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FORUM

Thromboinflammation in COVID-19: Can α_2-macroglobulin help to control the fire?

Rainer Seitz[1] | **Lutz Gürtler**[2] | **Wolfgang Schramm**[3]

[1]Paul-Ehrlich-Institut, Langen, Germany

[2]Max von Pettenkofer Institut, Ludwig-Maximilians University (LMU), Munich, Germany

[3]Ludwig-Maximilians University (LMU) and Rudolf Marx Stiftung, Munich, Germany

Correspondence
Rainer Seitz, Paul-Ehrlich-Institut (retired), Sofienstraße 70, D-63225 Langen, Germany.
Email: Rainer-Seitz@t-online.de

Abstract

The complex COVID-19-associated coagulopathy appears to impair prognosis. Recently, we presented the hypothesis that children are to some extent protected by higher α_2-macroglobulin (α_2-M) levels from severe COVID-19. In addition to endothelial cells, thrombin, and platelets, neutrophil granulocytes also appear to play an important role. Neutrophils extrude extracellular nets, which are histone- and protease-coated web-like DNA structures; activate coagulation and platelets; and release radicals and proteases such as elastase. The unique phylogenetically ancient and "versatile" inhibitor α_2-M contributes particularly during childhood to the antithrombin activity of plasma, binds a broad spectrum of proteases, and interacts with other mediators of inflammation such as cytokines. It is suggested that the scope of basic research and clinical studies would include the potential role of α_2-M in COVID-19.

KEYWORDS

COVID-19, α_2-macroglobulin, endothelial cells, extracellular traps, neutrophil, thromboinflammation

1 | INTRODUCTION

Hypercoagulability appears to worsen prognosis[1,2] in coronavirus disease 2019 (COVID-19). The coagulopathy in COVID-19 shows features distinct from disseminated intravascular coagulation (DIC), thrombotic microangiopathy (TMA), or the hemorrhagic coagulopathy seen in Marburg virus infections; bleeding complications and the typical consumption and depletion of components such as platelets and fibrinogen are infrequent, whereas thrombi in venous or arterial macro- and microcirculation predominate.[3]

Severe infections can trigger disturbances of hemostasis, and an activation of blood coagulation can fuel inflammation with thrombin as a gas pedal driving innate immunity,[4] a mutual enhancement leading to so-called thromboinflammation.[5] The pathophysiology of the complex interactions of endothelial cell damage, hypoxia, host defence systems (eg, complement; innate and acquired immunity; cytokine storm), and components of hemostasis in COVID-19 has recently been reviewed.[6]

A recent study found plasma levels of fibrinogen, the natural anticoagulants protein C and antithrombin III (ATIII), and platelet counts normal or even increased, confirming a pattern distinct from DIC, while factor VIII and notably von Willebrand factor (VWF) increased progressively with care intensity.[7] Incidence of thromboembolism and laboratory findings were recently reviewed,[8] and several guidance documents are available.[9,10]

Many recommendations deal with modalities and intensity of anticoagulation with unfractionated or low molecular weight heparins,

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or oral anticoagulants. A study in Milan[11] found a 60% reduction of mortality and clinical deterioration of COVID-19 and a 70% reduction of venous thromboembolism with high enoxaparin doses (127 patients) compared to standard prophylaxis (151 patients), while 3% of patients on high enoxaparin dosages had non-fatal major bleeding. Another large study including 3842 inpatients in the United States[12] found that particularly patients with severe COVID-19 benefit from D-dimer–dependent anticoagulation and that apixaban is effective in decreasing mortality in this disease. However, a 67-center cohort study including 3239 critically ill adults with COVID-19 in the United States[13] found that patients receiving therapeutic anticoagulation had a similar risk of death as those who did not. Apparently, we do not yet have a generally accepted, evidence-based concept for anticoagulation in COVID-19.

Maybe in this extremely complicated setting one should dig deeper and consider some ancient data. Recently, we presented in this journal the hypothesis that their higher level of α_2-macroglobulin (α_2-M) would protect children from severe COVID-19.[14] Building on this hypothesis, this article explores potential interactions of α_2-M with blood coagulation, endothelial cells, and neutrophil extracellular traps (NET).

