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REVIEW ARTICLE

COVID-19: A collision of complement, coagulation and inflammatory pathways

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Abstract

COVID-19 is frequently accompanied by a hypercoagulable inflammatory state with microangiopathic pulmonary changes that can precede the diffuse alveolar damage characteristic of typical acute respiratory distress syndrome (ARDS) seen in other severe pathogenic infections. Parallels with systemic inflammatory disorders such as atypical hemolytic uremic syndrome (aHUS) have implicated the complement pathway in the pathogenesis of COVID-19, and particularly the anaphylatoxins C3a and C5a released from cleavage of C3 and C5, respectively. C5a is a potent cell signalling protein that activates a cytokine storm—a hyper-inflammatory phenomenon—within hours of infection and the innate immune response. However, excess C5a can result in a pro-inflammatory environment orchestrated through a plethora of mechanisms that propagate lung injury, lymphocyte exhaustion, and an immune paresis. Furthermore, disruption of the homeostatic interactions between complement and extrinsic and intrinsic coagulation pathways contributes to a net pro-coagulant state in the microvasculature of critical organs. Fatal COVID-19 has been associated with a systemic inflammatory response accompanied by a pro-coagulant state and organ damage, particularly microvascular thrombi in the lungs and kidneys. Pathologic studies report strong evidence of complement activation. C5 blockade reduces inflammatory cytokines and their manifestations in animal studies, and has shown benefits in patients with aHUS, prompting investigation of this approach in the treatment of COVID-19. This review describes the role of the complement pathway and particularly C5a and its aberrations in highly pathogenic virus infections, and therefore its potential as a therapeutic target in COVID-19.

KEYWORDS

complement C5, COVID-19, cytokines, leukotriene B4, thrombin

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1 | INTRODUCTION

The morbidity and mortality associated with severe acute respiratory syndrome (SARS) coronavirus-2 (SARS-CoV-2) infection, the cause of coronavirus disease 2019 (COVID-19), is characterized by acute respiratory failure triggered by several pathogenic mechanisms. Observational and pathological studies in patients with COVID-19 have implicated the systemic cytokine effects of infection (commonly referred to as a cytokine storm with an elevation of associated hyper-inflammatory clinical markers)[1] and a pro-coagulant, thrombotic milieu.[2-4] Despite the severity of hypoxia, lung mechanics are well preserved in many cases and characterized only by high respiratory compliance and shunt fraction, reflected in the absence of typical features of acute respiratory distress syndrome (ARDS) such as hyaline membrane formation and type 2 pneumocyte proliferation.[5] However, those with aberrant mechanics of severe disease progress to ARDS typified by diffuse alveolar damage.[6,7] Often, a hypercoagulable inflammatory state[8] precedes or accompanies this, characterized by microangiopathic pulmonary changes, which may not be detectable using conventional radiographic imaging, despite emerging imaging evidence that macrovascular venous thromboses are common when actively sought in critically ill patients with COVID-19.[9,10] Consequently, the observed pulmonary hypoperfusion may benefit from different investigative and treatment approaches.[9]

It appears likely that the severe COVID-19 respiratory syndrome encompasses local and systemic pathological responses, with different clinical characteristics determined by the effects of SARS-CoV-2 on inflammation and coagulation and, in some patients, leading to the characteristic features of classical ARDS.

Here we describe the role of systemic complement activation that is likely to underpin many of the effects of COVID-19 and the associated hypercoagulability and inflammation, and why inhibition of complement, chiefly C5, with or without other anti-inflammatory interventions, may be an important therapeutic strategy.

