C1 esterase inhibitor and the contact system in COVID-19

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Summary
Coronavirus disease 2019 (COVID-19) is frequently associated with severe systemic consequences, including vasculitis, a hyperinflammatory state and hypercoagulation. The mechanisms leading to these life-threatening abnormalities are multifactorial. Based on the analysis of publicly available interactomes, we propose that severe acute respiratory syndrome coronavirus-2 infection directly causes a deficiency in C1 esterase inhibitor, a pathogen-specific mechanism that may help explain significant systemic abnormalities in patients with COVID-19.

Keywords: contact system, serpins, virology, complement, SARS-CoV-2.

Severe manifestations of coronavirus disease 2019 (COVID-19) include acute respiratory distress, cardiovascular affection, multi-organ involvement1 and coagulopathy,2–4 which may be compatible with disseminated intravascular coagulation (DIC).5,6 The causal mechanisms of the systemic manifestations associated with COVID-19 are attributed to the cytokine storm accompanying severe inflammatory syndrome,7–9 direct viral disruption of endothelial integrity,10 release of coagulation factors by inflammasome-activated macrophages,11 liver dysfunction,12 anti-phospholipid antibodies,13 hypertension, hypoxia, stress from mechanical ventilation, limited mobility of the patients, or a combination of factors.

While examining the published interactomes of severe acute respiratory syndrome coronavirus (SARS-CoV)14 and SARS-CoV-215 proteins [hereafter, CoV-1 and CoV-2, respectively] with human proteins, we noted that C1 esterase inhibitor [C1-INH, encoded by the serine proteinase inhibitor (serpin) family G member 1 (SERPING1) gene] is an interactor for seven distinct CoV-1 proteins and polypeptides, encoded by open reading frame (ORF) 3b, ORF7b, ORF14, non-structural protein (nsp)2ab, nsp13ab, nsp14ab and nsp8ab (Fig. 1A). These CoV-1 proteins are highly similar to their orthologous CoV-2 proteins (Figure S1B). Along with interferon and innate immune signalling components, such as interferon regulatory factor 3 (IRF3), transmembrane protein 173 (TMEM173), TANK binding kinase 1 (TBK1), inhibitor of nuclear factor kappa B kinase subunit epsilon (IKBKE), tripartite motif containing 25 (TRIM25), mitochondrial antiviral signalling protein (MAVS) or DExD/H-box helicase 58 (DDX58), C1-INH is one of the proteins with the highest connectivity in the merged CoV-1 and CoV-2 interactomes (Fig. 1C), suggesting a relevant role for these interactions in the life cycle of CoV-1 and, by similarity, CoV-2.

Of the SERPING1 CoV interactors, nsp8, together with nsp7, functions as a primase that forms part of the RNA polymerase complex,16 nsp13 is a helicase,17 nsp14 is a proofreading exoribonuclease18 and an S-adenosyl methionine (SAM)-dependent (guanine-N7) methyl transferase.19 The protein encoded by ORF3b antagonises interferon responses through as yet uncharacterised molecular mechanisms,20,21 the protein encoded by ORF7b bears a transmembrane domain through which it localises to the Golgi complex and whose expression upon viral infection can lead to aberrant localisation of cell-surface glycoproteins,22 and ORF14 encodes a 70 (CoV-1) or 73 (CoV-2) amino acid protein for which no function has been described. We speculate that the likelihood of a direct interaction of with SERPING1 is lower for nsp7, nsp13 and nsp14, given their above-described functions, although the formation of multimolecular complexes remains a possibility.

The 105-KDa glycoprotein C1-INH, a serine protease inhibitor bearing a conserved serpin domain, is the main inhibitor of coronavirus disease 2019 (COVID-19).
It is also the primary inhibitor of the activated Factors XII (FXIIa) and XI (FXIa) and activated plasma kallikrein\textsuperscript{23} and, more modestly, of plasmin, tissue-type plasminogen activator (tPA) and thrombin (Fig. 2). 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 protease-1 and -2 (MASP1 and MASP2), 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.\textsuperscript{24} C1-INH is the most heavily glycosylated plasma protein, and bears a sialyl Lewis\textsubscript{x}-related moiety through which it binds to endothelial cell-surface selectins E and P, in competition with the binding of leucocytes,\textsuperscript{25} exerting, as such, an anti-inflammatory function.

Through covalent bond formation with the complement components C1s, C1r, MASP1 and MASP2 and reversible binding to C3a24, 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 to cell lysis. Of note, severe acute respiratory distress syndrome (ARDS) in SARS was found to be associated with excessive activation of C3.26 Further, MASP2 has been found to be a target of the nucleocapsid (N) protein of Middle East respiratory syndrome (MERS)-CoV, SARS-CoV and SARS-CoV-2,27 and a blocking antibody to C5a has shown benefit in patients with COVID-19 with severe lung injury.