2 | ENDOTHELIAL CELLS AND NEUTROPHIL EXTRACELLULAR TRAPS

The causative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) depends on binding of its viral spike (S) proteins to the receptor ACE2 for cell entry and the host serine proteases trypsin, transmembrane protease serine subtype 2 (TMPRSS2), and furin for S protein priming.[15,16] SARS-CoV-2 is able to dock on lung alveolar epithelial cells and vascular endothelium, and may induce intense endothelitis,[17] comprising viral elements within endothelial cells, accumulation of inflammatory cells, and evidence of endothelial and inflammatory cell death. Whether the virus makes its way from primary contact with respiratory epithelium directly to vascular endothelial cells or by hematogenic or lymphogenic transport remains to be elucidated; this process may take place in inflamed lung tissue in which both cell types are situated in close vicinity, and might involve viral and host cell proteases.

Neutrophils appear to play an important role in severe COVID-19 pathology,[3] with pronounced formation of NETs, related to disease severity.[18] NET, a major immune mechanism to capture pathogens, are histone- and protease-coated web-like DNA structures released by neutrophils in response to a variety of stimuli. Detailed histopathological analysis of four fatal COVID-19 cases[19] revealed widely distributed NET-infiltrating lung areas, arteriolar microthrombi, and neutrophil-rich inflammatory areas of lung interstitium, alveoli, or bronchioles, often co-localized with occluding fibrin-rich deposits. NET can expose tissue factor (TF), and recently it was shown that neutrophils and NETs even promote clot formation without the addition of other triggers of coagulation.[20] In a recent review on the prominent role of NET in severe COVID-19,[21] the authors propose to search for therapeutics targeting NET, and among the candidates are protease inhibitors inactivating neutrophil elastase and peptidyl arginine deiminase type 4 (PAD4), which are key enzymes for the formation of NET. Normally, neutrophil elastase is controlled by the specific inhibitor α_1-antitrypsin (α_1-AT) and by α_2-M.

Neutrophil granulocytes and their proteases were suspected more than 40 years ago to be involved in the pathogenesis of disturbances of hemostasis in sepsis and leukemia.[22] Inflammatory cells and proteases released by them, such as human neutrophil elastase, are thought to play an important role in extravascular fibrin formation and fibrinolysis.[23] In an open clinical study,[24] patients with septic shock and DIC received natural inhibitors (ATIII concentrate plus fresh frozen plasma as a source of other inhibitors such as α_2-M), and initially high levels of a marker of granulocyte activation (elastase-α_1-AT complex) decreased with clinical improvement, a finding underlining the importance of the interaction of neutrophils with blood coagulation.

3 | POTENTIAL PROTECTIVE ROLE OF α_2-M

The inhibitor α_2-M is a phylogenetically very ancient mediator of innate immunity, structurally related to C3 and C4 complement components. It is abundant in plasma and tissue fluids and binds a broad spectrum of proteases and other proteins such as cytokines. In complex settings such as thromboinflammation in COVID-19, this "versatility" of α_2-M may be particularly important.

The structure of α_2-M was elucidated in the 1980s.[25] Its function and physiological role have recently been nicely reviewed.[26] The 720 kDa protein α_2-M is formed by the assembly of four 180 kDa subunits into a tetrameric quaternary structure. A bait region containing numerous protease cleavage sites is responsible for the highly diverse range of proteases interacting with α_2-M. Cleavage of the bait region in close proximity to a reactive thioester bond results in covalent trapping of proteases within a steric cage, which prevents access to high-molecular substrates and receptors (eg, thrombin access to fibrinogen or platelet receptors). When α_2-M is loaded with proteases (transformed α_2-M), it is cleared from circulation after binding to its receptor low density lipoprotein receptor-related protein (LPR1). Numerous proteins, including growth factors, cytokines, and misfolded proteins can bind noncovalently to α_2-M. The function of α_2-M is regulated by leukocyte myeloperoxidase-produced hypochlorite, which induces α_2-M dissociation into dimers with reduced protease binding but enhanced chaperone activity. Compared to native α_2-M, extent of binding and spectrum of ligands of transformed α_2-M and α_2-M dimers is altered; for instance, α_2-M dimers show enhanced binding to some cytokines (ie, tumor necrosis factor alpha, interleukin [IL]-2, and IL-6) and misfolded proteins. During inflammation, extracellular protease activity and the generation of hypochlorite are both elevated; therefore, it is plausible that protease-transformed α_2-M and hypochlorite-induced α_2-M dimers are concomitantly generated in vivo.[26]