2 | PATHOGENIC VIRUS INFECTIONS ACTIVATE COMPLEMENT

The complement pathway is fundamental to innate host immunity, with a critical role in defense against pathogens. Products of the complement pathway are present and activated across the alveolar–capillary membrane. The alveolar epithelium acts as the first barrier to inhaled pathogens, while the vascular endothelium amplifies the response. The complement system remains mildly active under normal homeostatic conditions but is intensified by endothelial stress caused by virus infections, bleeding, and trauma.[11] The trigger for complement can be either direct or indirect through the involvement of antigen–antibody complexes or toll-like receptors. Beyond normal homeostasis, unregulated complement activation triggers damage to host processes. It has been frequently described in rare microangiopathic conditions and following pathogenic virus infections that result in acute lung injury (ALI) and injury to other organs, such as H1N1, H5N1, and H7N9 influenza; SARS-CoV, Middle-East respiratory syndrome (MERS)-CoV; and now SARS-CoV-2 (COVID-19).[12] The mechanism of systemic activation of complement likely involves binding of the lectin pathway of complement component mannan-binding lectin (MBL) via the SARS CoV-1 spike protein.[13] This systemic activation is also likely to be true for SARS CoV-2 by the demonstration of co-localization of SARS CoV-2 spike protein with mannan-binding protein-associated serine protease 2 (MASP2) in human lungs.[5] While many complement products are involved in immune modulation and cell signalling, the critical anaphylatoxins discussed here are C3a and C5a.

Essentials

- COVID-19 and thrombo-inflammatory disorders such as atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH) share similar pathologic features.
- Thrombotic inflammation and microangiopathy are linked by uncontrolled complement activation; so complement inhibition strategies could reduce thrombo-inflammation and acute lung injury.
- Complement inhibitors with proven efficacy in other rare disorders need urgent investigation in COVID-19.

3 | C5a INDUCES AN ADAPTIVE IMMUNE RESPONSE WITH A CYTOKINE STORM

Complement is activated by three key pathways (lectin, alternative, and classical), leading to the production of C3 and C5, which are further cleaved to release C3a and C5a, by their respective convertases. C5 cleavage also produces the lytic membrane attack complex (MAC, or C5b-9) required for bacterial opsonization.[14]

C5a is a potent cell signaling protein that directly activates T cells by binding its ligand, the C5a receptor (C5R). C5a initiates an adaptive immune response by activating T and B cells and other pro-inflammatory cells such as neutrophils to release cytokines, including tumor necrosis factor (TNF), interleukin (IL)-1β, IL-6, and IL-8. This hyper-inflammatory phenomenon or "cytokine storm" occurs within hours of infection. Its presence in H5N1 and SARS infections, and now in COVID-19,[13,15,16] is widely recognized. A similar but distinct systemic inflammatory disorder is described in other diseases known collectively as secondary hemophagocytic lymphohistiocytosis and macrophage activation syndrome (MAS).[17] These inflammatory syndromes have recently been compared with COVID-19 pneumonia.[18] Interventions aiming to reduce complement (as measured by C3b2 complexes) and inflammation in these other diseases led to significant clinical improvements; thus, attenuating complement responses after infection may have therapeutic potential.[19] Murine models of infection support this: after infection with SARS-CoV-1, knockout

mice deficient in C3 had significantly reduced neutrophil infiltration and levels of inflammatory cytokines in the lungs and serum compared with non-deficient mice, despite a comparable viral load in the lungs.[13] In cells from SARS-CoV-1 infected patients that express angiotensin-converting enzyme 2 (ACE-2), an increased inflammatory response shown by higher levels of TNF-induced IL-6 was related to the degree of ALI.[20] Inhibition of TNF with etanercept protected mice infected with lethal H1N1 from ALI, suggesting that reducing the inflammatory response post-infection may reduce lung injury.[21]

4 | C5a PROMOTES A PRO-INFLAMMATORY RESPONSE BY ADDITIONAL MECHANISMS

Following infection, C5a stimulates inflammation, microvascular thrombosis, fibrinolysis, and an innate immune response.[12] H5N1-infected mice demonstrate ALI mirrored by high levels of C5a in bronchoalveolar lavage fluid (BALF).[22] Patients who died from H5N1-induced ARDS had significantly higher levels of C5a in the BALF and serum than survivors.[23] Once activated, C5a causes a plethora of pro-inflammatory effects orchestrated through different mechanisms.

