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Complement cascade in severe forms of COVID-19: Recent advances in therapy

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The complement system is an essential component of the innate immune system. The three complement pathways (classical, lectin, alternative) are directly or indirectly activated by the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). In the most severe forms of COVID-19, overactivation of the complement system may contribute to the cytokine storm, endothelial inflammation (endotheliitis) and thrombosis. No antiviral drug has yet been shown to be effective in COVID-19. Therefore, immunotherapies represent a promising therapeutic in the immunopathological phase (following the viral phase) of the disease. Complement blockade, mostly C5a-C5aR axis blockade, may prevent acute respiratory distress syndrome (ARDS) from worsening or progression to death. Clinical trials are underway.

Keywords: COVID-19 · SARS-CoV-2 · complement · C5a · cytokine storm

Introduction

We are currently in the grip of a pandemic of coronavirus disease 19 (COVID-19), a disease caused by infection with a new coronavirus, SARS-CoV-2, with devastating consequences for health and the economy. Less than a year since its appearance in China in the winter of 2019, more than 30 million cases and more than a million deaths have been recorded. In more than 85% of cases, COVID-19 is a benign disease. The vast majority of patients are asymptomatic or present a paucisymptomatic illness characterized by fever, a flu-like syndrome, anosmia (a loss of the sense of smell) and ageusia (a loss of the sense of taste). However, in 10 to 15% of cases, hospitalization is required, mostly due to hypoxemic pneumonia. The most severe form of the disease is severe acute respiratory distress syndrome (SARS), necessitating the admission of the patient to an intensive care unit (5 to 10% of hospitalized patients). An understanding of the pathophysiology of the disease, and particularly of its severe forms, is essential for the development of targeted treatments capable of attenuating the respiratory exacerbation.

The complement system was discovered at the end of the 19th century. It is part of the innate immune system and can be activated by three different pathways: the classical antibody dependent pathway, the lectin pathway and the alternative

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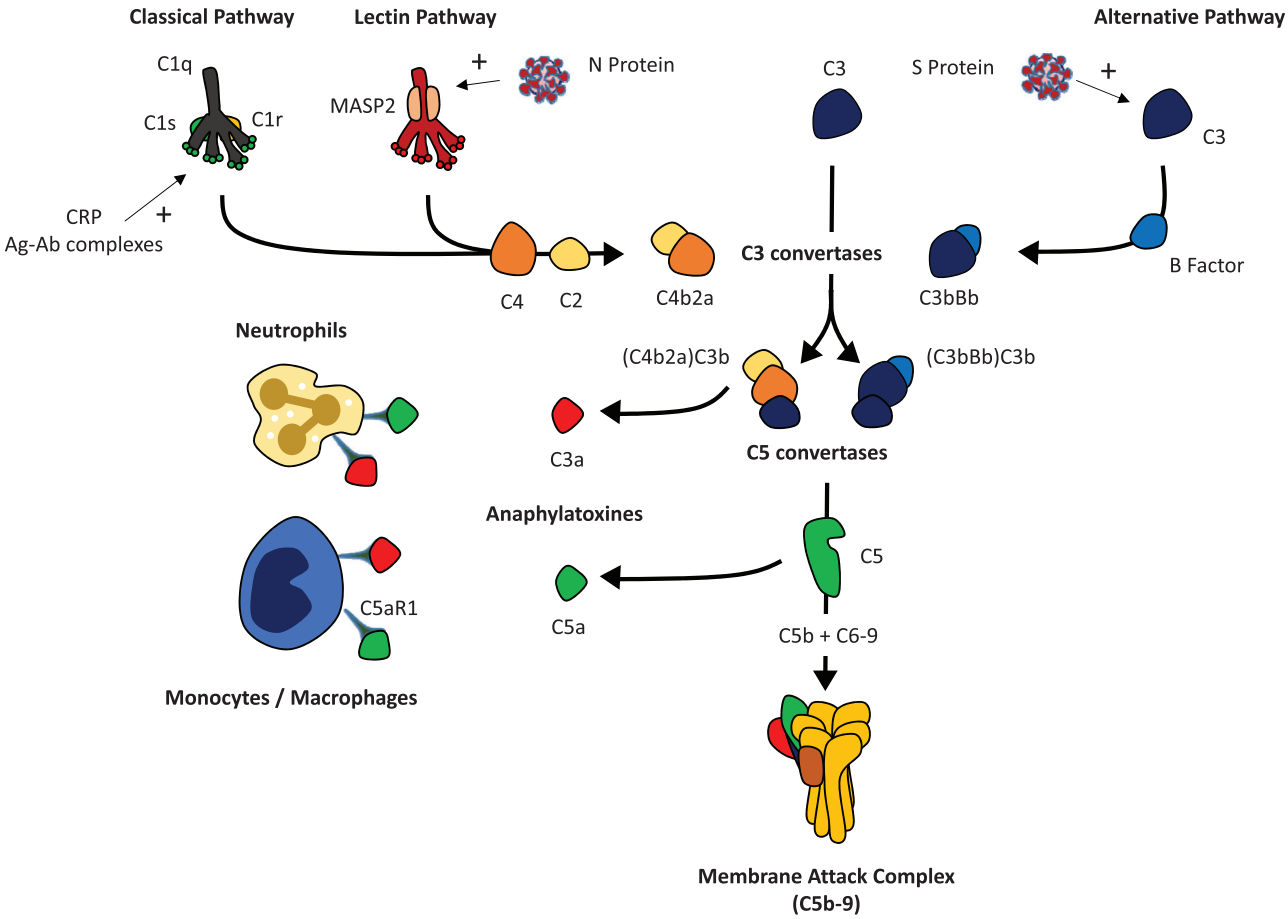


