COVID-19, microangiopathy, hemostatic activation, and complement

Wen-Chao Song[1,2] and Garret A. FitzGerald[1,2,3]

[1]Department of Systems Pharmacology and Translational Therapeutics, [2]Institute for Translational Medicine and Therapeutics, and [3]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

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 health care systems. Although severe respiratory disease is a dominant feature, strokes, venous thrombosis, renal failure, cardiomyopathy, and 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 a low blood platelet count (thrombocytopenia) and elevated plasma fibrin degradation products (D-dimers) (7, 8), is highly suggestive of complement activation.

Complement and its dysregulation in human disease

The complement system is part of the innate immune system and can be activated via three separate pathways: the Ab-dependent classical pathway, the mannose-binding lectin (MBL) pathway, and the alternative pathway (Figure 1 and ref. 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 and ref. 9). Complement activation also primes adaptive immune responses. For example, the adjuvant effect of C3 activation in eliciting a robust Ab 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 2 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 multiorgan 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% to 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. Although complement, like other innate immune system components, helps to control initial bacterial or viral infections, there is a risk of complement becoming detrimental in later stages of the infection because of overactivation directly induced 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 proinflammatory activities in leukocyte activation and trafficking and indirectly by synergizing with other innate immune sensors, such as TLRs, to amplify inflammation (16). Indeed, in a Chinese cohort of patients with COVID-19, 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

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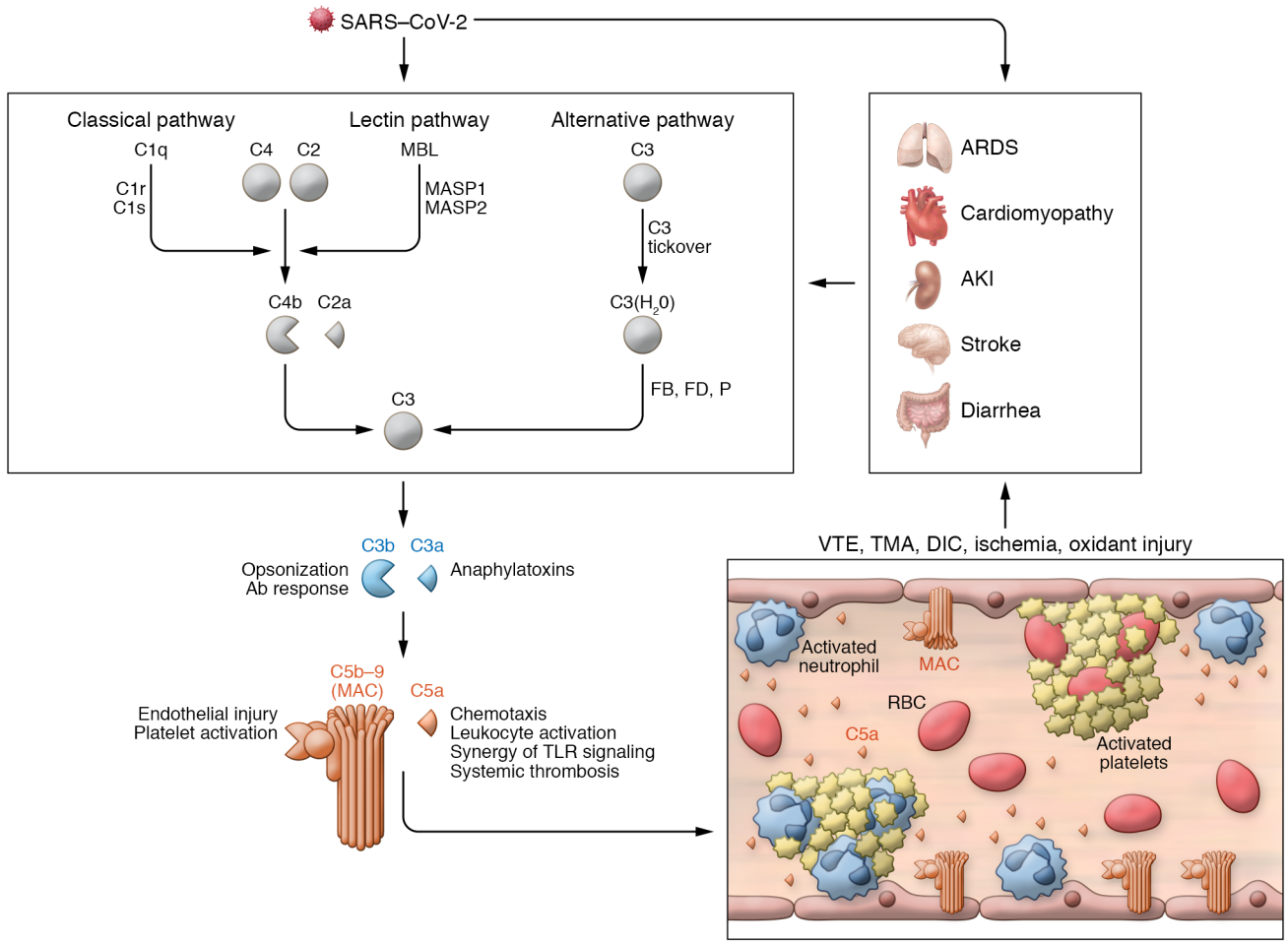