The presence of pulmonary oedema is another feature in patients with COVID-19 with ARDS, and aberrant activation of the kallikrein–bradykinin (BK) system has been proposed as an explanatory mechanism.\textsuperscript{28} A further connection with aberrant activation of the contact system in COVID-19 is provided by the emergence of cases of Kawasaki disease (KD)-like syndrome in SARS-CoV-2-positive children,\textsuperscript{29} characterised by skin rash, gastrointestinal affection, myocarditis and shock syndrome. Laboratory tests indicated ongoing fibrinolysis, with elevated blood D-dimer levels. KD is the most frequent childhood vasculitis\textsuperscript{30} and is frequently accompanied with excessive thrombocytosis,\textsuperscript{31} and evidence of activation of the classical complement pathway.\textsuperscript{32} The plasma contact system is a pro-coagulant and pro-inflammatory protease cascade that occurs on the surface of endothelial cells.\textsuperscript{33} Upon contact with surface-bound negatively charged polymers such as polyphosphate (polyP), proteoglycans or RNA, FXII is activated to catalyse the proteolytic activation of plasma kininogen to kallikrein which, in turn, converts high-molecular-weight kininogen to BK\textsuperscript{23} (Fig. 1D). FXII also binds to the endothelial cell surface through interactions with the urokinase receptor or integrins (through fibronectin-like domain), exerting signalling 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), (C1s).\textsuperscript{23}
yielding des-arg-9 BK, 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. 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.

Upon contact activation, FXIa initiates the coagulation cascade through sequential activation of Factors X, IX and prothrombin (Factor II), resulting in the polymerisation of fibrin from fibrinogen. Incidentally, Factor Xa cleaves the spike protein (S) of CoV-1 at the S1–S2 boundary, enhancing the fusion of viral particles to cell membranes. It has not yet been determined if the CoV-2 spike protein, which is cleaved at the S1–S2 boundary by transmembrane protease, serine 2 (TMPRSS2) and furin, 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. 2). 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. However, except in anecdotal cases with concurring pro-coagulant events, thromboembolism is generally not observed in HAE. In this regard, because patients severely deficient in FXII, high-molecular weight kininogen or prekallikrein display normal haemostasis, the intrinsic coagulation pathway was considered not to have a function in physiological haemostasis. 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 polyP (stored in platelets in complex with Ca^{2+}) or RNA. The interaction of several CoV-1 (and, by similarity, CoV-2) 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 BK 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-CoV-2 can infect...
multiple human cell types through the engagement of ACE2, expressed on the surface of alveolar type 2 pneumocytes, enterocytes, kidney epithelial cells and endothelial cells,\textsuperscript{42} causing extensive cell death and tissue destruction. Viral destruction of endothelial cells,\textsuperscript{10} 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.\textsuperscript{43} As such, a deficit in available C1-INH caused by interacting CoV-2 or CoV-1 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.\textsuperscript{44} C1-INH replacement therapy has long been used in acute and prophylactic treatment of HAE,\textsuperscript{45} and could be useful in the management of COVID-19-associated coagulopathy. Further, heparin and sulphated glycans amplify the inhibitory functions of C1-INH on the contact system,\textsuperscript{46} which, in addition to the activities of heparin on coagulation factors, could help explain the beneficial effects of heparin in the management of patients with COVID-19.

Relevantly, a recent limited observational study in five patients with COVID-19 with severe pneumonia reported a rapid improvement in their clinical and laboratory parameters after treatment with C1-INH (Ruconest, Pharming, Leiden, The Netherlands). The reported outcomes were resolution of fever within 48 h in four of the patients, accompanied with decreased blood C-reactive protein and interleukin 6, followed by rapid full recovery and discharge. The remaining patient had a slightly delayed recovery (https://www.pharming.com/news/pharming-reports-encouraging-results-use-rucconest-covid-19-patients). The fact that replenishment of C1-INH prompts the recovery of patients with severe COVID-19 not responding to a given standard-of-care (hydroxychloroquine and lopinavir/ritonavir) strongly suggests that such patients have C1-INH depletion. To our knowledge, our hypothesis provides the first mechanistic explanation for such a deficiency. On these bases, we propose C1-INH replenishment as a useful adjunct in the management of COVID-19.

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**Competing interests**

The authors declare no competing interests.

**Author contributions**

Timothy M. Thomson conceived and designed the study; Timothy M. Thomson and Emily Toscano-Guerra performed analyses and prepared the figure; Timothy M. Thomson wrote the manuscript; Rosanna Paciucci and Ernesto Casis provided critical information and assessment.

**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Supplementary Figure 1B.

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