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REVIEW ARTICLE

Complement system as the potential therapeutic target in the management of COVID-19 patients

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ABSTRACT

The emerging COVID-19 caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has been raised as a global health challenge. Despite the breakthrough in the development of the COVID-19 vaccine, it still continues as a serious crisis, worldwide. The aberrant immune responses are strongly associated with the severity of the disease and an increased rate of morbidity and mortality among COVID-19 patients. The complement cascade activation is mediated by classical, lectin, and alternative pathways which could induce an inflammatory state during the COVID-19 infection. The growing body of research suggests that complement system activation plays an important role in the immunopathogenesis of SARS-CoV-2. Therefore, the blockade of complement cascades may be an effective approach to prevent the multi-organ complications of COVID-19. In this review, we will highlight the role of the complement system in the immunopathology of COVID-19, emphasizing the potential therapeutical targets to ameliorate COVID-19 infection.

Keywords: SARS-CoV-2; Immune Response; Complement System; Inflammation; Vaccine

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1. Introduction

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as the cause of coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China in late 2019. The emerging COVID-19 has become a global problem and international emergency concern since 11th March 2020[1]. During the last decades, epidemics of the coronaviruses (CoVs) have occurred as a severe acute respiratory syndrome (SARS) in 2002 and the middle east respiratory syndrome (MERS) in 2012 with a fatality rate of 10% and 36%, respectively[2,3]. CoVs are enveloped and single-stranded RNA viruses that can affect different physiological systems such as the respiratory system, gastrointestinal system, cardiovascular system, central nervous system (CNS), and neuroendocrine system in birds, mammals, and humans[4]. The main structural proteins of SARS-CoV-2 including spike (S), envelope (E), membrane (M), and nucleocapsid (N) are encoded by specific genes in the open reading frame (ORF)-1 downstream regions[5]. In general, recognition and

binding to the target receptor is a crucial event in viral entry to the host cell. Among the different receptors identified as a mediator of cellular entry, the angiotensin-converting enzyme (ACE)-2 is the main target receptor for the SARS-CoV-2 virus[6]. The ACE2 receptor plays an important role in the renin-angiotensin-aldosterone system (RAAS) which mediates the regulation of blood pressure and electrolyte homeostasis[7]. Remarkably, the ACE2 receptor is highly expressed by several organs in the human body including the lung, heart, brain, kidney, liver, and adrenal glands[8,9]. Furthermore, extracellular matrix metalloproteinase inducer (EMMPRIN), known as CD147, is expressed by epithelial and neuronal cells and has been considered a novel invasion route of the SARS-CoV-2 virus[10,11]. In general, the viral entry to the host cells is mediated by the cleavage of S protein into the S1 and S2 subunits. The proteolytic priming of S protein is orchestrated by the transmembrane protease, serine 2 (TMPRSS2), and furin molecule as the cofactors for ACE2 and CD147, respectively[12,13].

Although the pathogenesis mechanism of SARS-CoV-2 still is unclear; however, this virus could influence the host cell through different mechanisms including; (I) direct viral damage to the host cells and tissues, (II) RAAS disruption, as a result of decreased expression of ACE2 and reduced cleavage of angiotensin I and II, (III) endothelial dysfunction and activation of the intrinsic coagulation pathway, (IV) immune dysregulation which includes excessive inflammation due to the inhibition of interferon (IFN) signaling mediated by the virus, lymphocyte depletion especially T cells, excessive production of proinflammatory cytokines, particularly interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) and complement activation[7,14–16]. Comprehensively, activation of the innate immune system as well as humoral and cellular immune responses are considered the immunopathogenesis mechanism of COVID-19 infection[17–20]. In this sense, the complement system as the first line of innate immune defense plays a fundamental role in the prevention of bacterial and viral infection[21]. Based on the recent evidence, complement activation plays a critical role in the pathogenesis of SARS-CoV and SARS-CoV-2 infection. Moreover, the activation of complement pathways is strongly associated with the severity of disease[22,23]. Hence, this article aims to highlight the role of the complement system in the COVID-19 and the potential approaches to target the complement activation and immune-inflammatory processes.