Figure 1. Potential mechanisms of complement-mediated pathology in COVID-19. SARS-CoV-2 virus may directly activate the complement pathways. Damaged host tissues could also secondarily activate complement via any of the three pathways: the classical pathway, the lectin pathway, or the alternative pathway. Complement activation generates the anaphylatoxins C3a and C5a and the MAC C5ab-9. The C5a anaphylatoxin can cause neutrophil and monocyte activation, promote the formation of NET and platelet-leukocyte aggregates, and stimulate neutrophil degranulation and the release of tissue factor to trigger the extrinsic coagulation pathway. These C5a effects may contribute to a hypercoagulative state leading to VTEs and disseminated intravascular coagulation (DIC). The MAC C5b-9 can cause direct endothelial injury and platelet activation, leading to TMA. Capillary and blood vessel occlusion by TMA and VTEs eventually results in tissue ischemia and oxidant injury, contributing to multiorgan failure in COVID-19. AKI, acute kidney injury; FB, complement factor B; FD, complement factor D; P, properdin.

(IgM) Abs that recognize viral antigens or neoantigens 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 MBL-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 (VTEs) 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 an FH point mutation and developing both VTEs and TMA have partially elucidated this question (21). Blocking C5 in the mutant mice rescued the renal and ocular TMA as well as VTE phenotypes 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 back-

ground, the mice avoided VTEs, 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 VTEs (22, 23). Thus, at least in this murine model, microvascular injury and capillary thrombosis are mediated by the MAC pathway, whereas systemic thrombophilia depends on the C5a/C5aR pathway. The mechanism by which C5aR activation induces VTEs remains unclear. However, given that neutrophils and monocytes prominently express the C5aR on their cell surface, downstream contributors such as cytokine production (16), neutrophil extracellular traps (NETs) 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 anticomplement 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 in patients with COVID-19 (27–30). As controlled clinical trials of complement inhibitors are initiated or considered for COVID-19, it will be necessary to ask which interventions are most likely to be safe and effective at various stages of disease evolution.

C5 blockade may benefit patients who are in critical condition requiring ICU care. Here, there is evidence from mice and humans consistent with tissue damage mediated by both C5a and C5b-9 (6, 17, 22, 23). In patients with less severe disease 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 versus anti-C5a mAbs, 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 with COVID-19 are 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 among these drugs 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.

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Address correspondence to: Wen-Chao Song, Perelman School of Medicine, 421 Curie Boulevard, Room 1254 BRBII/III, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. Phone: 215.573.6641; Email: Songwe@pennmedicine.upenn.edu. Or to: Garret A. FitzGerald, Institute for Translational Medicine and Therapeutics, Smilow Center for Translational Research, West Pavilion, 10th Floor, Room 116, 3400 Civic Center Boulevard, Building 421, Philadelphia, Pennsylvania 19104-5158, USA. Phone: 215.898.1185; Email: garret@upenn.edu.

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