While C5a directly activates T cells by binding to the C5aR, other cells such as neutrophils, endothelial cells, and platelets are activated by enhanced expression of key cellular adhesion molecules on their surfaces, including selectins (eg, P-selectin-targeting platelets), integrins (eg, targeting extracellular matrix through fibronectin and fibrinogen), and members of the immunoglobulin superfamily (eg, intercellular adhesion molecule-1 [ICAM-1], which is also the receptor for the major group of rhinoviruses). This upregulation of adhesion molecules promotes a variety of cell signaling and pro-inflammatory functions following infection.[24] Other cells such as lung macrophages are also activated to produce pro-inflammatory cytokines such as TNF. Subsequent blockade of adhesion molecules such as CD18, ICAM-1, and P-selectin significantly reduced neutrophil accumulation and ALI in a murine model.[24-26]

C5aR expression increases on antigen-presenting dendritic cells after influenza infection, allowing the promotion of CD8 T-cell activation by C5a.[27] Unsurprisingly, C5aR blockade reduces the numbers of virus-specific CD8 T cells following influenza infection.[28] C5a activates neutrophils and mast cells to release pro-inflammatory cytokines such as IL-12 and TNF,[29] and also activates macrophages and endothelial cells, which promote vascular leakage,[30] and the release of enzymes and oxidants.[31] Complement-induced cytokines such as IL-12 further influence CD8 T-cell activation, and TNF accelerates T cell transit through vascular endothelium, facilitated by the increased levels of adhesion molecules such as ICAM-1.[32]

Excess C5a also stimulates neutrophils to generate reactive oxygen species such as hydrogen peroxide.[31] These products of oxidative stress induce a wide variety of pro-inflammatory effects, including cytokine release and cell death by apoptosis and necrosis. The sequelae in mice infected by influenza included alveolar destruction with pneumonia. This oxidative stress following infection can be reduced by specific C5a blockade[33] or directly by antioxidant treatment.[34]

Neutrophil extracellular traps (NETs)—networks of fibers composed of DNA, histones, and proteins released from neutrophils that bind viral proteins, bacteria and, fungi—have an important role in innate immunity.[30] They are abundant in lung capillaries, are pro-coagulant, and trap platelets in a mesh. However, following influenza infection C5a stimulates excess NET production, which results in collateral epithelial and alveolar damage, increased permeability of the alveolar–capillary barrier, and release of pro-inflammatory cytokines.[31,35] NETs therefore have an essential role in inflammation, infection, and thrombosis (Figure 1). In summary, uncontrolled C5a activation following infection produces a pro-inflammatory environment that propagates lung injury.

5 | C5a PROMOTES AN IMMUNE PARESIS WITH LYMPHOCYTE EXHAUSTION

Lymphocyte exhaustion is another substantial C5a-mediated effect that is seen after significant infection, resulting in functional impairment of antigen-specific lymphocytes in the presence of a persistently high viral load[36] and a strong predictor of mortality.[37] This leucopenia and impaired antigen-presenting capacity have been described in H7N9 influenza infection;[38,39] fatal infection was associated with remarkably low blood T cells and subgroups.[40] C5a further induces apoptosis of thymocytes (hematopoietic progenitor cells in the thymus that mature into T lymphocytes), resulting in an overall reduction in T cells and immune paresis.[39] Furthermore, in SARS-CoV-1 and MERS-CoV infections, C5a-induced IL-6 and IL-8 release further reduces the T-cell-presenting ability of dendritic cells and their ability to produce anti-viral cytokines such as interferon (IFN)-α, -β, and -γ and IL-12. Thus, along with up-regulation of pro-inflammatory chemokines, there is a profound counter-reduction in anti-inflammatory activity, supporting the idea that C5a-mediated immune paresis has a critical role in the ALI seen in patients infected with viruses such as H7N9, SARS-CoV-1, MERS-CoV, and now SARS-CoV-2.[12]