2. Pathogenesis of multiple organ dysfunction in SARS-CoV-2 infection

Remarkably, the SARS-CoV-2 virus could damage different physiological systems. According to SARS-CoV relevant documents, the plethora of viral load and the inflammatory mediators as well may potentially affect multiple organs[24–26]. Taking this into consideration, we briefly describe the underlying mechanism of the SARS-CoV-2 and discuss systemic and organ-specific involvement in the COVID-19 patients (**Figure 1**).

2.1 Lung

Respiratory system involvement is the most important clinical manifestation of COVID-19 infection. It has been demonstrated that direct cytotoxicity of SARS-CoV-2 leads to alveolar epithelium injury[27]. In general, the activation of type II pneumocytes by the SARS-CoV-2 virus results in proinflammatory cytokine production including IL-6, ge inflammatory protein (MIP)1-α, MIP1-β, monocyte chemoattractant protein (MCP)-1, and interferon gamma-induced protein (IP)-10[28]. The pro-inflammatory cytokine production possibly attracts the CD3-positive/CD20-negative T lymphocytes as well as the activated CD68-positive macrophages to the alveolar spaces[29]. Moreover, the proliferation of interstitial fibroblasts and the secretion of fibrin and extensive proteins induce hyaline membrane formation[30]. Thickened alveolar septum due to bilateral inflammation and hyperplasia can lead to fibrosis[7]. Accumulation of granulocytes,

