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Implications of Complement Imbalance in COVID-19: A Molecular Mechanistic Discussion on the Importance of Complement Balance

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ABSTRACT

Two central questions in COVID-19 treatment which should be considered are: "How does the imbalance of the complement system affect the therapeutic approaches?" and "Do we consider complement inhibitors in therapeutic protocols? ". The complement system is a double-edged sword since it may either promote immune responses against COVID-19 or contribute to destructive inflammation in the host. Therefore, it is crucial to regulate this system with complement inhibitors. In this manuscript, we discuss the molecular mechanisms of complement and complement inhibitors in COVID-19 patients. We searched the terms "COVID-19", "Complement", "Complement inhibitor", "SARS-CoV-2", and all complement fragments and inhibitors from 2000 to 2022 in PubMed and google scholar and checked the pathways in "KEGG pathway database". Complement is not well-appreciated in the treatment protocols despite its multiple roles in the disease, and most of the preventive anti-inflammatory therapeutic approaches did not include a complement inhibitor in COVID-19 therapeutic protocols. In this review article, we discussed the most recent studies regarding complement components mediated interventions and the mechanism of these interventions in COVID-19 patients. Since the control of the complement system overactivation is associated with a better prognosis in the initial stages of COVID-19, heparin, anti-thrombin, C1-inhibitor, montelukast, and hydralazine can be effective in the initial stages of this viral infection. Recombinant complement activation (RCA) proteins are more effective in regulating complement compared to terminal pathway therapeutic approaches such as the C3a and C5a inhibitors.

Keywords: Complement System Proteins, Complement Inactivating Agents, Severe Acute Respiratory Syndrome Coronavirus 2

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an inflammatory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) and initially appeared in December 2019 in Wuhan, China (1). Since June 29th 2021, there have been recorded more than 181 million cases with SARS-CoV-2 infection and approximately 4 million COVID-19 fatalities. These reports make SARS-CoV-2 an important subject for further studies (2). This virus imbalances several mechanisms such as renin–angiotensin–aldosterone system (RAAS), inflammatory response, complement, and coagulation (3). The complement system is an important mediator of innate immune response over-activated in the lungs of COVID-19 patients. Gao et al. suggested that the N protein induces lung injury via activating mannose-binding protein-associated serine protease 2 (MASP-2) in the mannose-binding lectin (MBL) pathway of this system (4, 5). Further studies indicated higher tissue expression of MBL in COVID-19 patients, confirming the activation of the complement via the lectin pathway (6). Another research conducted by Satyam et al. reported the accumulation of C1q, C4d, Factor H, and C3d, which are the components of classical and alternative pathways, in the lung tissue of COVID-19 patients (7). Additionally, the spike protein stimulates the alternative pathway of the complement directly (8). There is an increasing concern that the complement overactivation plays an essential role in lung and other organ dysfunction in COVID-19 patients. The complement components play an essential role in the development of diffused thrombotic microangiopathy (TMA), organ dysfunction, and thrombocytopenia in COVID-19 patients (3). In several infectious diseases such as Kawasaki disease the complement is activated via MASP-1, just like SARS-CoV2. MASP-1 activates complement in COVID-19 patients, particularly in young ones. Intravenous immunoglobulin (IVIG) treatment is effective in Kawasaki via interfering MASP-1. Based on these findings, IVIG might be a useful therapeutic approach in COVID-19 patients (9). Several studies and clinical trials were done in COVID-19 patients, for instance, Synopsis Inhaled budesonide which is a selective glucocorticoid and is used to manage adult and childhood asthma (10). Synopsis Inhaled budesonide remains in the airways as ester so it can be considered as a synthetic glucocorticoid and it has been confirmed for use once a day in asthma (11). Additionally, it decreases vascular endothelial growth factor secretion and expression in the lung tissue. Interestingly, vascular endothelial growth factor (VEGF) increases in COVID-19 patients in a severe stage which is an alarm for their microvascular dysfunction (12). A study in France supported the efficacy of intravitreal (IVT) anti-vascular endothelial growth factors (anti-VEGF) in the COVID-19 (13). However, a specific and efficient therapeutic protocol based on the complement management could not be presented yet. Based on previous studies, the management of the complement activation is very essential in COVID-19. Notably, inhibiting the complement system potentially reduces the immune system's ability against various viral and bacterial pathogens. This is another interpretation of the complement system in COVID-19 as a double-edged sword. Hence, identifying the specific behaviors and functions of different complement mediators in COVID-19 could be the key to developing effective and novel therapeutic strategies. In this review, we present an update on the molecular pathways of the complement activation and the mechanisms of specific medicines that target the complement components in COVID-19. Furthermore, we discuss the probable choices for developing therapeutic protocols for COVID-19.

