COVID-19, Microangiopathy, Hemostatic Activation and Complement

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The COVID-19 pandemic caused by the SARS-CoV-2 virus has swept the globe with devastating societal consequences and has placed tremendous stress on healthcare systems. Although severe respiratory disease is a dominant feature, strokes, venous thrombosis, renal failure, cardiomyopathy, coronary and systemic vasculitis have complicated the clinical phenotype (1-5). Indeed, besides typical features of acute respiratory distress syndrome (ARDS), patients with COVID-19 exhibit a thrombotic necrotizing injury of the lung capillaries (6). This clinical pattern, together with low blood platelet count (thrombocytopenia) and elevated serum fibrin degradation products (d-dimers) (7, 8) is highly suggestive of complement activation.

**Complement and its dysregulation in human disease**

The complement system is a part of the innate immune system and can be activated via three separate pathways: the antibody-dependent classical pathway, the mannose-binding lectin (MBL) pathway, and the alternative pathway (Figure 1)(9). Activated complement can produce direct effector functions by target opsonization with cleaved complement component 3 (C3) and C4 fragments, promotion of inflammation with C3a and C5a, and direct cell lysis with the assembly of the membrane attack complex (MAC) C5b-9 (Figure 1)(9). Complement activation also primes adaptive immune responses. For example, the adjuvant effect of C3 activation in eliciting a robust antibody response is well established (9).

Our understanding of inappropriate complement activation in human disease derives mainly from genetic mutations of complement proteins—either loss of function mutations in regulatory proteins that protect host tissues under homeostatic conditions or gain of function mutations resulting in resistance to regulatory protein surveillance (10). Paroxysmal nocturnal
hemoglobinuria (PNH), for example, is caused by deficiency of two complement regulatory proteins on affected blood cells, decay-accelerating factor (DAF, also known as CD55) and CD59, and is characterized by complement-mediated hemolysis and multi-organ thrombosis (11). The thrombotic microangiopathy (TMA) of the atypical hemolytic uremia syndrome (aHUS) leads to thrombocytopenia, hemolytic anemia, and renal failure (12). However, thrombotic events in aHUS are not confined to the kidney; 3-10% of patients have cardiac complications due to coronary microangiopathy and patients often develop complications involving other organs (12). The most frequent mutations in aHUS are found in the regulatory protein factor H (FH), and such mutations lead to MAC-mediated endothelial injury and capillary thrombosis, which are the hallmarks of TMA (13). Both PNH and aHUS are caused by inadequate control of complement activation and successfully treated with Eculizumab, a humanized anti-C5 mAb (9).

**Evidence and mechanism of complement activation in COVID-19**

It is highly plausible that complement activation plays a role in the pathogenesis of COVID-19. Pathogen infection is a common external trigger of increased complement activation. While complement, like other innate immune system components, helps to control initial bacterial or viral infections, there is a risk for complement to become detrimental in later stages of the infection due to over-activation directly by the pathogen, or secondarily via damaged host tissues. Runaway complement activation may overwhelm host cell regulatory mechanisms, particularly in individuals with genetic predispositions to subclinical complement regulation insufficiency.

The clinical phenotypes of COVID-19 likely reflect direct effects of virus-mediated physical damage to tissue integrity as well as maladaptive host immune responses. For example,
endothelial cells express high levels of angiotensin converting enzyme 2 (ACE2)—the receptor for SARS-CoV-2—and viral infection can disrupt endothelial cell function directly or evoke an inflammatory or other immune response (14). These last mechanisms are critical in the cytokine storm that complicates the most severe phenotype of COVID-19 (15). The anaphylatoxins C3a and C5a are likely major contributors to cytokine storm syndrome, both directly through their intrinsic pro-inflammatory activities in leukocyte activation and trafficking, and indirectly by synergizing with other innate immune sensors, such as Toll-like receptors, to amplify inflammation (16). Indeed, in a Chinese cohort of COVID-19 patients, plasma C5a levels reflected the severity of the disease (17). Anecdotally, treating two of these patients with an anti-C5a mAb coincided with resolved fever and increased oxygen saturation (17).

It is likely that complement is activated in COVID-19 via multiple pathways, both by SARS-CoV-2 itself and by damaged tissues and dying cells at later stages of the disease (Figure 1). For example, natural (IgM) antibodies that recognize viral antigens or neo-antigens exposed on damaged host tissues could trigger the classical pathway (18). The lectin pathway could also activate in response to viral components such as the nuclear protein (N protein), as demonstrated in a recent study for SARS-CoV in which the N protein directly interacted with and activated mannose-binding lectin-associated proteases 2 (MASP2) (17). Finally, dying cells in multiple ischemic organs likely shed lipid-anchored membrane complement regulatory proteins, such as DAF and CD59, and lose glycosaminoglycans for FH binding (19, 20), allowing complement activation by the alternative pathway to occur unimpeded.