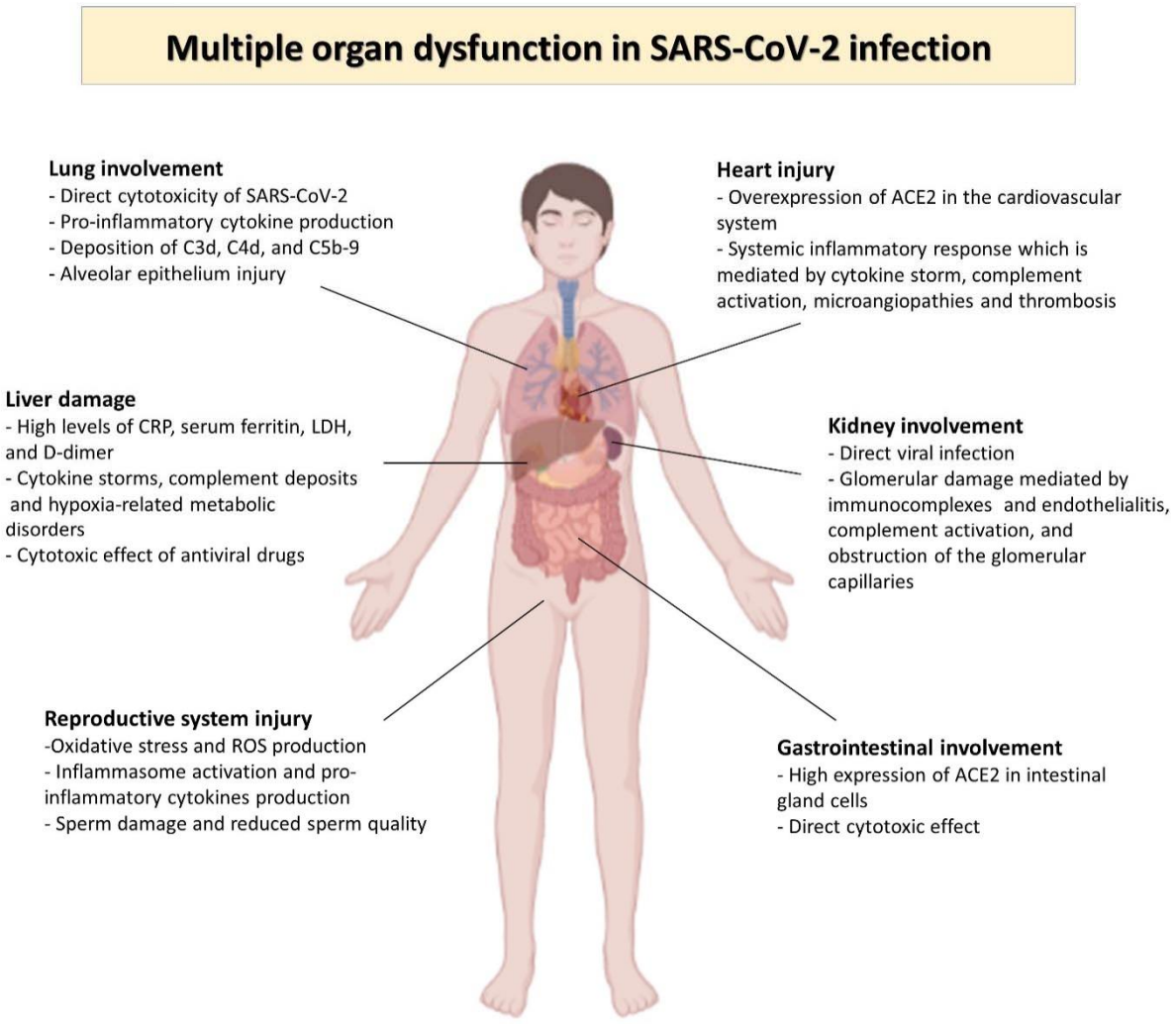

Figure 1. The multi-organ dysfunction in COVID-19 patients. SARS-CoV-2 infection influences different organs and tissues in the human body including the lung, heart, kidneys, liver, gastrointestinal tract, and reproductive system. Direct cytotoxicity, inflammatory responses, and complement deposits could mediate the tissue injury during the COVID-19 infection.

especially neutrophils that most likely occur as a consequence of bacterial infection, has also been reported in some SARS-CoV-2 infected cases[7]. Also, polynuclear giant cells have been found in the alveolar tissue as a result of viral changes especially in SARS and MERS infections[31]. Interestingly, the histopathological analysis of post-mortem biopsies has demonstrated the deposition of complement components including C3d, C4d, and C5b-9 during COVID-19 infection. It has been suggested the complement-mediated lung injury in SARS-CoV-2 infected patients[32]. Possibly, these mechanisms are the main factors responsible for some clinical symptoms including lung damage and acute respiratory distress syndrome (ARDS)[33].

2.2 Heart

It is postulated that the SARS-CoV-2 does not directly infect heart cells; however, evidence has reported the isolation of this virus from myocardial tissues[34,35]. Considerably, the inflammation during the COVID-19 infection has a deleterious effect on the cardiovascular system, which is not only due to the immune system disruption and inflammatory responses but also is highly related to the overexpression of ACE2 in the cardiovascular system[36]. The most important cause of myocardial injury is the systemic inflammatory response which is mediated by cytokine storm, complement activation, and thrombosis[37]. Furthermore, excessive C5a production through the activation of complement pathways may cause significant dysfunction in cardiomyocytes[38]. Besides, the underlying cardiovascular disease could increase the mortality rate among

the COVID-19 infected patients[39]. Hence, it may be perceived that complement components and thrombotic microangiopathies are contributed to cardiovascular dysfunction and increased mortality rate during the COVID-19 infection.

2.3 Kidney

According to the histopathological findings, the ACE2 receptor is highly expressed at the border of proximal tubular cells of the kidney by which SARS-CoV-2 may directly infect kidney cells[40]. Interestingly, a significant accumulation of CD68-positive and nucleoprotein (NP)-positive macrophages have also been observed in all patients with COVID-19 that is associated with acute kidney injury (AKI)[41]. Besides, it has been demonstrated that the cytokine storm may play an important role in AKI[42]. On the other hand, possible glomerular damage mediated by viral antigen immunocomplexes results in the development of focal segmental glomerulosclerosis in infected patients with SARS-CoV-2[43]. Subsequently, dysfunction of the vascular vessels in the kidney occurs due to the viral particles, lymphocytic subendothelial inflammation, also known as endothelialitis, complement activation, and obstruction of the glomerular capillaries in the kidney[44]. Taken together, direct viral infection, complement activation, and inflammation are major causes of COVID-19-associated AKI.