Figure 1. The complement system. The classical pathway is initiated by the binding of the C1 complex to antibodies or endogenous ligands. This complex cleaves C4 and C2 to form the classical C3 convertase (C4b2a). The lectin pathway is activated by the binding of the MBL-MASP complex to the pathogen surface. This complex mediates the cleavage of C4 and C2 and thus lead to the generation of a C3 convertase (C4b2a) identical to that formed in the classical pathway. The alternative pathway acts as a surveillance system, maintaining a low level of activation of the system through a process known as "tickover". Tickover is the spontaneous hydrolysis of C3 to generate C3(H_2O) in the fluid phase. C3(H_2O) can bind to factor B, which is then cleaved by factor D to form the alternative C3 convertase (C3bBb). These three pathways lead to the formation of a C3 convertase capable of cleaving C3 to generate C3a, an anaphylatoxin, and C3b, leading to the formation of the C5 convertase (C4b2a3b or C3bBb3b). C5 is then cleaved to generate C5a, an anaphylatoxin, and C5b, which initiates the assembly of the terminal complement pathway. This terminal pathway leads to the formation of the membrane attack complex (MAC, C5b-9), which is responsible for pathogen lysis. The complement system is tightly regulated by serum (C1inh, FI, C4BP, FH, clusterin, vitronectin) and membrane (CR1, MCP, DAF, CD59) proteins. MBL-MASP: mannose-binding protein-mannose-binding lectin serine protease; C1inh: C1 inhibitor; C4BP: C4-binding protein; MCP: membrane cofactor protein; DAF: decay-accelerating factor.

pathway (**Figure 1**). The complement cascade is a succession of proteolytic reactions with a common endpoint: the formation of a C3 convertase, which cleaves the key product of the system, C3, to generate C3a and C3b. The features of this cascade are: (i) the formation of the final product, C5b-9 or the membrane attack complex (MAC), which is directly involved in pathogen elimination, and (ii) the release of anaphylatoxins: C3a and C5a. These molecules facilitate the recruitment of myeloid cells (monocytes/macrophages, polymorphonuclear neutrophils) to the site at which the cascade is occurring. These cells participate in the local innate immune response and inflammation.

Certain proteins regulate the complement cascade (e.g. C1 inhibitor, factors H and I, CD55, CD59), attenuating any activation that might prove deleterious. Like the immune system, complement plays an ambivalent role in human disease. It is essential to combat infections, but its disproportionate activation causes tissue lesions, as observed in lupus, vasculitis and thrombotic microangiopathy, for example.

The complement system, and the anaphylatoxin C5a, seems to play a major role in the severe forms of COVID-19, as discussed in detail in this review.

General pathophysiology of severe forms of COVID-19

Schematically, COVID-19 has two successive phases: an initial viral invasion phase followed by a second phase, the immunopathological phase, involving an uncontrolled immunological response causing pulmonary, and sometimes systemic, inflammation (**Figure 2**).