**Complement-mediated coagulopathy and microangiopathy in COVID-19**

Complement activation may also contribute to hemostatic activation leading to coagulopathy and explain microvascular injury, pathological features that are widely reported in
COVID-19 (4-7). In the atypical ARDS that complicates COVID-19, substantial deposits of complement C4d, MASP2, and C5b-9 have been reported in the lung and in the dermal microvasculature (6). Thus, there is evidence of systemic thrombophilia as well as microvascular injury, in parallel with elevated plasma C5a and endothelial C5b-9 deposits in COVID-19.

Eculizumab effectively inhibits C5 to block venous thromboembolic events (VTE) in PNH and TMA in aHUS (9). Until recently, the relative contributions of the C5a and C5b-9 pathways to these pathologies remained unknown. Experiments in a mouse model carrying a FH point mutation and develops both VTE and TMA has partially elucidated this question (21). Blocking C5 in the mutant mice rescued the renal and ocular TMA as well as VTE phenotype, and prevented thrombocytopenia, hemolytic anemia, renal failure, stroke, and sudden death (22). On the other hand, when the researchers deleted the C5a receptor (C5aR) in the same mutant FH background, the mice avoided VTE, but were still susceptible to TMA in the kidney and eye (22, 23). Conversely, blocking C5b-9 by C6 gene deletion rescued the mutant mice from TMA injury, but had no impact on the incidence of VTE (22, 23). Thus, at least in this murine model, microvascular injury and capillary thrombosis is mediated by the MAC pathway, whereas systemic thrombophilia depends on the C5a/C5aR pathway. The mechanism by which C5aR activation induces VTE remains unclear. However, given that neutrophils and monocytes prominently express C5aR on their cell surface, downstream contributors such as cytokine production (16), neutrophil extracellular traps leading to platelet-leukocyte aggregates (24, 25), and tissue factor release to trigger the extrinsic coagulation pathway (26) could all have played a role.
Considerations in anti-complement therapy for COVID-19

The approved drugs Eculizumab and RUCONEST, a C1 inhibitor (C1INH), and experimental anti-C5a and anti-C3 drugs have been administered and well tolerated under compassionate use to COVID-19 patients (27-30). As controlled clinical trials of complement inhibitors are initiated or considered for COVID-19, one question is: which are the interventions most likely to be safe and effective at various stages of disease evolution?

C5 blockade may benefit patients who are in a critical condition requiring ICU care. Here, there is evidence from mice and humans consistent with tissue damage mediated both by C5a and C5b-9 (6, 17, 22, 23). In less severe patients who require hospitalization but not ICU care, an argument could also be made for prescribing medications that block C5, although the anti-C5a approach would carry a lower risk of Neisseria bacterial infection, which is normally controlled by MAC (9). Ideally, this question of comparative safety and efficacy would be addressed by a randomized, controlled comparison of anti-C5 vs anti-C5a, perhaps enriching patient selection by including those with elevated d-dimers and indices of complement activation. Vaccination and prophylactic use of antibiotics could mitigate the risk of Neisseria infection and consequent meningitis (9, 27). However, the relative safety and comparative advantage of such strategies in patients sick with COVID-19 is unknown. A disadvantage of the current anti-C5 or C5a mAbs is the high dosage requirement (9, 17), which may create pharmacokinetic uncertainties in the setting of hemodynamic instability. In addition to these terminal complement inhibitors, drugs targeting earlier steps or a specific pathway of the complement cascade are also in clinical development (9). Such drugs may achieve more complete inhibition of complement than anti-C5 or anti-C5a, but their benefit and risk profiles also require careful consideration.
Conclusions

In summary, there are persuasive lines of evidence implicating complement activation in COVID-19. These rest on the consistency of the clinical phenotypes with both human syndromes and mouse models of complement activation. Pharmacological inhibitors of complement activation include approved drugs and others in late stages of development. Selection amongst them and the timing of treatment will depend on controlled trials that define the relative importance of distinct complement pathways, the efficiency of drug administration and the risk of opportunistic infection.
Figure 1. Potential mechanisms of complement-mediated pathology in COVID-19. SARS-CoV-2 virus may directly activate the complement pathways. Damaged host tissues could also secondarily activate complement via all three pathways: the classical pathway (CP), the lectin pathway (LP) or the alternative pathway (AP). Complement activation generates anaphylatoxins C3a and C5a and the membrane attack complex C5b-9. The C5a anaphylatoxin can cause neutrophil and monocyte activation, promote formation of neutrophil extracellular trap (NET) and platelet-leukocyte aggregates, and stimulate neutrophil degranulation and release of tissue factor to trigger the extrinsic coagulation pathway. These C5a effects may contribute to a hypercoagulative state leading to venous thromboembolic events (VTE) and disseminated intravascular coagulation (DIC). The membrane attack complex C5b-9 can cause direct endothelial injury and platelet activation, leading to thrombotic microangiopathy (TMA). Capillary and blood vessel occlusion by TMA and VTE eventually result in tissue ischemia and oxidant injury, contributing to multi-organ failure in COVID-19. Complement factor B (FB); Complement factor D (FD); Properdin (P)
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