6 | COMPLEMENT AND COAGULATION FACTORS DIRECTLY INTERACT

The extrinsic and intrinsic coagulation pathways cooperate with complement through a variety of bidirectional interactions that help maintain the homeostasis of coagulation and fibrinolysis.[41] Pathogenic virus infections disrupt this balance and, in COVID-19 for example, lead to an overall thrombo-inflammatory state. For example, coagulation factors IXa, Xa, XIa, and plasmin effectively cleave C3 and C5, generating C3a and C5a.[42,43] C5a has a further pro-coagulant role in the activation of tissue factor from multiple cellular sources, including endothelial cells[44] and neutrophils.[45]

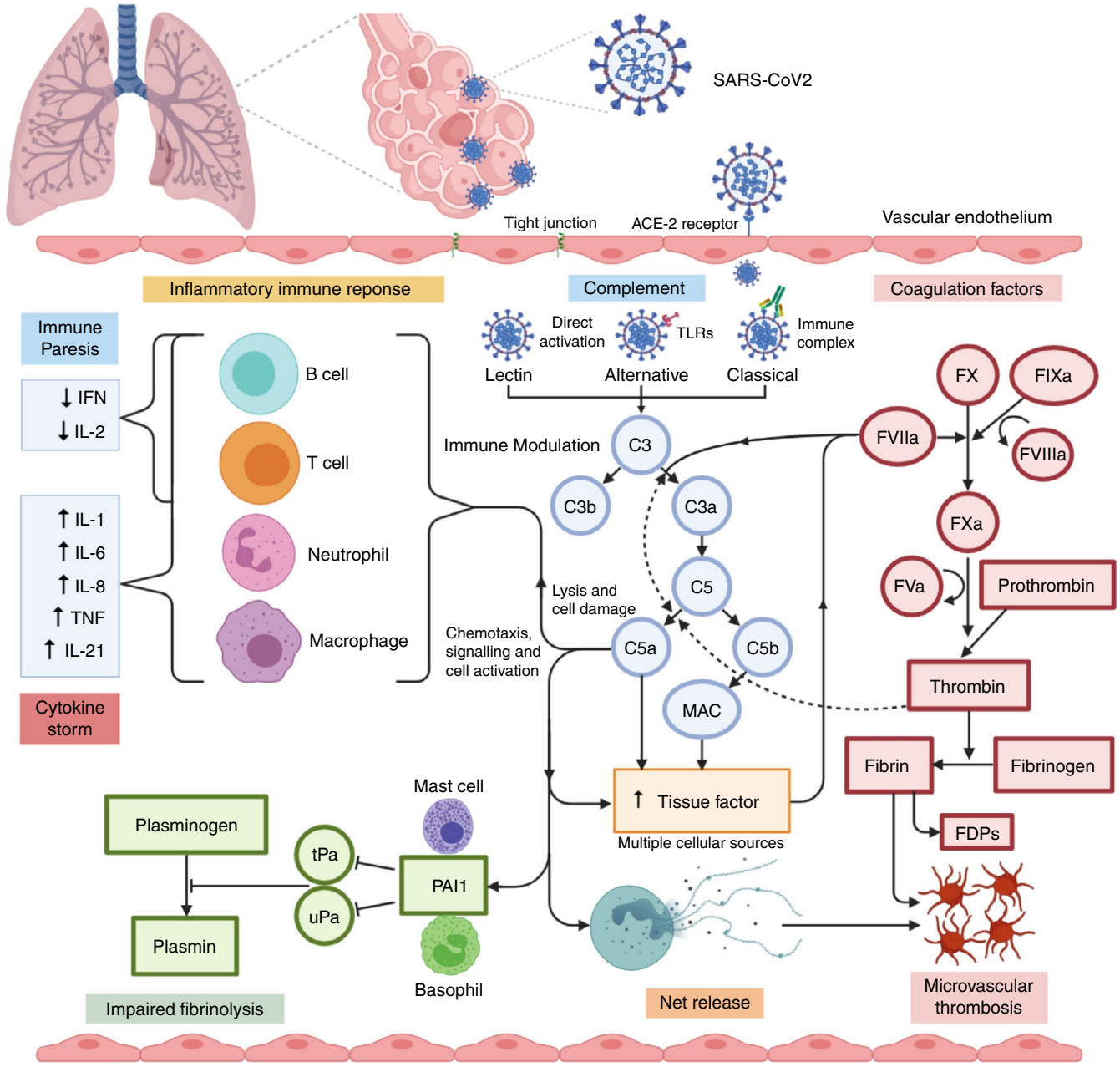

FIGURE 1 Interactions among the complement, coagulation, and inflammatory pathways following COVID-19 infection, based on knowledge of previous highly pathogenic virus infections. Complement is activated through three different pathways following virus infection, eventually leading to increased levels of the critical anaphylatoxins C3a and C5a, which have central roles in the innate immune response; C5a in particular influences chemotaxis, and cell signaling and activation. This contributes to the release of pro-inflammatory cytokines from effector cells, with a parallel reduction in anti-viral cytokines and lymphocyte reduction. C5a also impairs fibrinolysis by increasing the release of PAI1 from cellular sources including mast cells and basophils. C5a activation of tissue factor leads to a pro-coagulant activity, and critical clotting factors such as factors VIIa, IXa, and XIa complex cleave the parent C3 and C5 molecules into the anaphylatoxins C3a and C5a. C5a activity causes NETs to release pro-coagulant mediators, contributing to microvascular thrombosis and endothelial damage. C, complement (a suffix indicates activated complement); F, factor; FDP, fibrinogen degradation products; IFN, interferon; IL, interleukin; MAC, membrane attack complex; PAI1, plasminogen activator inhibitor 1; tPA, tissue plasminogen activator; TNF, tumour necrosis factor; uPA, urokinase

Tissue factor binds factor VII, generating factor Xa, thrombin, and fibrin (see Figure 1), resulting in a net pro-coagulant state in the microvasculature of many critical organs. Circulating blood glycoproteins such as fibronectin, fibrinogen, and von Willebrand factor also bind NETs following C5a-stimulated release from neutrophils. This fibrinous lattice contributes to further platelet aggregation; coagulation; and, eventually, thrombus formation.[11]

