The state of complement in COVID-19

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Abstract | Hyperactivation of the complement and coagulation systems is recognized as part of the clinical syndrome of COVID-19. Here we review systemic complement activation and local complement activation in response to the causative virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and their currently known relationships to hyperinflammation and thrombosis. We also provide an update on early clinical findings and emerging clinical trial evidence that suggest potential therapeutic benefit of complement inhibition in severe COVID-19.

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the largest pandemic disease of humans in the past century. Thus far, approximately 225 million cases and more than 4.5 million deaths have been attributed to COVID-19 globally. The clinical manifestations of COVID-19 can be highly variable, ranging from mild upper respiratory tract symptoms in most cases to severe, life-threatening, multi-organ disease. Up to one-third of patients affected by COVID-19 will develop persistent symptoms, and some will have permanent end-organ dysfunction pursuant to tissue fibrosis. Despite the emerging epidemiological data, the underlying pathogenesis of COVID-19 and its optimal treatment are still poorly understood. Undoubtedly, detailed mechanistic understanding of this disease will enable more effective therapies for its treatment, save lives and preserve healthy tissues.

The complement system is an ancient, evolutionarily conserved and non-redundant component of immunity. It is classically viewed as a liver-derived and plasma-operative system constantly scanning the blood and interstitial fluids for invading pathogens and self-derived noxious antigens. Pathogen sensing triggers one or several complement activation pathways (the lectin pathway, the classical pathway or the alternative pathway) and cleavage-mediated activation of the key components C3 and C5 by C3 and C5 convertases, respectively, into bioactive C3a and C3b and C5a and C5b. C3a and C5a are anaphylatoxins that mediate the general inflammatory reaction, C3b is a major opsonin that induces tagging and phagocytic uptake of pathogens and C5b seeds the formation of the membrane attack complex (MAC; C5b–9), which directly lyses pathogens. Owing to its central role in the detection and removal of pathogens, complement should intuitively be protective during SARS-CoV-2 infection. However, several lines of evidence implicate this system as a key component in the pathogenesis of COVID-19, and emerging trial data suggest clinical benefits from targeting this system therapeutically. Thus, complement, like many components of the immune system, could be viewed as a ‘double-edged sword’, with its dysregulation leading to harmful effects. Of course, how and why the normally protective complement system becomes pathogenic in COVID-19 is not yet known, but one could speculate that, consistent with the Goldilocks principle, some complement activation is beneficial in the initial response to the virus, but too much, sustained for too long, propagates disease.

Through parallels with other coronaviruses, through systems biology approaches and through detailed pathological and clinical observations, researchers and clinicians now realize the importance of complement in pathophysiology and see the potential therapeutic benefit of complement inhibition.

Here we review the emerging roles of complement in COVID-19-associated thromboinflammation, propose a key contribution from lung intracellular complement as well as systemic complement activation, and finally provide an update on eagerly awaited clinical trial results.

Complement in severe COVID-19

Multiple lines of evidence implicate hyperactivation of the complement system in the pathophysiology of COVID-19. Mechanistically, SARS-CoV-2 itself can activate the complement system either directly through the lectin pathway, the classical pathway and/or the alternative pathway or cause endotheliopathy (endothelial cell injury and dysfunction) and thromboinflammation (inflammation associated with coagulation and thrombosis), which in turn activate the complement system. Specifically, the spike and nucleocapsid proteins of SARS-CoV-2 can be directly recognized by lectin pathway components, leading to complement activation, and IgG and IgM antibodies directed against the receptor-binding domain of the spike protein initiate classical pathway activation.

Furthermore, SARS-CoV-2 spike protein may directly dysregulate the alternative pathway of complement activation by binding heparan sulfate and competing with factor H, which is a negative regulator of complement activity.

These observations are consistent with the long-known observation that complement activation is a frequent feature of the pathophysiology of acute respiratory distress syndrome, induced by infection or other triggers. For example, increased anaphylatoxin concentrations are found in serum from patients with influenza virus H1N1 infection of the lungs. Similarly, patients with severe COVID-19 have high circulating levels of C5a and of the terminal activation fragments, soluble C5b–9, which correlate with clinical severity, as well as high levels of processed fragments of C3, itself an independent risk factor for death. Likewise, data from the UK Biobank support genetic single-nucleotide variants in genes encoding the complement regulators decay accelerating factor (DAF; also known as CD55), complement factor H and complement component 4-binding protein-a (C4BPa) as risk factors for morbidity and death from SARS-CoV-2.
infection. Significant microvascular deposition of mannose-binding lectin serine protease 2 (MASP2; the primary enzymatic initiator of the lectin pathway of complement activation), C4d and C5b–9 has been reported in the skin and lungs of patients, the last two complement components co-localizing with SARS-CoV-2 spike protein. Reports of children with vascular lesions and hypercoagulability in the context of COVID-19 (REFS 27,28) (the so-called multisystem inflammatory syndrome in children (MIS-C)) also suggest complement involvement through co-triggering of the complement and coagulation cascades. A key study has shown that elevated plasma levels of C5b–9 are common in MIS-C, suggesting systemic complement activation in COVID-19. Characteristic endothelial cytormorphological changes observed in patients29,30 are also pathognomonic of complement-mediated injury induced by C5b–9 (REF 33). Finally, early clinical findings
and emerging trial evidence also indicate potential benefits of complement-targeting therapies in COVID-19, as reviewed later.