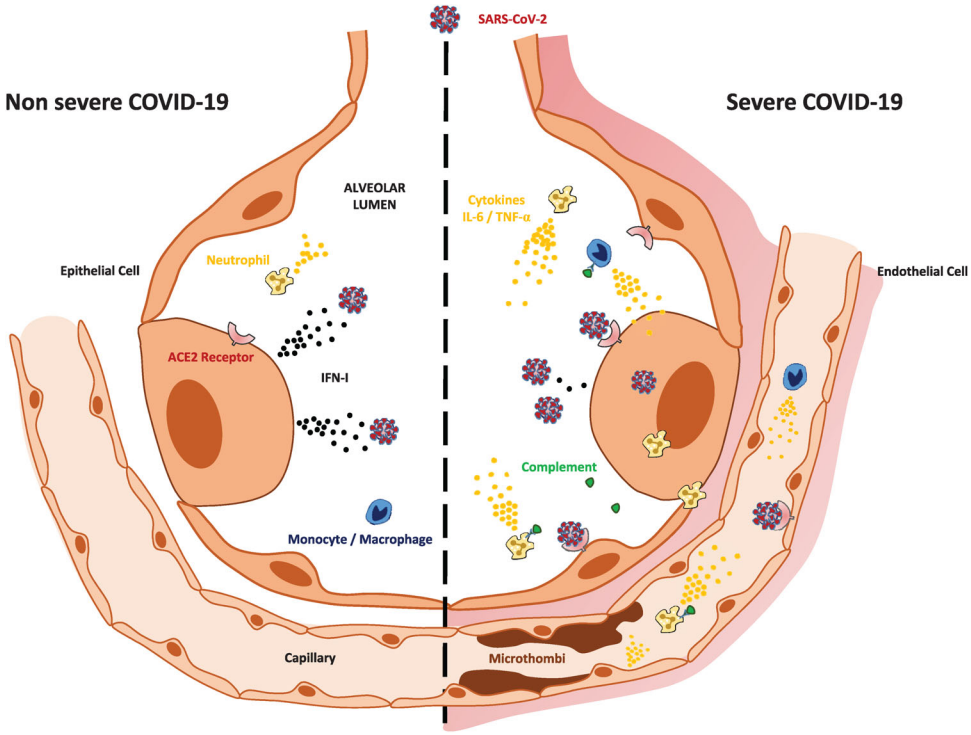


Figure 2. Model of COVID-19 immunopathology: progression to severe COVID-19 is associated with transition from an epithelial to an endothelial disease.
-Non severe COVID-19 is characterized by rapid viral clearance and efficient interferon (type I interferon - IFN-I) response. Epithelial involvement remains isolated (epithelial disease).
-In severe COVID-19, SARS-CoV-2 reaches the endothelium (endothelial disease). Viral persistence overactivates complement cascade. C5a may recruit myeloid cells leading to cytokine storm. Endothelial inflammation (endotheliitis) promotes microthrombi formation.

Viral invasion phase

SARS-CoV-2 is the name of the virus responsible for COVID-19 (CO for "corona," VI for "virus," and "D" for "disease," and "19" because it was first detected in December 2019). SARS-CoV-2 is an enveloped RNA virus with a diameter of 125 nm belonging to the *Coronaviridae* family (so-named because the viruses seem to be surrounded by a "crown" when observed under the microscope), and genus *Betacoronavirus* [1]. SARS-CoV-2 has a genome sequence 80% identical to that of SARS-CoV-1 and 96% identical to that of a bat virus (RaTG13). The genome of SARS-CoV-2 contains about 30,000 bases, making it one of the longest known RNA virus genomes.

Transmission between humans occurs principally via the respiratory route (respiratory droplets), and more rarely through direct (handshakes, hugs, etc.) or indirect (contaminated surfaces) contact. The viral RNA can be detected in contaminated individuals one to three days before the appearance of symptoms, with viral load peaking on the day of symptom onset and then gradually decreasing over a period of 1–2 weeks, on average. The viral infection begins with the binding of viral particles to target cells. SARS-CoV-2 infects the lungs by binding and then entering respiratory epithelial cells (type II alveolar pneumocytes). The spike (S) protein on the surface of the virus binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the cells [2]. ACE2 is a peptidase expressed by most tissues, but particularly in the airways, including the lungs, the kidneys, brain, heart and endothelia. This accounts for the tropism of the benign ("mild" respiratory infection) and severe (pneumonia, renal insufficiency, encephalitis, myocarditis and thrombosis) forms of the disease.