Further serine proteases from the lectin pathway also amplify complement activation. Mannan-binding lectin serine protease 1 (MASP)-1 cleaves complement C2 and C4 following virus infections

and is also able to cleave fibrinogen and factor XIII and therefore activate coagulation.[43] MASP-1 also activates MASP-2, which can bind to the surface of mannose, and other carbohydrates on the surface of bacteria, fungi, and viruses to facilitate phagocytosis. It also amplifies complement activity by cleaving C4 and C2.[14] Highly pathogenic viruses such as H5N1 and H3N2 also interact directly with endothelial cells to promote a pro-coagulant, pro-inflammatory microvascular state by causing plasma exudation, increasing tissue factor levels and activating other inflammatory cells.[46-48]

Activated platelets have an essential, although less frequently recognized, role in innate immunity following virus infection.[49-51] They attract T cells, B cells, dendritic cells, and neutrophils through cell signaling, by the release of pro-inflammatory mediators such as C–C motif chemokine ligand 5 (CCL5; also known as RANTES) and IL-1β,[52] or by direct interactions with adhesion molecules and their mutual ligands on inflammatory cells such neutrophils.[53] For example, P-selectin is a C3b-binding protein on activated platelets that results in increased C3a, C3b, and C5b-9 (MAC) production following infection.[54] Continued activation of platelets by virus infection can then lead to intravascular thrombosis, with paradoxical thrombocytopenia as a result of exhaustion of platelet stores.[55] Inhibition of protease-activated receptor-1 reduces platelet overactivation by thrombin in mice infected with influenza A, reducing inflammation and improving survival.[56]

Complement can also inhibit fibrinolysis. Thrombin cleaves C5 to generate C5a, which increases the activity of the serine protease plasminogen activator inhibitor 1 (PAI1) on mast cells, basophils, endothelial cells, and fibroblasts. PAI1 inactivates both tissue plasminogen activator and urokinase, preventing the conversion of plasminogen to plasmin, the active fibrinolytic enzyme.[11]

These observations suggest several mechanisms by which virus infections may activate coagulation and thrombotic microangiopathy through interactions with complement and pro-inflammatory cells and cytokines.