The findings of studies of patients align with findings in animals responding to related pandemic betacoronaviruses. For example, Middle East respiratory syndrome coronavirus infection in mice causes extensive complement deposition in lung tissues, where the damage can be reduced by inhibition of distal complement components44. In the same model, C5aR blockade limits the thickening of alveolar septa and prevents interstitial oedema and haemorrhage in the lungs45. Similarly, the complement components C3, C1r and C1s are all part of a pathogenic pulmonary gene signature associated with lethality in mouse models of SARS-CoV infection39, and deletion of C3 in these mice protects against disease46. Indeed, primary infection with SARS-CoV upregulates complement genes in ferrets, including the lectin pathway components MASP1 and ficolin 1 (REF. [5]). SARS-CoV itself interacts with mannose-binding lectin (MBL) to activate complement C3 proximally via the lectin pathway on virus-infected cells in a similar manner to SARS-CoV-2, the nucleocapsid protein of which aggravates lung injury through MASP2-mediated complement hyperactivation47. The lectin pathway contributes to the excessive inflammatory response associated with other pandemic viral infections, as MBL-deficient mice develop less severe disease when infected with influenza A virus strain H1N1 or strain H9N2/G1 compared with wild-type mice48.

Although the detrimental contributions of complement to COVID-19 are now recognized, the underlying molecular mechanisms driving pathology are less clear. Recent data indicate that the long-acknowledged but still poorly understood functional connection between systemic complement and the coagulation cascade and also the intrinsic production of complement by lung cells are involved and may thus be promising novel therapeutic targets.

**Thromboinflammation in COVID-19**

SARS-CoV-2 infection of the respiratory epithelium triggers an inflammatory response driven by the host innate immune system that in 20–30% of hospitalized patients results in endothelial injury and the activation of coagulation and thrombosis49, a phenomenon called ‘thromboinflammation’44. These events start in the lung, but may extend to several other organs, and can clinically manifest themselves as renal, hepatic and heart dysfunction and eventually multi-organ failure50,51. Thrombosis (venous thrombosis, pulmonary embolism and arterial thrombosis) is indeed a major complication of COVID-19. Despite routine thromboprophylaxis, almost 10% of patients hospitalized with COVID-19 have thrombotic complications52,53, and clotting-associated complications are associated with death54–56. Indeed, pulmonary artery thrombosis and microangiopathy in pulmonary tissue were observed in up to 79% of patients who died with COVID-19 as the primary cause and were associated with the presence of diffuse alveolar damage57, which supports the role of thrombotic phenomena in the development of COVID-19-related lung damage. Patients with COVID-19-associated coagulopathy have highly elevated levels of plasma D-dimer58, a fibrin degradation product that is present in the blood after activation of fibrinolysis. In a retrospective study of patients from Wuhan, China, D-dimer levels greater than 1 µg ml⁻¹ at admission to hospital predicted an 18-fold increase in the odds of dying following SARS-CoV-2 infection59.

Endothelial cell injury and dysfunction (also termed ‘endotheliopathy’) play a central role in COVID-19-associated thrombosis and coagulopathy. Endothelial cells and microvascular pericytes express entry receptors for SARS-CoV-2 (REFS [60,61]), and endothelial tropism of SARS-CoV-2 has been suggested by autopsy studies and single-cell RNA sequencing atlases55,56. Here, SARS-CoV-2 binding to its host entry receptor, angiotensin-converting enzyme 2 (ACE2), blocks ACE2-mediated production of angiotensin (1–7), thus preventing vasorelaxation and antithrombotic and anti-inflammatory effects on endothelial cells and enhancing the harmful effects of angiotensin II, exacerbating endothelial cell activation and injury62. However, the evidence for direct SARS-CoV-2 infection of endothelial cells remains a matter of debate63–65. Nevertheless, the levels of markers of endothelial cell activation and injury, including von Willebrand factor (vWF), angiopoietin 2, soluble P-selectin and soluble thrombomodulin, are elevated in the circulation of patients with severe COVID-19 in intensive care units66–68, and vWF and thrombomodulin levels correlate with mortality68,69. Extensive endothelialitis with inflammatory cells adhering to the endothelium and platelet-rich fibrin thrombi have been reported in large and small arterial vessels in the lungs, heart, liver, small bowel, kidneys and other organs of patients who have succumbed to COVID-19 (REFS [70,71]).