An important physiologic function of α_2-M is maintaining hemostatic balance; in addition to ATIII, α_2-M contributes about 25% to the antithrombin activity of plasma of adults, and participates in the regulation of fibrinolysis.[27] It is interesting that α_2-M plasma levels show an age-dependent variability[28]; children show markedly elevated levels, normal adult levels are reached in the mid-twenties. Experiments assessing thrombin generation confirmed that the amount of thrombin bound to α_2-M in children is considerably higher than in adults.[29] Young siblings with inherited ATIII deficiency appear to be protected from thrombotic complications until they reach their thirties. An old observation of two families with inherited ATIII deficiency, which was "compensated" in the young siblings by high α_2-M levels, came to our mind in relation to the clinical observation that children often have a milder and rarely fatal course of COVID-19.[30] This led us to the hypothesis previously published in this journal[14] that during SARS-CoV-2 infection the higher α_2-M level in childhood may contribute to the more favorable course of COVID-19 in children.

In addition to its role in hemostasis and inflammation, direct interactions of α_2-M with viruses, in this case SARS-CoV-2, would be interesting. Equine and guinea pig sera were found to be potent inhibitors of influenza virus, and their inhibitory activities resided entirely in their α_2-macroglobulins,[31] containing 4-0-acetyl-N-acetylneuraminic acid (4-0-Ac-NeuAc) which has been found in few species. Proteases are important for cell entry and replication of viruses. An interaction with α_2-M was tested for human immunodeficiency virus type 1 (HIV-1), and a cleavage was shown in the bait region, but not in the receptor binding region of α_2-M that is responsible for clearance.[32]

Consistent with having an ancient origin in innate immunity,[28] α_2-M participates in the regulation of inflammatory mediators. In the context of the endotheliitis in COVID-19 is intriguing that α_2-M was shown by immunofluorescent-staining techniques to form a thin, continuous lining on the luminal surface of endothelial cells.[33] The authors concluded that "the demonstration that α_2-M is localized at the interface between the vessel wall and the circulating blood suggests that this protein may play a vital role in protecting the vascular endothelium by modulating various protease-generating reactions which take place adjacent to the endothelial surface."

4 | CONCLUSIONS

The complexity of COVID-19-associated coagulopathy and DIC is comparable. Endothelial cells, thrombin, platelets, and particularly neutrophil granulocytes appear to play an important role. NET support killing of pathogens, but also induce cell and tissue damage and inactivate protective host proteins. The phylogenetically ancient, unique, and "versatile" inhibitor α_2-M binds a broad spectrum of proteases forming a cage, which keeps captured proteases apart from their physiologic high-molecular substrates and receptors,[28] and interacts with many other proteins such as cytokines.

The significance of α_2-M for maintaining the hemostatic balance and moderating innate immunity would support the hypothesis[14] that children are to some extent protected by their higher α_2-M level from severe course of COVID-19. A first step to test this hypothesis would be clinical studies assessing α_2-M plasma levels in different age cohorts and clinical risk groups. However, just measuring α_2-M plasma concentration would probably not be sufficient, because important features of structure (eg, native, transformed, or dimeric α_2-M) and function (cage-like or noncovalent binding, chaperone function) would not be assessed. In this context, assays capturing integrity and functionality of α_2-M should be developed and validated. For example, an elegant study determining complexes of α_2-M with activated protein C found a correlation with the risk of venous thrombosis.[34] Beyond that, more basic research is needed into physiology and interactions of α_2-M, particularly with NET. Should further research find evidence for a potentially protective role of α_2-M in COVID-19, this might even stimulate considerations to increase its level by substitution.

In the past months excellent and multifaceted research has been communicated around the world, starting from China as the first-hit country. We should strongly stimulate and encourage exchange of ideas and cooperation of all involved disciplines, such as eg, clinical medicine, virology, epidemiology, immunology, biochemistry, and hemostaseology. The above-explained hypothesis would need to be confirmed by considerable clinical and experimental work. However, in a pressing acute situation during which we are losing many lives, it should be justified to mobilize all available sources of knowledge, in order to identify fire fighters who might help us to extinguish the fire of thromboinflammation in COVID-19.

CONFLICTS OF INTERESTS

No author has conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

R. Seitz and W. Schramm wrote the first draft; all authors contributed to the concept, literature search, conclusions, and editing of the final version.

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