7 | COMPLEMENT INHIBITION REVERSES COMPLEMENT-ACTIVATED THROMBOTIC MICROANGIOPATHY

Two rare but potentially lethal human diseases are associated with complement overactivation and are associated with the dysfunctional coagulation and thrombotic microangiopathy seen in COVID-19.[14] In atypical hemolytic uremic syndrome (aHUS), defects in the alternative complement pathway (sometimes genetically determined) lead to excessive complement activation, causing systemic thrombotic microangiopathy and thrombocytopenia. Renal tissue is particularly susceptible to deposition of circulating active complement fragments in the glomeruli, resulting in significant inflammation-induced tubular and microvascular injury[57-59] and, ultimately, renal failure. Inhibition of C5 with a humanized monoclonal antibody has been shown to reverse the renal failure and reduce the progression of thrombotic microangiopathy in patients with aHUS.[60]

Paroxysmal nocturnal hemoglobinuria (PNH) is a genetic disorder due to rare mutations that result in the absence of complement inhibitory proteins. Uncontrolled complement production by the alternate pathway causes destruction of red blood cells, and intravascular hemolytic anemia and disordered coagulation. Complement lysis of white cells increases tissue factor in plasma that also leads to thrombosis.[61] Thus, PNH is a chronically disabling disease with poor outcomes. However, as in aHUS, anti-C5 approaches have transformed the prognosis.[62] A humanized anti-C5 antibody treatment for example significantly reduces intravascular hemolysis and hence transfusion requirements, while also improving symptoms and quality of life.[63]

Experience in these two rare diseases indicates that C5 inhibition reverses thrombotic angiopathy; in addition, a significant proportion of patients have a genetic predisposition through the absence of complement inhibition, suggesting that susceptibility to microangiopathy in patients with severe COVID-19 may be due to a genetic predisposition to excessive complement activation.[5,64,65]

8 | SEVERE COVID-19 INFECTION IS ASSOCIATED WITH COMPLEMENT ACTIVATION

Several observational studies of patients with severe COVID-19 describe a systemic inflammatory response that is accompanied by a pro-coagulant state, suggested by elevated fibrin degradation products such as D-dimer, elevated platelets, and abnormally short clotting times.[66] There is strong evidence of multiple organ damage, with the lungs and kidneys most frequently affected. Patients who have died from COVID-19 have diffuse microvascular thrombi in many organs.[64]

A recent pathological study from New York reported the strong presence of complement components on immunological staining of lung tissue in five patients with purpuric rash and respiratory failure who died of COVID-19. Abnormalities of the coagulation cascade were evident in all five cases, with elevated hemolytic activity of the complement system and serum C3 and C4 (where measured).[5] Signs typical of classic ARDS were distinctly absent on histological examination.[5] Instead, significant mural and luminal fibrin deposition was seen, with neutrophil accumulation and only a minimally inflamed capillaritis. Further staining confirmed significant microvascular deposition of MASP-2, C4d, and C5b-9 (MAC); the latter two components were co-localized with COVID-19 spike glycoproteins in the lung and skin in two patients.

Further evidence comes from a study of patients who died from COVID-19 that also reported the strong presence of complement components such as MASP-2, C3 and C5b-9 (MAC) in lung tissue[67] (data only available as a non–peer-reviewed pre-print at time of writing). These support the earlier findings of Magro et al.[5] The signal was present in a variety of cells (eg, alveolar epithelial and inflammatory cells) and non-cellular sources (eg, necrotic cell debris, alveolar spaces, and exudates). These pathological changes were accompanied by elevated serum levels of C5a. MASP-2 (the serine protease that amplifies complement activation) was shown to bind the

nucleocapsid protein region of SARS-CoV-1, MERS-CoV, and now SARS-CoV-2, providing a strong link between abnormal complement activation and lung inflammation and likely other organ injuries. In confirmatory experiments, blockade of MASP-2 nucleocapsid protein and C5 (as part of a clinical trial in two patients) significantly reduced complement levels and lung injury.[67] These findings are also consistent with reports from New York.[5]