Molecular Mechanism of the Complement Activation in COVID-19

The complement cascade is initiated via various pathways in COVID-19. N protein

activates MASP-2 via MBL pathway (3), leading to C2 and C4 cleavage just like a classical pathway, and C3 convertase (C4b2a) assembling taking place. C3 convertase cleaves C3 to C3a (anaphylactic and chemotactic component) and C3b, assembling C5 convertase (C3bC4b2a). C5 convertase breaks down the C5 component to C5a (anaphylactic and chemotactic component) and C5b which is the initiator element of complement membrane attack (MAC) of the complement, also known as C5b-9 (14). Studies on the complement pathways indicated that MASP-2 interacts with MASP-3 and then affects factor B associated with C3, then it will be cleaved by factor D into Ba and Bb in an alternative pathway. Finally, this sequence leads to the complement cascade signals' continuation (15). We should emphasize this important point that the Bb fragment can cleave additional C3, and once C3b is produced, it binds to factor B and generates more C3-convertase. Additionally, lectin and classical pathways can initiate alternative pathway via C3b generation binding to factor B. Once we ,deeply, focus on the complement pathways, we will find out that C3 is embedded at the core of the complement pathways (16). Although MBL binding and lectin pathway activation by the N-protein has drawn some attention, MBL levels in human blood are really low, generally about 1 g/mL, lower than those of the other complement proteins like C3 or C4. The total activation of the complement during SARS-CoV-2 infection in humans may thus not be greatly influenced by MBL binding and lectin pathway activation by the N-protein alone. The complement activation and immunological dysregulation during COVID-19 may, however, be influenced by additional complement activation pathways, such as the alternative route. (17, 18). Note that additional lectins such as ficolins and collections, as well as the other complement proteins and regulators, may still be active in the complement reaction to SARS-CoV-2 even if MBL may not play a substantial role in the complement activation in COVID-19 (18, 19).

The classical pathway is activated by immunoglobulin (Ig) M, specific isotypes of IgG that are fixed to complement, and several proteins such as the C-reactive protein. Then the interaction of this complex with C1q initiates the classical pathway of the complement cascade (20). Additionally, factor XII can cleave C1s and lead to the activation of classical complement pathway in COVID-19 patients (21) (Fig. 1).

Implications of the Complement Overactivation in COVID-19

The complement has been defined as an essential effector for the clearance of apoptotic cells in radiotherapy, which might play an anti-inflammatory and tolerogenic role. Based on a murine study, using tumor-targeted complement inhibitors during radiotherapy improves the outcome noticeably, which might be linked to balancing apoptotic cells and inflammation (22). Several studies on the mouse model confirmed the activation of the complement cascade in the lung as early as the first day in SARS-CoV (23). Lage et al. conducted a study on circulating blood monocytes of COVID-19 patients and discovered that the number of monocytes increased in COVID-19 patient. During an acute infection, the levels of monocyte membrane-bound C1q, C3, and CD55 correlated with plasma inflammatory indicators such as C-reactive protein (CRP) and serum amyloid A. So this finding reflects the fact that systemic complement activation is linked to monocytes in various ranges of severe COVID-19 disease (24). Additionally, SARS-COV-2 induces endothelial inflammation and dysfunction and affects various organs such as the lungs, intestine, cardiac system, heart, and kidney. The elevated endothelial injury biomarkers are detectable in severe COVID-19 patients and molecular biomarkers of endothelial dysfunction, such as the von Willebrand factor (vWF), have been found to increase in the acute respiratory distress syndrome (ARDS) condition in COVID-19 patients (25). Endothelial dysfunction may