2.4 Gastrointestinal system

Gastrointestinal symptoms are considered one of the primary manifestations in patients with COVID-19. There is direct virus-mediated tissue damage to the gastrointestinal tract due to the high expression of ACE2 in gastrointestinal epithelial cells[45]. The interaction between ACE2 and SARS-CoV-2 results in cellular damage and destruction of physical barriers in the gastrointestinal tract. It has been revealed a high number of plasma cells, lymphocytes infiltrate the stomach, lamina propria, duodenum, and rectum of COVID-19 infected patients which could mediate gastrointestinal tissue damage which activate inflammatory pathways. It has been revealed a high number of plasma cells infiltrate to the stomach, lamina propria, duodenum, and rectum of COVID-19 infected patients which could mediate gastrointestinal tissue damage[46]. This inflammation can trigger a response against the normal microbial flora and exacerbate dysbiosis, causing serious damage to the intestinal mucosa. With the role of ACE2 in the tryptophan absorption, the production of antimicrobial peptides is disrupted after infection with SARS-Cov-2. Complement has been shown to have a protective effect in patients with SARS-CoV-2 infection, but complement, like other components of the immune system, can have devastating effects if left unregulated. Usually, after widespread inflammation and activation of the complement system during the acute phase of the disease leads to problems with the host cells. Mechanisms through which complement can cause pathogenesis in patients: causing more inflammation and exacerbating it, leukocyte recruitment, MAC formation, and lysis of virus-infected cells.

2.5 Liver

Liver dysfunction is mainly reported in patients with severe COVID-19 which is mediated by the immune activation and severe inflammatory responses caused by SARS-CoV-2. The high levels of C-reactive protein (CRP), serum ferritin, lactate dehydrogenase (LDH), and D-dimer indicate an exacerbated inflammation in COVID-19 patients[47]. Cytokine storms cause hypoxia-related metabolic disorders, which is another potential mechanism for liver damage. Complement deposits have been detected in the hepatic artery and portal vein of the liver. Therefore, it has been suggested there is a significant correlation between complement activation and liver damage during the COVID-19[48]. Besides, the SARS-CoV-2 virus has a direct cytotoxic effect on liver cells[49]. Mild lymphocytic infiltration in hepatic lobules, diffuse necrosis, and centriolar sinus dilation has also been reported in these patients[50]. In addition, the liver, as a key organ of detoxification, is exposed to liver damage from high-dose antiviral drugs, antibiotics, or steroids, such as those involved with other viral

infections[51]. The virus also binds directly to ACE2 in epithelial cells of the bile duct or cholangiocytes[52]. Hence, the immune system hyperactivation mediated by the activation of complement pathways has been proposed as the main contributor to liver dysfunction.

2.6. Reproductive system

The novel SARS-CoV-2 virus invades the target cells through the interaction with the ACE2 receptor. It has been demonstrated the expression level of ACE2 in the female reproductive tract and ovaries is very low; however, ACE2 is highly expressed on seminiferous tubules, Leydig cells, Sertoli cells, and spermatogonia[53]. Besides, data has revealed the correlation between androgen receptors and TMPRSS2 that affects the pathogenesis of COVID-19[54]. Therefore, it seems the male reproductive system, especially testes are most susceptible to SARS-CoV-2 infection and damage[55]. Interestingly, SARS-CoV-2 induces oxidative stress and the production of reactive oxygen species (ROS) in the host cell which can be mediated through different mechanisms[56]. Besides, SARS-CoV-2 directly activated the oxidant-sensitive pathways which lead to the production of ROS. ROS is a potent signaling molecule and mediator of inflammation that can activate the NLRP3 inflammasome and NF-kB related pathways[57]. Based on the reported results of SARS-CoV infections, ROS production contributed to the activation of complement cascades, excessive cytokine production, and uncontrolled inflammatory responses. For instance, hydrogen peroxide upregulated the gene expression of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α. All these cytokines may trigger immune-inflammatory cells including macrophages, neutrophils, and endothelial cells that lead to NADPH oxidase activation. Consequently, ROS production and cytokine storm are leading components of an amplification loop that can exacerbate the destructive pathogenesis of SARS-CoV-2[58]. Consequently, the impact of oxidative stress and ROS production on the male reproductive system is exploited by sperm membrane peroxidation and reduced sperm quality[59].