The immune system can detect the virus, and may play a dual role in COVID-19. In more than 85% of cases, a proportionate immune response eliminates the virus and the patient experiences only a few mild symptoms or remains asymptomatic. In 10 to 15% of cases, the immune response is disproportionate, and too intense. An immunopathological phase thus follows the viral invasion phase, with the patient developing a severe form of the disease. Pulmonary inflammation leads to pneumonia, or even SARS. The inflammation may also affect other organs. Type I interferons (IFN-I), cytokines secreted by the epithelial and immune cells following recognition of the virus, play an important role in antiviral immunity. An impaired IFN-I response has been described in severe forms of COVID-19 [3]. Anti-IFN-I autoantibodies and genetic variants impairing the IFN-1 response have been reported [4]. No antiviral strategy (hydroxychloroquine, remdesivir, lopinavir/ritonavir) has yet been shown to be effective, but studies are currently underway to evaluate the efficacy of IFN-alpha and IFN-beta treatments during the viral invasion phase, for improving virus clearance and preventing progression to a severe form (the immunopathological phase) [5].

Immunopathological phase and the cytokine storm

Excessive inflammation in response to viral infection is responsible for the serious forms of the disease, ranging from hypoxemic pneumonia to SARS, sometimes with the failure of other organs. This reaction highlights the need for a "correct balance" of immune responses to infection: the infection spreads in cases of immunodeficiency, on the other hand, if the immune response is excessive, hyperinflammation leads to organ lesions. This excessive inflammation is often described as a "cytokine storm" [6]. Pro-inflammatory cytokines are first released by the innate immune system (principally by myeloid cells: monocytes/macrophages/polymorphonuclear neutrophils), following the recognition of the virus by their receptors. The viral motifs (known as pathogen-associated molecular patterns, or PAMPs) are recognized by pathogen recognition receptors (PRRs). For example, viral RNA can be recognized by Toll-like receptors 7 and 8 (TLR7 and TLR8). Once the PRRs have been activated by the virus, an intracellular cascade is triggered, leading to the expression of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1β [7]. IL-6 is a cytokine synthesized by immune cells, but also by other types of cell, including endothelial cells. It activates innate immune cells, such as polymorphonuclear neutrophils and monocytes, and the cells of the adaptive immune system, including Th17 lymphocytes and follicular Th cells. IL-6 is useful for the immune response, but may be harmful if secreted in excessive amounts, causing inflammation and tissue lesions [8]. Not only is the cytokine storm associated with severe forms of COVID-19, but its persistence during the disease is also correlated with the disease severity [9].

If the immune system fails to eliminate the virus at the epithelial barrier, the viral invasion progresses toward the endothelium. The endothelial cells bear ACE2, which act as a receptor for the S protein of SARS-CoV-2 [10]. The resulting endothelial inflammation is known as endotheliitis or endothelialitis [11, 12]. Endotheliitis is associated with thrombotic phenomena in the context of COVID-19, such as microthromboses [13], macrovascular thromboses, and pulmonary embolism [14].

Immunotherapies targeting cytokine storm

Several immunotherapies directly targeting the cytokine storm are being evaluated for use in COVID-19. Glucocorticoids are the only drugs shown to date to have clinical benefits in terms of mortality (dexamethasone in the RECOVERY trial [15]). These molecules inhibit the transcription of genes encod-ing pro-inflammatory cytokines, such as IL-6, IL-8, and TNF-α. Immunomodulatory treatments, such as a monoclonal antibody targeting the IL-6 receptor (tocilizumab) have also been evalu-ated [16,17]. The efficacy results obtained for such treatments are discordant [12, 18], precluding their recommendation for use in routine practice. The EMPACTA study (**NCT04372186)** that evaluate the efficacy and safety of tocilizumab in 379 hos-pitalized participants with COVID-19 pneumonia, who were not receiving mechanical ventilation, claimed to have found a lesser need for orotracheal intubation in hospitalized patients, but with no improvement in survival [19, 20]. The COVACTA study (NCT04320615), evaluating the efficacy, safety, pharmacodynamics, and pharmacokinetics of tocilizumab compared with a matching placebo in combination with standard of care in 450 hospitalized patients in intensive care, showed no significant improvement in terms of respiratory problems [21]. Other targeted anti-cytokine therapies, such as anakinra, an antagonist of the IL-1 receptor, yielded no significant benefits [22]. Even if treatments could successfully attenuate the cytokine storm, this poses a problem because they may also inhibit the action of the immune system. If introduced too early in the disease (during the viral invasion phase), they might impair virus elimination, thereby worsening the prognosis.