Furthermore, diffuse microvascular thrombosis with endothelial cell injury and rarefaction have been observed most often in pulmonary alveolar capillaries69,70, as well as in kidney glomerular and peritubular capillaries71,72, and in myocardial capillaries73, accompanied by deposits of complement activation products within damaged microvasculature74,75.

Histological examination of lungs, hearts and kidneys from patients with severe COVID-19 shows fibrin and intense C5b–9 immunostaining in the glomeruli and cardiac microthrombi, respectively76,77,78. The pattern of microvascular lesions and C5b–9 deposits, along with elevated levels of lactate dehydrogenase and low levels of haptoglobin and schistocytes in peripheral blood, that have all been observed both in adults and children with severe COVID-19 (REFS [70,79]), are similar to complement-mediated thrombocytic microangiopathy (also known as atypical haemolytic uraemic syndrome), a prototypic disease of complement C5-mediated endothelial injury79,80. The report of two cases of atypical haemolytic uraemic syndrome relapse triggered by COVID-19, one of which was in a patient with a heterozygous R139W gain-of-function point mutation resulting in formation of a hyperactive C3 convertase, and cured by the anti-C5 antibody eculizumab79,80, indirectly supports a pathogenetic role of the complement terminal pathway in COVID-19-associated thromboinflammation.

The terminal complement components C5a and the MAC can promote endothelial injury and dysfunction through multiple processes81. C5a recruits and activates neutrophils, monocytes and macrophages82. Furthermore, both C5a and the MAC induce chemokine release and upregulation of adhesion molecules on endothelial cells, events that support transmigration of neutrophils and macrophages83,84. C5a and the MAC also promote platelet adhesion by stimulating the exocytosis of P-selectin and vWF multimers from endothelial cells, tissue factor expression and shedding of thrombomodulin from cell surfaces85,86, which trigger the coagulation cascade. Activated platelets and C3 subsequently activate the recruited neutrophils to release neutrophil extracellular traps (NETs), generating extracellular DNA and histones that are highly toxic to endothelial cells and accumulate in microthrombi of patients with
COVID-19 (REFS 133,135). The release of NETs (NETosis) is a unique form of neutrophil cell death that is intimately linked with the complement and coagulation cascades85. C5a receptor 1 (C5aR1) and C5aR2 activation generates extracellular histones, which are major components of NETs and activate the intrinsic coagulation pathway86–88. NETs act as a scaffold for thrombus formation by inducing platelet aggregation, while platelets in turn induce NET formation86–88. The cumulative results of these events are vascular injury and loss of endothelial antithrombogenic properties, with massive formation of blood clots. Consistent with this, exposure of cultured microvascular endothelial cells to SARS-CoV-2 spike protein induced leukocyte recruitment, C3 and Csβ–9 deposition and platelet adhesion and thrombus formation on cell surfaces. Thrombus formation was halted by complement inhibitors92. The observation that the signature of complement deposition across different organs in patients with severe COVID-19 mimics the signatures found in complement-driven thrombotic disease40 supports the notion that complement is a driving pathogenic force in severe COVID-19. Furthermore, it is possible that complement also contributes to SARS-CoV-2 vaccine-induced thrombotic complications (BOX 1).

Thus, collectively, the available evidence places the consequences of C5 activation among the drivers of COVID-19-associated thromboinflammation and highlights the potential of inhibitors targeting C5, C5a or C5aR1 to prevent and control thromboembolic complications in this debilitating infectious disease.

**Intracellular complement**

Complement circulating in plasma is produced and secreted by the liver89. However, complement components are also produced by both immune42,93 and non-immune42,94 cells. In T cells, complement is produced both on activation through the T cell receptor and via ICAM1–LFA1 interactions during diapedesis into tissues92,95. The latter scenario is relevant to COVID-19 as it represents a source of local complement in tissues, where plasma complement is absent, and is a requirement for the bioenergetic reprogramming necessary for optimal effector function of immune cells92,95. Airway epithelial cells (AECs) also produce C3 and have the capacity to take up some C3 from exogenous sources90. In the context of cellular stress, for example from serum starvation, intracellular C3 protects AECs from apoptosis96, although the mechanism is currently unknown.