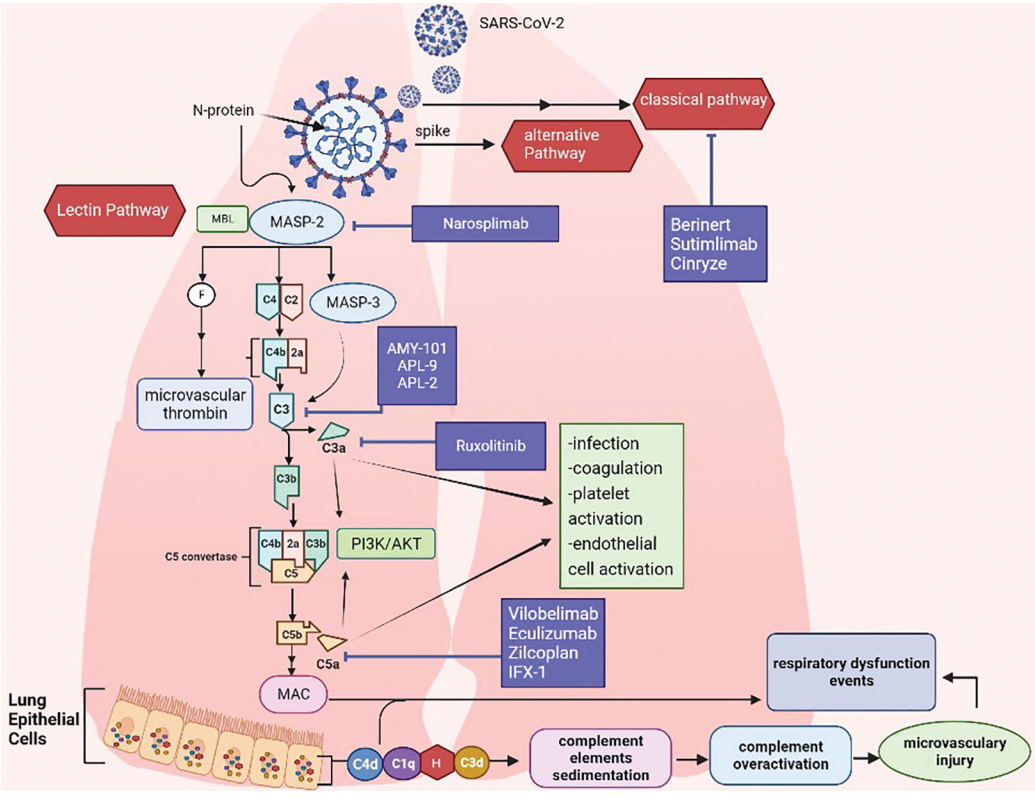

Fig. 1. The complement cascade is initiated via various pathways in COVID-19. N protein activates the MBL pathway. Then it leads to C2 and C4 cleavage just like the classical pathway, and C3 convertase (C4b2a) assembling occurs. C3 convertase cleavage C3 to C3a (Anaphylactic and chemotactic component) and C3b, which assembles C5 convertase (C3bC4b2a). C5 convertase breaks down the C5 component into C5a (Anaphylactic and chemotactic component) and C5b, the initiator element of MAC of the complement, also known as C5b-9. MASP-2 interacts with MASP-3 and then affects factor B associated with C3, then it will be cleaved by factor D into Ba and Bb in an alternative pathway. The lectin and classical pathways can initiate the alternative pathways via C3b generation binding to factor B. The classical pathway is activated by IgM, specific isotypes of IgG that are fixed to the complement, and several proteins such as C-reactive protein. Then the interaction of this complex with C1q initiates the classical pathway of the complement cascade. MASP1/2 can interact with thrombin (F2) and leads to microvascular thrombin production. C3a and C5a increase inflammation. Mannose-binding lectin (MBL); Complement membrane attack (MAC); Immunoglobulin (Ig); Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Mannose-binding protein-associated serine protease (MASP); Phosphatidylinositol 3-kinase /protein kinase B (PI3K/AKT)

be explained by undiagnosed organ damage such as multifocal individual cardiomyocyte injury, fibrinoid necrosis of small vessels, and areas of perivascular inflammation in the lungs and intestine. Hemorrhage in organ failure during viral infection is caused by endothelial dysfunction. Hemorrhage leads to microvascular leak, inflammation, a pro-coagulant condition, and organ ischemia (26). Numerous investigations have revealed a tight connection between organ dysfunction and cytokine release syndrome (CRS), also known as cytokine storm. One of the vital factors is IL-6, whose level is strongly connected with the disease's severity. The molecular processes behind CRS in COVID-19 are influenced by the actions of the virus S- and N-proteins as well as their capacity to activate nuclear factor kB (NF-kB) by blocking its inhibitory components. The interaction of IL-6 with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon responses are two additional pathways connected to IL-6. Since IL-6 plays such an important role, tocilizumab trials are precious studies (27).