Involvement of the complement cascade in the immunopathological phase of severe forms of COVID-19

SARS-CoV-2 activates complement

It has been shown that the complement cascade is directly activated by SARS-CoV-2. The viral N protein (encoding the nucleocapsid) activates the lectin pathway via the proteases associated with MBL-2 (MASP2) [23], while the S protein activates the alternative pathway [24]. At more advanced stages of COVID-19, the classical pathway may also be activated, by immune complexes and C-reactive protein, for example [23]. The role of the complement cascade in the elimination of coronavirus is poorly understood, but its deleterious role in the severe forms of COVID-19 is emerging.

Complement contributes to the formation of tissue lesions in severe forms of COVID-19

Mouse models of viral infection have revealed a pathogenic role of the complement cascade in the generation of tissue lesions, particularly in the lungs [25]. Complement has deleterious effects through its participation in both the cytokine storm and endotheliitis as mentioned above.

The C5a anaphylatoxin may also promote and maintain inflammation during the immunopathological phase of COVID-19. By binding to its receptor, C5aR1 (CD88), on the surface of myeloid cells, C5a mediates the recruitment of these cells to the lungs [26]. These monocytes/macophages and polymorphonuclear neutrophils then secrete pro-inflammatory cytokines (IL-6, TNF-α), which contribute to the cytokine storm. Serum and alveolar C5a concentrations are correlated with the severity of COVID-19. The macrophages found in the pulmonary lesions of patients who died from COVID-19 express C5aR1 strongly. The C3a anaphylatoxin, expressed further upstream in the complement cascade, may play the same role as C5a in COVID-19. Indeed, the