In the context of SARS-CoV-2 infection of AECs, expression of complement genes is among the biological pathways most highly induced by the virus97. Here, virus sensing triggers type I interferon signalling through the JAK–STAT machinery and induces direct regulation of complement genes, notably including C3 and CFβ (encoding complement factor B)100. Factor B drives the assembly of an inducible, intracellular alternative complement activation convertase, which processes C3 to C3a. Accordingly, SARS-CoV-2-infected cells produce C3a, and this can be neutralized by culture with ruxolitinib, an inhibitor of JAK1 and JAK2, or a cell-permeable inhibitor of factor B100. Importantly, immune cells close to SARS-CoV-2-infected AECs respond to the milieu of heightened local C3 activation and upregulate genes induced by C3 fragment receptors (C3aR and CD46), generating proinflammatory mediators, a signature not detected in circulating immune cells100. Whether increased C3 production and intracellular C3a generation by AECs triggered by SARS-CoV-2 infection has a cell-intrinsic effect has not yet been explored. Collectively, AEC-derived complement is likely to represent a substantial local source of complement in the context of SARS-CoV-2 and is unlikely to be neutralized by drugs targeting complement components in the circulation.

Given the contributions of canonical (plasma-circulating) and non-canonical (intracellular) complement to human immunological health101,102, it may be worth exploring whether changes in complement activity with age may be a risk factor for severe COVID-19 (BOX 2).

**Therapeutic perspectives**

Early in the first wave of the COVID-19 pandemic, a number of case reports and small-scale clinical interventions that were not randomized nor properly controlled advocated the use of complement-inhibiting therapies103–109, very similar to reports advocating the wide-scale use of cytokine antagonists110. What has become clear over the past 18 months is that initial enthusiasm needs to be followed by rigorously executed clinical trials of appropriate sample size across many countries before conclusions can be reached and clinical practice altered. For COVID-19 therapy, it is emerging that timing is everything. The disease runs a bimodal course with an early viral phase comprising mainly upper airway symptoms, followed in approximately 20% of patients by dyspnea and hypoxia, sometimes progressing to severe acute respiratory...
distress syndrome. Despite the considerable numbers of patients who are eligible and willing to participate in clinical COVID-19 trials, only 20% of patients progress to severe disease. Thus, the biggest hurdle to obtaining definitive answers with various intervention trials in severe COVID-19 has been to select the right timing and the right patient who might benefit from the intervention, on the basis of either clinical or biochemically easy to measure (point of care) biomarkers. It is very likely that the hyperinflammation that characterizes severe disease is driven by several molecular and cellular pathways that tend to self-amplify, so in advanced disease, targeting only one pathway and hoping for improvement is likely to be futile. Early intervention without causing immunosuppression is probably key. Currently, however, biomarkers predicting those who will go on to develop life-threatening COVID-19 (prognostic biomarkers) among the less ill and that could predict those who will respond to therapy (theragnostic biomarkers) are still lacking. One notable exception is the serum concentration of soluble urokinase plasminogen activator receptor, which strongly predicts clinical responsiveness to blockade with the IL-1 receptor antagonist anakinra. In the specific case of complement inhibition studies, it is still complicated in most hospitals to get point-of-care assessments of the complement cascade and in a timely manner. Clinical practice is also rapidly changing during this pandemic, and use of the broad anti-inflammatory drug dexamethasone has become the standard of care, potentially making it harder to replicate earlier findings regarding new compounds in small-scale studies in larger randomized trials that now include dexamethasone as the standard of care. As an example, despite many negative clinical outcome trials, the effectiveness of interfering with IL-6 in COVID-19 became clear only from a meta-analysis of more than 30 randomized intervention trials, in which it was shown that IL-6 reduces 28-day mortality mainly in the most severely ill patients. Counterintuitively, the clinical benefit was, however, seen mainly in those receiving dexamethasone or other steroids, potentially because these patients had a worse prognosis. Specifically, with regard to complement, other considerations include whether components of the complement system themselves should be targeted (extracellularly or within cells) or whether mechanisms inducing complement production and/or activation should be targeted instead (for example, using JAK inhibitors or factor B inhibitors; see earlier). These considerations are important because AEC-derived complement is likely to represent a substantial local source of complement in the context of SARS-CoV-2 and is unlikely to be normalized by drugs targeting complement components in the circulation. Cell-permeating drugs targeting complement intracellularly (such as factor B inhibitors) or upstream components inducing complement gene transcription (such as JAK inhibitors) could potentially be more efficacious in severe COVID-19.