Several studies on SARS-CoV-2 confirmed that complement overactivation is the key player in respiratory dysfunction events. sC5b-9 complex and C4d extremely enhanced in COVID-19 patients with respiratory complications and C4d, sC5b-9, and C5a associate with antiviral antibodies, but not with viral load (28). C3 is the central component of the complement system. Studies on C3 ($C3^{-/-}$) mice indicated significantly less respiratory failure despite the equal viral loads in the lung of the mouse models for SARS-CoV infection. Remarkably fewer polymorphonuclear neutrophils (PMNs) and inflammatory monocytes were detected in the lungs of $C3^{-/-}$ mice in comparison with their control model, and further studies confirmed the lower cytokine levels in both the lungs and the serum of $C3^{-/-}$ mice. This study is only a small piece of great evidence that complement inhibition can be an essential therapeutic approach in coronavirus infection (23). C3a is a critical component of the complement in COVID-19 patients and can be inhibited by Ruxolitinib which is a JAK1/2 inhibitor. Ruxolitinib can regulate all complement gene transcripts so it can potentially be applied in severe COVID-19 cases (29). Recent next-generation sequencing studies on COVID-19 patients identified critical variants in the complement system related to severe COVID-19. These variants are rs2547438 (C3), rs2250656 (C3), rs1042580 (THBD), rs800292 (CFH) and rs414628 (CFHR1). This study reveals the impact of the complement on the COVID-19 (30). Additionally, C3b which is a section of the C3, after the engagement of factor B, starts the alternative pathway. Complement receptor immunoglobulin (CRIg) is a phagocytosis mediator that exists on the macrophage surface and has an inhibitory effect on alternative pathway via binding to C3b and inhibiting convertase activation. This knowledge can help us develop novel therapeutic approaches targeting complement (31). C5a is a chemotactic fragment for PMNs, eosinophils, and macrophages, and it can increase the risk of ARDS and pulmonary dysfunction by increasing PMN aggregation. C-activated plasma is a good indicator of PMN-aggregating activity, reflecting C5a levels. Interestingly, C5a might be a good predictor of ARDS, and corticosteroids are shown to decrease PMN aggregation in ARDS (32). ARDS was reported in several cases of COVID-19 disease. This pulmonary complication was followed by a significant amount of terminal complement components C5b-9 (membrane attack complex), C4d, and MASP-2, in the microvasculature, reflecting the systemic activation of the complement pathways. Furthermore, the co-localization of COVID-19 spike glycoproteins with C4d and C5b-9 was reported in the interalveolar septa and the cutaneous microvasculature of several patients of COVID-19. So complement overactivation can lead to microvascular injury in severe COVID-19, via its crosslink to coagulation pathways (33). Furthermore, the anaphylactic and chemotactic components, C3a and C5a, are potent effectors of acute lung injury, so the pharmacological inhibition of their receptors (C5aR and C3aR) decrease pulmonary inflammation (34). C3a and C5a mediate critical actions such as inflammation, coagulation, platelet activation (which is the nexus between inflammation and coagulation), leukocyte recruitment, and endothelial cell activation in COVID-19 (35) (Fig. 2).

C3a

C3 is a key component of the complement system, as it is where all three complement activation pathways merge to generate C5 convertase (36). Along with its downstream element, C5a, the complement activation product C3a is frequently referred to as a pro-inflammatory mediator. However, new research suggests that C3a has an anti-inflammatory effect based on *in vivo* studies. C3a acts in direct opposition to C5a in the acute inflammatory response, avoiding the build-up of neutrophils in injured tissues by independently controlling their mobilization (37). Respiratory virus-induced over-