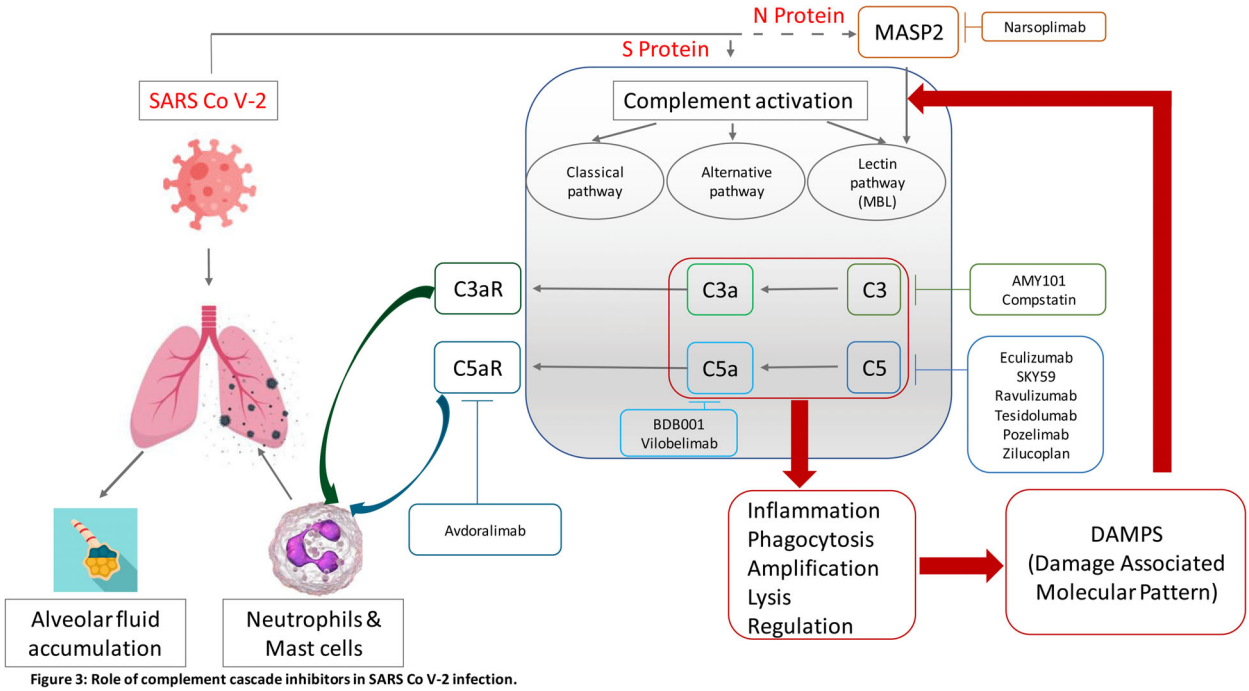


Figure 3: Role of complement cascade inhibitors in SARS Co V-2 infection.

Figure 3. The role of inhibitors of the complement cascade in SARS-CoV-2 infection. SARS CoV-2 initially affects the respiratory tract and the alveolar cells, via the ACE2 receptors. SARS-CoV-2 then may interact directly with MASP2, activating the lectin pathway. The classical and alternative pathways, which are integral parts of the innate antiviral response, are also activated and, in turn activate the C3 fraction of complement, leading to the secretion of C3a and C5a. These anaphylatoxins trigger the cytokine storm, mast cell degranulation, and the activation of leukocytes and the infiltration of these cells into pulmonary alveoli. The complement cascade is also activated via DAMPs, and it renders the lungs more fragile, following the deposition of complement proteins. The inhibition of complement proteins could decrease the severity of pathological conditions in COVID-19 patients. Adapted from ref. [45] with permission.

inhibition of C3, which blocks the generation of C3a and C5a, has been shown to decrease pulmonary inflammation [27]. C3, C5, and convertases are thus currently considered potential targets of COVID-19 [28, 29, 30]. Finally, there is evidence consistent with tissue damage mediated by both C5a and C5b-9 [13, 23, 31].

In addition to the typical features of ARDS, patients with COVID-19 present necrotizing thrombotic lesions of the pulmonary capillaries. Complement is associated with endotheliitis in severe forms of COVID-19. Histological deposits of MASP-2, C4 and MAC, and macrophages overexpressing C5aR1 have been associated with endotheliitis lesions and microthrombi [32].

Complement activation may also contribute to hemostatic activation leading to coagulopathy and explain microvascular damage, pathological features widely reported in COVID-19 [14]. Complement and coagulation (contact phase or intrinsic coagulation pathway) are closely linked [33]. In mouse models of complement regulatory protein deficiencies [34], the microvascular lesions observed are mediated by MAC, whereas the macrovascular thromboses are more dependent on the action of C5a. The mechanism by which the hyperactivation of C5a-C5aR1 induces thromboses remains unclear. The polymorphonuclear neutrophils may play a role, through their extracellular traps (NETs) [35] and the release of tissue factors (activation of the extrinsic coagulation pathway) [36].

Finally, the association of MBL with thrombotic processes in COVID-19 severe forms coagulopathy has been suggested. Thus, pharmacological targeting of the complement system in particular of the MBL pathway could be a new treatment option for thrombosis in COVID-19. Laboratory tests of MBL levels could be useful in identifying COVID-19 patients at risk for thromboembolic events [37].

Targeting complement in severe forms of COVID-19

Complement blockade in severe forms of COVID-19 would constitute a promising rationale for attenuating both the cytokine storm and endotheliitis (**Figure 3**). The complement cascade can be blocked at several different points, with the drugs listed in Table 1. The tolerance and efficacy of these drugs for this indication are currently being evaluated, and none has yet been approved for use in the treatment of COVID-19.

Anti-C3a drugs (such as AMY-101) or inhibitors of the lectin pathway are in development. Inhibition of the C3 fraction, which is more proximal than the C5 fraction, could reduce the inflammatory response [29]. Nevertheless, it could also reduce antiviral response and prevent immunity to additional infectious diseases.