3. SARS-CoV-2 elicited inflammatory pathways

In general, the SARS-CoV-2 virus entry into the host cells is triggered through the ACE2 receptor which is identified as the most important mediator of the viral invasion[60,61]. The innate immune system is immediately activated as the first line of defense. The potential invader is recognized through extra- or intra-cellular pattern recognition receptors (PRRs) expressed by neutrophils, monocytes, macrophages, natural killer (NK) cells, epithelial, and endothelial cells. These receptors including toll-like receptor (TLR), retinoic acid-inducible gene I (RIG-I)-like receptor (RLR), and NOD-like receptor (NLR) are activated by a diverse range of viral components. Besides, SARS-CoV-2 infection may cause inflammatory cell death by which the damage-associated molecular patterns (DAMPs) are released[62,63]. Following the PRR activation, different transcriptional factors and signaling pathways may be triggered inside the cell which includes nuclear transcription factor κB (NF-κB), and interferon regulatory factors (IRFs)[64,65]. Thus, the innate immune responses for viral elimination initiates by interferon responses and different cytokine/chemokine production such as IL-1β, IL-2, IL-6, IL-7, TNF-α, CCL-2, CCL-3, CXCL8, and CXCL10[66,67]. Immune dysregulation and various inflammatory pathways are key underlined mechanisms in the pathogenesis of SARS-CoV-2[68–70]. Excessive and generalized cytokine production named cytokine storm or cytokine release syndrome (CRS) occurs as a consequence of unrestricted systemic inflammation and may cause multiple organ damage[69]. Inflammatory cytokine production, especially high levels of IL-1β, IL-6, and TNF-α significantly related to the severity of COVID-19 infection[71,72]. Based on the evidence, it can be perceived that different signaling pathways including Janus kinase/signal transducer and activator of transcription (JAK/STAT), tyrosine kinases, mitogen-activated protein kinase (MAPK), and mammalian target of rapamycin (mTOR) are the important mechanisms associat-

ed to COVID-19 pathogenesis[73]. Interestingly, innate immune mediators may infect by the SARS-CoV-2 virus and release pro-inflammatory cytokines which may exacerbate the hyperinflammation. For instance, the SARS-CoV-2 virus directly infects the macrophages through CD147 or EMMPRIN[10,74]. Moreover, these cells may be infected by phagocytosis of the virus-infected cells and produce pro-inflammatory cytokines, especially IL-6 and TNF-α. On the other hand, complement cascade is roughly activated by the first days of infection as a humoral part of the innate immune responses and can be limiting or aggravating in different phases of the COVID-19 infection[75]. DCs, a mediator between innate and adaptive immune responses, play an important role in antigen presentation, cytokine production, and specific T cell priming. Significantly, following the DC activation, adaptive B and T cell immune responses initiate 7–14 days after the onset of symptoms[76]. Regarding the SARS-CoV infection, both innate and adaptive immune receptors including TLR and Fc receptors (FcRs) may stimulate the aberrant inflammatory responses[77].

4. Complement pathways

The recognition of danger signals is a fundamental bio-mission of the complement system which is comprised of more than 40 naturally inactive proteins and serine proteases. In general, the complement cascade can be activated through the identification of specialized molecules by three main organized pathways including classical, lectin, and alternative pathways which results in opsonization, cell lysis, and inflammation[78] (**Figure 2**).

The complement classical pathway is activated through the interaction of the C1 complex by antigen-antibody complexes. The C1 complex containing C1q hexamer, C1s, and C1r modular serine proteases is the first trigger of the classical cascade which is predominantly coupled with the Fc domain of IgM and IgG antibody. Subsequently, autocatalytic activation of C1s and C1r leads to C2 and C4 cleavage into large (C2a and C4b) and small fragments (C2b and C4a). The binding of C4b and C2a to the C1 complex which is known as C3 convertase, leads to the C3 cleavage into C3b and C3a anaphylatoxin[79]. Correspondingly, the complement cascade can be activated by the lectin pathway which is identified as an antibody-independent mechanism. Mannose-binding lectin (MBL), ficolins or collectins in complex to MBL-associated serine proteases (MASPs) are key contributors to the activation of the lectin pathway by interaction with carbohydrate ligands.

Recently, the extrinsic pathway is recognized as the complement pathway which contains proteases and proteolytic enzymes of the coagulation cascade including thrombin, plasmin, kallikrein, and factor XIIa[80]. Hence, activation of the extrinsic pathway contributes to polymerization of fibrin, and activation of platelets which is leading to blood clot formation and thrombosis[81].