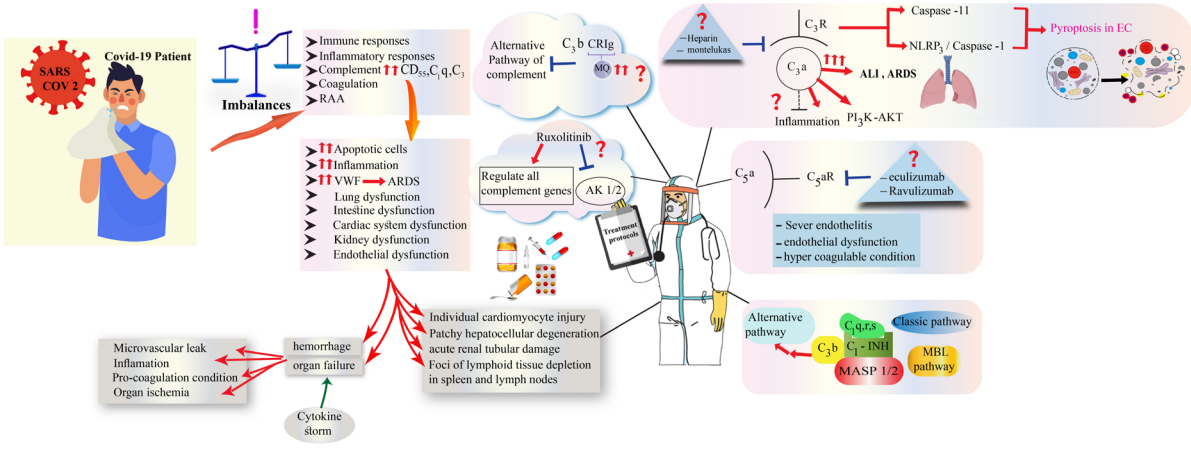

Fig. 2. COVID-19 patients face various imbalances in various systems such as immune responses, complement, and coagulation. This imbalanced condition has various consequences such as ARDS, lung dysfunction, intestine dysfunction, kidney dysfunction, and epithelial dysfunction that lead to complicated conditions such as individual cardiomyocyte injury, patchy hepatocellular degeneration, and organ failure. The complement has an impressive role in complicated conditions and physicians should handle the condition by balancing the immune responses especially the complement activation. Here, we suggest several interventions which can be useful in balancing immune responses in COVID-19 patients and they can empower COVID-19 therapeutic protocols. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Renin–angiotensin–aldosterone (RAA); Acute lung injury (ALI); Acute respiratory distress syndrome (ARDS) ; Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3); Complement receptor immunoglobulin (CRIg); Coronavirus disease-2019 (COVID-19); Phosphatidylinositol 3-kinase /protein kinase B (PI3K/AKT); Epithelial cell (EC); Complement component 3 receptor (C3R); Mannose-binding protein-associated serine protease (MASP); Mannose-binding lectin (MBL); C1 esterase inhibitor (C1-INH)

inflammatory reactions, often known as "cytokine storm," are caused primarily by immunological dysregulation manifested as pro-inflammatory cytokine production. Peroxisome proliferator-activated receptors (PPAR) play a crucial role in antagonizing fundamental inflammatory pathways such as NF-kB, AP1, and STAT, in addition to influencing lipid and glucose metabolism. Their function in controlling inflammatory responses induced by lung pathogens is becoming more well-known, paving the way for new anti-diabetic and lipid-lowering therapeutic uses (38). C3a inhibits PPAR-dependent activation of CD36, FABP4, and LXR genes, as well as the response to the LXR ligand TO901317, via the ERK1/2 signaling pathway (39), and the main point regarding the CD36, FABP4, and LXR genes is that they have anti-inflammatory functions. Based on these facts C3a inhibitors can have anti-inflammatory effects (40, 41). C3a levels are much higher in acute lung injury (ALI), and is linked to organ failure and poor prognosis in sepsis. Recent studies indicated that the C3a–C3aR axis inhibition might prevent pyroptosis in pulmonary epithelial cells (ECs) by blocking both the NLRP3/caspase-1 and caspase-11 pathways. These findings suggest that inhibiting the C3A–C3AR complement axis might prevent pulmonary vascular EC pyroptosis, which could be a therapeutic target for ALI (42). Additionally, several studies indicated that C3a-C3aR signaling activates PI3K-AKT signaling pathway (43). In a recent investigation of SARS-CoV, which is closely linked to SARS-CoV-2, researchers discovered that activating complement component C3 worsens symptoms in SARS-CoV-associated ARDS. Despite equal virus loads in the lungs, C3-deficient animals infected with SARS-CoV showed reduced respiratory dysfunction, which was linked to decreased lung infiltration of neutrophils and inflammatory monocytes, as well as lower levels of cytokines and chemokines in the

lungs and sera. This shows that inhibiting C3 may also help with SARS-CoV-2 infection's inflammatory lung consequences (44). Additionally, C3a plays a critical role in the pathophysiology of infection-related lung injury, and a high C3a level in the blood can be used as a predictive element for predicting ARDS development (45) (Fig. 2).