Targeting the C5 fraction of complement

The complement blocker eculizumab (Soliris) is a monoclonal antibody that inhibits C5 [38]. It is also the only drug approved

Table 1. Modulators of complement in trials for COVID-19

Molecule	Target*	Clinical trials performed/underway on COVID-19 (ClinicalTrials.gov)
Monoclonal antibodies		
Narsoplimab (OMS721)	MASP-2	-
AMY101	C3	NCT04395456
Eculizumab	C5	NCT04288713, NCT04346797, NCT04355494
Ravulizumab	C5	NCT04570397, NCT04390464, NCT04369469
BDB-001	C5a	NCT04449588
Avdoralimab (IPH5401)	C5aR1	NCT04371367
Vilobelimab	C5a	NCT04333420
Peptides / Proteins		
Contestat alpha	Active C1inh	NCT04414631, NCT04530136
APL-9	C3	NCT04402060
Zilucoplan	C5	NCT04382755

by the US Food and Drug Administration (FDA) for the treatment of paroxysmal nocturnal hemoglobulinuria (PNH). This disease is linked to a mutation preventing the binding of complement-regulating proteins (CD55 and CD59) to the surface of red blood cells. PNH can only be diagnosed if more than one lineage (i.e., erythroid AND neutrophils; or erythroid AND monocytic, etc.) is affected by the loss of GPI-anchored cell surface molecules. It results in excessive red blood cell destruction (hemolysis) by C5b-9. In a non-randomized proof-of-concept study, the potential efficacy of eculizumab for the treatment of severe forms of COVID-19 was demonstrated [39]. Indeed, by binding to the human C5 fraction, eculizumab inhibits the generation of pro-inflammatory molecules C5a and C5b-9 (MAC) [38]. In pathologies where complement is deleterious, such as autoimmune diseases, the use of inhibitory drugs such as eculizumab, reduces thrombotic events occurrence. Therefore, anti-C5 therapy with eculizumab might represent a potential strategy in the treatment of patients with confirmed diagnosis of SARS-Co V-2 infection with severe pneumonia or ARDS [39]. However, its use in intensive care settings is rendered difficult by the high risk of bacterial superinfection, particularly in the respiratory system. Blocking MAC would be associated with an intolerably high risk of sepsis. The blockade of anaphylatoxins or their mechanism of action thus constitutes a preferred avenue for the development of treatments.

Besides, C5 inhibition with the C5 blocker Ultomiris (ravulizumab) does not appear to be either the answer to the search for treatments for severe Covid-19. Enrollment in a Phase III study testing Ultomiris, among patients requiring mechanical ventilation has been paused after the independent data monitoring committee raised a lack of efficacy in an interim analysis. Among 122 patients (out of a planned enrollment of 270), there was no meaningful difference in survival at Day 29 [40].

Blocking the C5a-C5aR1 axis

To limit severe COVID-19 pathologies and reduce the risk of bacterial complications, one approach is to block the pro-inflammatory action of anaphylatoxin C5a without blocking MAC. At the start of the epidemic, a Chinese team reported an improvement in two patients with severe COVID-19 treated with the monoclonal antibody vilobelimab, which blocks C5a [41]. The PANAMO study (NCT04333420) highlighted the efficacy and tolerance of vilobelimab in severe forms of COVID-19 [42]. Even if the secondary outcome results in favor of vilobelimab are preliminary, they still support a phase 3 trial where C5a inhibition is investigated, using 28-day mortality as the primary endpoint [42]. The C5a antagonists vilobelimab and PMX-53 thus constitute therapeutic prospects for ARDS in SARS CoV-2 infection [43].

A randomized, controlled, double-blind trial (FORCE, *NCT04371367*) is evaluating the tolerance and efficacy of avdoralimab, a monoclonal antibody blocking the receptor of C5a, in severe forms of COVID-19 [44]. *In vitro*, avdoralimab attenuated the cytokine storm. *In vivo*, in a mouse model of acute lung injury, it decreased neutrophil and macrophage infiltration in the lungs.

Very recently, SARS-CoV-2 specific monoclonal antibodies have been used for treating patients with severe COVID-19 pneumonia as soon as they show a need for oxygen therapy $\geq$5 L/min or high flow oxygen therapy, or in COVID forms at ARDS stage, hospitalized in intensive care under high flow oxygen therapy, or invasive mechanical ventilation.

The results of the studies exploring how patients at risk and eligible for such therapies are still under analysis.

Conclusion

The complement cascade may play an important role in the immunopathological phase of COVID-19. It may participate in the cytokine storm, endothelial lesions and thromboses, through the action of anaphylatoxins, including C5a in particular. Clinical trials targeting the C5a-C5aR1 pathway are currently being evaluated in severe forms of the disease.

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Conflict of interest: Eric Vivier is Innate Pharma employee.

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