Finally, the activation of the classical, lectin, and alternative pathways lead to MAC formation and tissue damage. The complex of C3b fragment and C3 convertase results in the formation of C5 convertase which cleavage the C5 into a large fragment of C5b, and anaphylatoxin of C5a. Afterward, C5, C6, C7, C8, and C9 were irreversibly assembled in a row that constructed terminal Membrane Attack Complex (MAC), sub lytic pore construction, and lysis of potential insult. In addition, MAC-mediated cell death can release damaged molecular patterns (DAMPs), which in turn can result in further complement activation[81]. Taken together, the complement cascade act as a part of humoral innate immunity to defend against invasive pathogens in different ways through opsonization, anaphylatoxins, and MAC formation. On the other hand, anaphylatoxins such as C3a and C5a can activate neutrophils, mast cells, monocytes and macrophages, basophils, eosinophils, T cells, and B cells. Anaphylatoxins highly contributed to the development of important immune processes, including chemotaxis, NETosis, degranulation, production of cytokines, inflammation, production of ROS, and NETosis[82–84]. Moreover, the release of ROS causes endothelial damage, in-

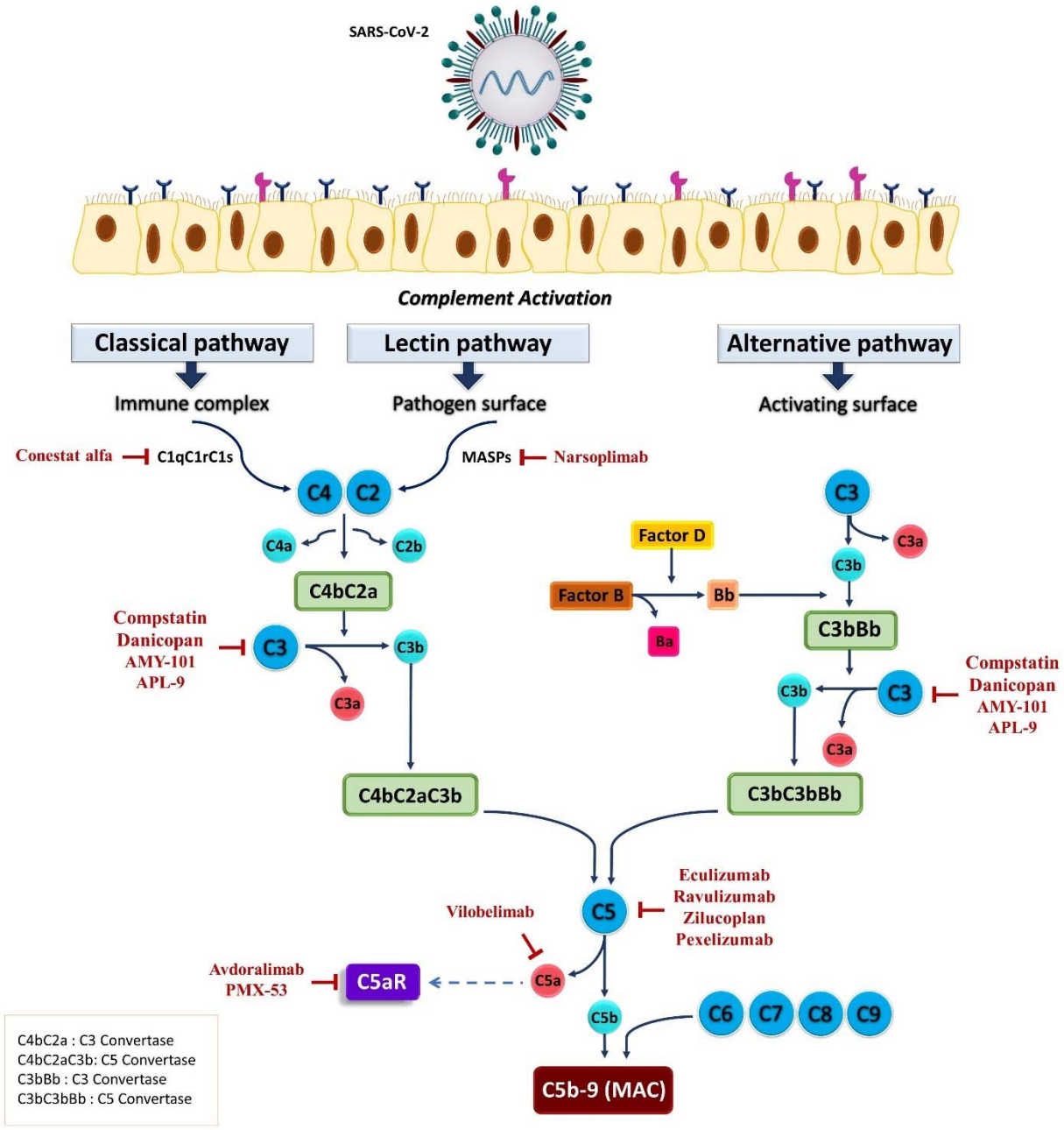

Figure 2. Complement activation pathways and suggested treatments for complement inhibition in COVID-19 patients. The classical pathway is triggered by the binding of the antigen-antibody complex with the C1 esterase containing C1qC1rC1s, which then forms C3 convertase (C4b2a). The lectin pathway is activated by binding of mannose-binding lectin (MBL) to the mannose-containing carbohydrate structures on bacteria or virus surfaces and then activates mannose-associated serine proteases (MASPs) to form the C3 convertase (C4b2a). The alternative pathway is activated directly by pathogen surfaces and also through spontaneous autoactivation, leading to the formation of a C3 convertase (C3bBb). The C3 cleaves to C3a and C3b by C3 convertase and then form C5 convertase (C4bC2aC3b and C3bC3bBb). C5 convertase cleaves the C5 and resulting in the generation of C5b-9 or membrane attack complex (MAC). Inhibiting the complement components with mentioned drugs could reduce the severity of the virus-mediated pathogenic conditions.

flammation and thrombosis, and reduces the function of the innate immune system, and dysfunction of the organ involved[85]. It also stimulates macrophages and neutrophils to produce strong proinflammatory conditions through the expression of TNF-α, IL-1β, and IL-6[86,87]. Ultimately, complement is severely involved in host defense and also regulates the immune system in several ways.

5. The role of complement system in COVID-19

The aberrant immune activation and dysregulated cytokine responses are key media-