C5a

C5a is the central complement protein anaphylatoxin implicated in sepsis and acute lung damage mediated via the CC-chemokine receptor 5 (46). C5a causes exaggerated early pro-inflammatory responses and activation of neutrophils and macrophages via the PI3K/Akt and MAPK signaling pathways, followed by the release of histones and reactive oxygen species (ROS), which cause endothelial damage, inflammation, and multi-organs dysfunction (47). Recent studies indicated that individuals with severe COVID-19 disease had considerably increased serum C5a concentration, stimulating leukocytes to produce a variety of inflammatory cytokines which leads to cytokine storm (48). Recent studies indicated that C5a plays a key role in COVID-19 patients' severe endotheliitis, endothelial dysfunction, and hypercoagulable condition (49). Exposure to tissue factors starting the intrinsic coagulation pathway is one of the main factors that contribute to the coagulation cascade. In both circulation and tissue form, C5a has been found to boost tissue factor activity (50). As the C5-C5aR axis produces C5a and MAC, Zilucoplan could be a noticeable therapeutic target and valuable medication for further studies in COVID-19 (51). The complement overactivation was studied in paroxysmal nocturnal hemogolobinuria (PNH) and recent studies indicated eculizumab or ravulizumab can be an effective treatment in PNH. Since COVID-19 presents a similar condition, eculizumab is a good targeted therapy in COVID-19 treatment (52, 53). C5 inhibition in COVID-19 patients leads to milder conditions than those untreated and it was studied in COVID-19 infection in a generalized myasthenia gravis patient (54), and in pregnant and postpartum adults. The patients did not face severe conditions and did not need hospitalization or supplemental oxygen and presented lower levels of inflammatory markers, reflecting that early complement inhibition can have protective effects (55) (Fig. 2).

Mechanisms of the Complement Inhibitors in COVID-19 Patients

C1- inhibitors

An essential multifunctional plasma glycoprotein called human C1-Inhibitor (C1INH), often referred to as C1-esterase inhibitor, has a special role in the regulation of the complement, contact, coagulation, fibrinolytic, and innate immune systems. C1INH engages in non-inhibitory interactions with several endogenous proteins, polyanions, cells, and infectious pathogens in addition to its inhibitory functions. Although C1INH is important for many physiological functions, it is best recognized for its role in the rare autosomal dominant disease hereditary angioedema (HAE), characterized by recurring acute bouts of increased vascular permeability and edema (56). The highlight point regarding C1INH is that C1INH binds to C1q and inhibits C1r and C1s of the classical pathway, C3b of the alternative pathway, and MASPs of the lectin pathway. C1INH affects all pathways of the complement system. Additionally, MASP-1/2 activates coagulation factors, and thus complexes between MASPs and antithrombin/C1-inhibitor are generated. Additionally, activated platelets and fibrin can activate MASP-1 and MASP-2 (57). To neutralize C1r and C1s and prevent additional classical complement activation, C1INH forms covalent connections with C1r and C1s (56). Early COVID-19 treatment with C1 esterase inhibition has shown some promise. Recombinant C1INH was administered in Switzerland to patients with COVID-19 who had not recovered despite being treated with hydroxychloroquine and antiviral drugs.

This reduced their fever and inflammatory biomarkers. Urwyler et al. reported that Conestat alfa was used intravenously in 3 doses of 4200 IU per 12 h after the initial 8400 IU dose. The report showed that five patients with severe COVID-19 pneumonia (lung involvement of 11 to 39 percent on a computed tomography scan of the chest) were treated. Inflammatory indicators and oxygen supplementation fell or stabilized in four individuals. Prior to conestat alfa treatment, C1INH levels were high. After the therapy, complement activation products' concentrations decreased (58). Larger, randomized studies will evaluate clinical outcomes in expanded trials using recombinant C1INH, icatibant, and lanadelumab—a kallikrein inhibitor. For example, patients with COVID-19 in the Netherlands had lower oxygen needs after taking the bradykinin receptor antagonist icatibant, which inhibits the contact pathway in thrombosis (59, 60) (Fig. 2).

We suggest that the cross-talk between the complement and the coagulation is a very critical point and physicians should consider this item in COVID-19 management. The decrease in serine protease inhibitors (antithrombin, protein C, and C1- inhibitor) can be used as biomarkers in COVID-19 prognosis. Therefore, antithrombin can be considered in the therapeutic protocols of COVID-19 as a multi-task agent that balances both the coagulation and the complement in COVID-19.