9 | INHIBITION OF C3 AND C5a REDUCES ACUTE LUNG INJURY DURING SIGNIFICANT VIRUS INFECTION

The evidence to date for the potential benefits of C5a blockade in reducing lung injury following virus infection comes from animal models. Nomacopan, a dual C5 and leukotriene B4 (LTB4) blocker, was more effective than the selective LTB4 blocker zileuton in reducing neutrophil, macrophage, and lymphocyte infiltration in mice infected with H1N1 influenza.[68] Nomacopan also improved survival when combined with the antiviral oseltamivir.[68] C5a blockade reversed ALI in mice infected with H5N1 influenza[69] and in monkeys infected with H7N9 influenza.[70] C5aR and C3aR inhibition reduced an array of pulmonary effects following intravenous injection of cobra venom in rodents, such as neutrophil accumulation, vascular permeability, and release of pro-inflammatory cytokines.[71] Nomacopan also significantly reduced levels of chemokines and cytokines, including IL-1α, IL-1β, IL-2, IFN-γ, IL-6, IL-10, IL-17, TNF, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein (MIP) 1α and -1β (reported to be raised in ARDS[1]) in a murine model of septic ARDS.[72] AMY-101, which blocks both C3a and C5a, prevents IL-6 release from cells such as macrophages expressing the C3aR.[62]

10 | THERAPEUTIC BENEFITS OF C5 BLOCKADE IN COVID-19

Complement-modulating treatments have an established role in the management of rare disorders such as aHUS and PNH[62] and are being investigated in the treatment of other disorders such as bullous pemphigoid, hidradenitis suppurativa, renal transplantation, and transplant-associated microangiopathies.[73] Anti-C3 and anti-C5 approaches show the most significant promise. Eculizumab (Solaris®, Alexion Pharmaceuticals) and nomacopan (Akari Therapeutics) are C5 inhibitors that have demonstrated efficacy in clinical trials in aHUS and PNH.[62] Eculizumab provided additional benefits as it also blocks LTB4. Treatment with eculizumab[74] and IFX-1 (InflaRx),[75] another anti-C5a monoclonal antibody, has recently been reported in a small number of patients with severe COVID-19, initially through a compassionate use program followed by research.[74] These trials are laudable and welcome efforts, although it is unclear which patients are most likely to benefit as there are no data on the prevalence and intensity of complement activation in COVID-19, whether it is related to more severe disease, and which complement pathways are activated (to know the best targets). C3 inhibition is currently not reported in COVID-19, whereas C5 is an appealing target given its orchestrating role in inflammation and coagulation—the critical features in COVID-19 pneumonia.

11 | CONCLUSION

Increased complement activation has been implicated in thrombotic microangiopathy, systemic inflammation, and hypercoagulation in rare disorders and pathogenic virus infections, now including severe COVID-19 that is not associated with classical ARDS. Complement inhibition may offer an effective treatment in patients with COVID-19.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

AJ Chauhan is lead author and guarantor of the review. He developed the idea, managed the references, drafted the article, and completed the figure. LJ Wiffen and TP Brown provided critical revisions. All authors provided interpretation and critical review of the article and gave their final approval of the version to be submitted.

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SEARCH STRATEGY

References for this review were identified through searches of PubMed for articles published from 1980 to May 2020, by use of the terms in the table in combination. Replacing SAR-CoV-2 with COVID-19 did not retrieve any significant additional references. We included articles published in English.

COVID-19 alone	>8000
COVID-19 and complement	25
COVID-19 and coagulation	71
COVID-19 and ARDS	150
COVID-19 and cytokine storm	86
COVID-19 and thrombosis	69
COVID-19 and microangiopathy	3
COVID-19 and acute lung injury	40