Indeed, although many trials are ongoing, some data already support beneficial efficacy of JAK inhibitors in the context of severe COVID-19. Finally, although the proliferation of preprint publications somewhat mitigates publication bias, it still remains likely that negative data may be under-reported.

Strategies to target complement in COVID-19 have mainly used available drugs that were already undergoing clinical testing or were already approved for clinical use in complement-mediated diseases, such as thrombotic microangiopathy associated with transplantation, atypical haemolytic uraemic syndrome, paroxysmal nocturnal haemoglobinuria, macular degeneration, complement-mediated nephropathies and myasthenia gravis. As a result, most of these have focused on targeting the C5 system. Initially, a few case series of C5-targeting therapies (eculizumab, a monoclonal antibody targeting C5, by Alexion Pharmaceuticals; BDB-001, a monoclonal antibody against C5a, by Staidson Biopharmaceuticals) were trialled in a total of 16 patients with severe to critical COVID-19, 14 of whom recovered. A subsequent proof-of-concept non-randomized trial in 40 patients with severe COVID-19 and controls showed a survival benefit in the group treated in an intensive care unit setting with eculizumab. On the basis of these studies, several randomized controlled trials (RCTs) have been initiated, and some have completed enrolment. Targeting C5 in COVID-19 has been trialled using the monoclonal antibodies eculfizumab (NCT04288713 and NCT04346797), ravulizumab (Ultomiris; NCT04570397 and NCT04369469) and tesidolumab (LFG316, Novartis), or using small protein or peptide antagonists of C5 such as nomacopan (Akari Therapeutics; trial about to start in the UK and Brazil) or zilucoplan (ZILU-COV trial (NCT04382755, UCB Pharma). Interim analysis of a large phase III trial with ravulizumab (NCT04369469) in patients with COVID-19 already receiving mechanical ventilation at randomization could not demonstrate clinical benefit, leading to a pause in the study. In the ZILU-COV trial, in contrast, 81 patients with COVID-19 and signs of hypoxia not yet requiring mechanical ventilation were randomized to receive 32 mg of zilucoplan subcutaneous daily for 14 days in addition to prophylactic broad-spectrum antibiotics.
(to cover the risk of meningococcal disease) or standard-of-care treatment and the same antibiotic regimen\textsuperscript{12,13}. Patients receiving zilucoseb had improved oxygenation parameters at day 15, accompanied by a lowering of circulating cytokine levels. At day 28 there was also lower mortality in zilucoseb-treated patients, although the study was underpowered to detect effects on mortality. Importantly, there was no risk of opportunistic infections in those receiving zilucoseb. This indicates that early timing of C5 blockade may be crucial. Trials are also studying the impact of blocking either C5a or C5aR. An initial RCT using the C5a-targeting antibody vilobelimab (NCT04333420, Innate Pharma) reported minimal improvement in oxygenation in 30 patients and controls, although there was a trend for increased survival in the vilobelimab arm\textsuperscript{12,13}. This study has now been extended to a large multicentre placebo-controlled RCT involving 390 patients receiving mechanical ventilation (NCT04333420) and is currently recruiting patients. Similarly, a large phase II/III RCT involving 368 participants is addressing the C5a-specific antibody BDB-001 (NCT 04449588, Staidson Biopharmaceuticals) and is in progress. Despite promising results of C5aR blockade involving 368 patients receiving mechanical ventilation (NCT04333420) and is currently recruiting patients. Similarly, a large phase II/III RCT involving 368 participants is addressing the C5a-specific antibody BDB-001 (NCT 04449588, Staidson Biopharmaceuticals) and is in progress.

Conclusions
Both clinical and basic science studies suggest that uncontrolled activity of the complement system may be a central player in the pathogenesis of COVID-19. Clinical trial evidence is now beginning to substantiate this suspicion. This clearly is an exciting development; however, it should still be viewed with caution because, similarly to IL-6-targeting therapies, it is likely that clinical trials will indicate that the benefits of complement-targeting therapies will depend on several confounders, namely severity of disease, timing of initiation and co-administered drugs. Thus, at present there is no compelling evidence to strongly advocate complement-targeting treatments for all patients presenting with severe COVID-19. This will likely change in the future as the niche for complement targeting becomes clearer and as we gain a better understanding of the distinct spatio-temporal contributions of complement following SARS-CoV-2 infection, its crossstalk with the coagulation system and the role of genetic polymorphisms in both systems.

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97. The authors declare no competing interests.