C3 Inhibitors

Excessive complement activation is a significant contributor to tissue damage in a wide range of clinical situations and immunological complex disorders. Complement overactivation causes tissue damage and a variety of clinical disorders, including Alzheimer's disease, heart failure, burn injuries, and immune-mediated diseases. Compstatin, a 13-residue peptide, is a potent inhibitor of complement component C3 activation and obstructs one of the most important and fundamental steps in the complement cascade (61). Heparin is an anticoagulant with anti-complement power. It can suppress classical and alternative pathways via engaging with C3 and inhibiting the formation of the C3 convertase (62). Low molecular weight heparin (LMWH) is well known among doctors and can be a good choice in the therapeutic approach to COVID-19 especially in the severe phase but resistant patients should also be considered (63). Montelukast is known as a cysteinyl leukotriene receptor antagonist basically used for aspirin-induced asthma, poorly controlled with inhaled corticosteroid monotherapy asthma, etc. Montelukast can target eosinophils, monocytes, and neutrophils so it can decrease neutrophil recruitment in the lung. These facts support the anti-inflammatory effect of the montelukast (64). Further studies indicated that montelukast seems to affect C3 and C4 indirectly (65). We suggest that adding montelukast to the therapeutic protocols of COVID-19 can be lifesaving, particularly in the initiation of the disease when the patient feels difficulty in his breathing. A study on baboons indicated that the complement components such as C3a and C5a might associate with the fibroproliferative response, and ARDS condition. Complement inhibitors such as compstatin, which is a C3 convertase inhibitor, can be an effective therapeutic approach against ARDS-induced fibroproliferation (66). Mastaglio et al. reported a successful treatment of a COVID-19 patient with severe ARDS with compstatin-based complement C3 inhibitor AMY-101 (67). By reducing both C3a and sC5b-9 production and inhibiting factor B consumption, C3 inhibition provided COVID-19 patients with a wide range of therapeutic approaches. The extensive inhibitory effect of C3 inhibition was linked to a more severe lymphocyte recovery, reduced neutrophil extracellular trap (NET) release, and accelerated serum LDH reduction (68). According to a study by Fang et al., COVID-19 patients with low complement C3 levels had

a worse prognosis (69). Additionally, Jiang et al. reported individuals with COVID-19, who have low complement C3 levels, are linked to a higher likelihood of clinical deterioration. They suggested that the serum C3 levels might be useful in the identification of patients who could benefit from complement inhibitors (70).

C3a, C5a Inhibitors

So far, several investigations have shown the anti-inflammatory effects of C5a and C3a inhibitors in various diseases (such as eculizumab and ravulizumab in hemolytic uremic syndrome) (71). However, long-term complement suppression is critical and should also be considered (72). Further studies confirmed that C5a inhibitors can decrease acute lung injury in animal models (73). Studies on COVID-19 disease suggested that the terminal pathway of the complement is over-activated in 64% of COVID-19 patients, which might be associated with disease severity. Inevitably, it is challenging to come to any firm conclusions regarding the effectiveness of complement blocking due to the limited number and variety of patients, as well as the lack of a control group. High doses of eculizumab doses such as 1,200 mg on days 1, 4, and 8 seem to be required in severe cases (74). Recent data indicated that C5a and IL-6 levels can be used as prognostic biomarkers for COVID-19-related ARDS, so they can be useful indications for regulating the eculizumab regimen in COVID-19 patients (75). Blockade of C5a is the terminal portion of the cascade and we should notice that some patients need the intervention in the first stages of the complement and before the extra production of anaphylactic components. We suggest alleviating C3a and C5a plus inhibiting C3 or C4 in the critical phase. For instance, montelukast plus C3a/C5a inhibitors can work strongly in the critical stage (Fig. 2).

C4 inhibitors

Hydralazine and Isoniazid have an inhibitory effect on C4 (76, 77). Based on recent studies, pyruvic acid calcium isoniazid is a metabolite of isoniazid and is listed as one of the top-scoring ligands for S-protein of SARS-COV-2 (78). Considerably, the drug-drug interaction of isoniazid and other anti-coagulants such as coumarin (bishydroxy-coumarin) and warfarin (79), and its hemorrhagic side effect(s) on specific patients (such as Waldenström's macroglobulinemia) should also be considered and well-studied (80). If we add this information to the complement section, we would find out that C4 belongs to the initiation stages of classic and lectin pathways.

Recombinant Complement Activation (RCA) Proteins Inhibitors

The complement system is the main noncellular system of innate immunity and it can label the target with ligands such as C3b,5 iC3b, and C3d for further interactions with the cellular innate immune system via CD35, CD21, CD11b, etc. C3 is an essential element in the complement system, and the complement system has complicated proteins that regulate the activity of C3 fragments. RCA proteins such as CR1 (CD35), DAF (CD55), factor H , etc. have a strong inhibitory effect on C3 and C5 convertases (81). Factor H is an essential element in the alternative pathway of complement. This element binds to C3b via three various short consensus repeats (SCR) domains and controls the complement activation via this interaction. Additionally, seventeen other SCR domains are responsible for binding to sialic acid and/or heparin and are responsible for host recognition (82). While the complement activation proceeds on the majority of other surfaces, mostly foreign surfaces, factor H blocks C3b amplification on self surfaces containing certain polyanionic carbohydrates (83). RCA proteins are suggested to be studied as candidates for complement regulation in COVID-19 patients, particularly in severe and critical phases.

