed, blood D-dimer levels can be elevated during angioedema attacks in HAE[30]. However, except in anecdotal cases with concurring pro-coagulant events[31], thromboembolism is generally not observed in HAE. In this regard, because patients severely deficient in FXII, HK or prekallikrein display normal hemostasis, the intrinsic coagulation pathway was considered not to have a function in physiological hemostasis. This seeming paradox was resolved by the finding that FXII is potently activated by activated platelets, platelet-shed vesicles and solid-phase bound (membrane-associated) negatively charged molecules, such as polyphosphate (stored in platelets in complex with Ca^{2+}) or RNA[32].

The interaction of several CoV1 (and, by similarity, CoV2) proteins with C1-INH suggests that this essential regulator of the contact system is inhibited during viral infection, leading to a propensity to activate the complement cascade, the bradykinin pathway and the intrinsic coagulation cascade. For the latter to promote thromboembolism and other clinical manifestations of pathological coagulation, a concomitant activation or destruction of platelets, or local release of negatively charged polymers may be required. SARS-CoV2 can infect multiple human cell types through the engagement of ACE2, expressed on the surface of AT2 pneumocytes, enterocytes, kidney epithelial cells and endothelial cells[33], causing extensive cell death and tissue destruction. Viral destruction of endothelial cells, either through direct cell disruption or indirectly through inflammatory mechanisms, activates the extrinsic coagulation pathway, initiated by interaction of platelets with collagen and tissue factor and initial production of thrombin. This is followed by amplified production of thrombin through the intrinsic pathway[34]. As such, a deficit in available C1-INH caused by interacting CoV2 or CoV1 proteins would be expected to prime the intrinsic coagulation pathway, leading to a pro-coagulant state that can overcome physiological anti-coagulant activities.

Depletion of C1-INH below certain thresholds increases the risk of angioedema

attacks in HAE[35]. Although a similar association has not been made between C1-INH deficiency and coagulopathies, we suggest that in pathophysiological scenarios with concomitant activation of the extrinsic pathway, such as in COVID-19, C1-INH could play a protective role. C1-INH replacement therapy has long been used in acute and prophylactic treatment of HAE[36], and could be useful in the management of COVID-19-associated coagulopathy. Further, heparin and sulfated glycans amplify the inhibitory functions of C1-INH on the contact system[37], which, in addition to the activities of heparin on coagulation factors, could help explain the beneficial effects of heparin in the management of COVID-19 patients.

Conclusions

Unlike all currently prevailing hypotheses, which focus on non-pathogen-specific mechanisms, we propose a pathogen-specific mechanism by which non-structural SARS-CoV proteins bind to C1 esterase inhibitor (C1-INH, encoded by SERPING1), potentially causing a deficiency in this regulator of the contact and complement systems. Although it requires experimental confirmation, such as protein-protein binding and C1-INH enzymatic assays, the plausibility of our hypothesis is supported by the multiplicity of interactions observed between CoV proteins and C1-INH. Importantly, our hypothesis provides a single mechanism that explains many clinical, pathological and laboratory findings associated with severe COVID-19 and opens up a whole new set of therapeutic options to tackle the most deadly manifestations of this viral disease.

Acknowledgments

Competing interests

The authors declare no competing interests.

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Figure legend

Figure 1. **a**. Cytoscape rendering of SERPING1 interactions retrieved from BioGrid. Viral proteins are in red, contact system and coagulation factors in dark blue. **b**. Similarities between CoV1 and CoV2 proteins, estimated from pairwise alignments (Supplementary Information). **c**. Cytoscape rendering of the nodes with the highest connectivity of human proteins with CoV1 and CoV2 proteins, merged from BioGrid interaction networks (https://thebiogrid.org). SERPING1 is shown in orange and edges of its interactions with CoV1 proteins are in red. Viral proteins are in red; innate immunity and interferon pathway components are in yellow. **d**. Schematic illustration of the contact, complement and renin-angiotensin systems. Green arrows represent activating functions and red stop rods represent inhibitory functions. Only selected reactions are represented, relevant to the discussion. <u>Contact system</u>: FXII, FXI: factors XII and XI; PK: prekallicrein; PKa: activated kallicrein; HK: high-molecular weight kininogen; B2R: bradykinin receptor B2; BK: bradykinin; BK1-5: bradykinin 1-5; PLG: plasminogen; PLM: plasmin; tPA: tissue-type plasminogen activator. <u>Renin-angiotensin system</u>: ACE: angiotensin-converting enzyme; ACE2: angiotensin-converting enzyme 2; AngI: angiotensin I; AngII: angiotensin II; Ang1-7: angiotensin 1-7; AT1R: angiotensin type 1 receptor; MAS-R: MAS receptor. <u>Complement system</u>: MBL: mannose-binding lectin; MSP1/2: mannose-associated serine protease 1 and 2. Other factors and components of the three systems are shown with conventional designations. SARS-CoV and SARS-CoV-2 predicted proteins are shown as red-filled rectangles. Also represented is the negative regulation of cell-surface ACE2 through internalization upon interaction and entry of the CoV1 and CoV2 spike proteins.

Figure 1