A Revision on Therapeutic Protocols is Needed: Hypothesis-based Discussion

C3 activation is a common fate between

Table 1. Complement-based studies in COVID-19 patients and their efficiency

Name	Effects on the complement	Study design and dosage	Result	Ref
Narsoplimab	Targets MASP-2, inhibits MBL pathway	Started within 48 h of CPAP initiation ,Narsoplimab 4 mg/kg was administered intravenously twice weekly for 2–4 weeks.	Decreases endothelial damage, thrombotic events and ARD	(90)
AMY-101	C3 convertase inhibitor	IV dose of 5 mg/kg mg/Kg/ day, given as primary loading dose administered in 6 h; 24-h continuous infusions, for a 14-days	Improvement	(67)
Eculizumab *	C5 inhibitor	900 mg of eculizumab in sever phase, Additional 1200 mg doses on days 13 and 20	D-dimers decrease, improvement	(91)
Zilucoplan	Inhibit C5	32,4 mg Zilucoplan for 14 days in sever phase	Improvement of oxygenation	(92)
Avdoralimab *	Anti- C5aR	…….....	Decrease lung edema, controls ARDS	(86)

Helmet-continuous passive airway pressure (CPAP); intravenously (IV); *Off-label trials; Mannose-binding protein-associated serine protease (MASP); Mannose-binding lectin (MBL); Acute respiratory distress syndrome (ARD); Acute respiratory distress syndrome (ARDS)

classical, alternative, and lectin pathways. C3 inhibitor AMY-101 was administered 5 mg/kg/daily to control inflammatory damage particularly ARDS in severe COVID-19 patients (44). However, trial research has not yet been conducted on this C3-inhibitor's improving effects on COVID-19 patients' clinical conditions. Intravenous immunoglobulins (IVIG) are utilized in many inflammatory diseases, and these immunoglobulins can regulate the complement activation. IVIG targets C1q, C4b, and C3b but it should not be considered a primary complement inhibitor. Noticeably, IVIG can be effective in COVID-19 patients, particularly in severe conditions (84). Science, and the generation of the aphylatoxins were confirmed in COVID-19 patients in the severe stage, suggesting systemic TMA via vigorous complement activation and its cross-link with coagulation. Complement regulator gene variants, associated with the risk of severe TMA and multi-organ injury were detected in African-Americans (85). Research on anti-complements' effectiveness in COVID-19 patients has recently received more attention. Although studies conducted on selected COVID-19 patients have indicated that Avdoralimab (Innate Pharma, Marseille, France), an anti-C5aR monoclonal antibody, can control lung inflammation associated with ARDS in COVID-19 via inhibiting C5a-mediated myeloid cell recruitment and activation in lung tissue, a recent clinical trial has reported the opposite. Although targeting complement mediators seems to be a practical approach for inhibiting inflammatory reactions in COVID-19 patients, more trial research is needed to present the potential of complement inhibitors with more certainty in improving the clinical condition of these patients. (86, 87). BERINERT is the only C1 esterase inhibitor (C1INH)/kallikrein 20 U/kg was used intravenously on days 1 and 4 (plus standard care; or icatibant 30 mg), three doses/ day for 4 days plus standard care based on the protocols of the COVID-19 clinical trials, and the results were satisfying (88). But we should consider the possible allergic reactions to it, such as hives, chest tightness, wheezing, difficulty breathing, etc. Additionally, the risk of transmittable infectious agents, such as

viruses should be mentioned (89). In Table 1 we presented a summary of complement-based studies in COVID-19.

CONCLUSION AND FUTURE PERSPECTIVE

Complement is a regulator of adaptive immunity which should be considered in COVID-19 therapeutic protocols. We suggest that anti-inflammatory treatment strategies cannot work well without the complement regulation. According to the COVID-19 complex nature, we need to include the complement inhibitors in our future protocols. Additionally, targeting C3a and C5a is considered as terminal complement pathway therapeutics approaches, and they might not be effective enough in COVID-19 treatment; therefore, we should consider the complement in COVID-19 patients at the beginning of the infection. We should study the effects of heparin, anti-thrombin, C1-inhibitor, and montelukast, more specifically in COVID-19 patients, and after confirming their positive impact on COVID-19, we can consider them in COVID-19 treatment protocols.

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AUTHORS' CONTRIBUTION

Z.D.Z. wrote the first draft, M.T. revised the immunologic section, H.M. designed the Tables, C.D. revised the pharmacologic section, H.K designed the Figs., N.S. upgraded the final version.

CONFLICT OF INTEREST

None declared.

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