tors of COVID-19. The complement system is regarded as the humoral arm of the innate immune defense in viral and bacterial infections. Although over-activated innate immune responses have been reported in several experimental and peer-reviewed papers since the epidemic outbreak of the SARS-CoV-2 virus; however, the conceivable role of the activation of complement cascade requires further genetic and immunological studies. Complement activation has already been confirmed in SARS and MERS that have a genetic resemblance with the novel SARS-CoV-2 virus[23]. In addition, evidence has indicated the role of complement activation in the pathogenesis of COVID-19 hospitalized patients. Dysregulated activation of the complement system during the SARS-CoV-2 infection causes serious damage to different organs[88]. Interestingly, several studies have shown that SARS-CoV-2 is capable of directly activating the complement system through the classical, alternative, and especially lectin pathways. The clinical results revealed that respiratory failure, neutrophil infiltration, and systemic inflammation in C3 complement-deficient mice significantly reduced in comparison to the wild type[23]. Recently, it has been revealed that the S protein is highly glycosylated and rich in D-mannose or L-fucose sugars. Therefore, it can be speculated that the SARS-CoV-2 possibly activates the lectin pathway through the epithelial cell receptors in the pulmonary alveoli[89]. It has been demonstrated that the complement gene expression has a significant increment in the SARS-CoV-2-infected cells[90,91]. Furthermore, the S and N proteins of SARS-CoV-2 are detected via MBL and MASP2 and lead to the activation of the lectin pathway[92]. Besides, MASP1 could cleavage fibrinogen and factor XIII that are two main factors of the coagulation cascade in clot formation[93]. MBL is strongly correlated with plasma D-dimer levels, confirming a clinical association with thromboembolism (TE)[94]. Comprehensively, it is reported that MBL causes thrombosis and abnormal coagulation in patients with severe COVID-19[95–98]. Activation of complement and deposition of its components in lung, kidney, heart, etc., directly related to the severity of the COVID-19 infection.

As already explained, the classical pathway is activated by antibodies. These antibodies are either specific to S and N proteins or natural or polyreactive antibodies produced by B-1 cells that react with the SARS-CoV-2 virus[99,100]. It has been shown that people with blood type O have a lower viral load due to the natural IgM antibodies against A and B antigens[101]. These polyreactive antibodies may reduce the viral load of the hosts by binding to the virus and activating the classical pathway[75]. However, by replicating the virus and increasing the viral load, a strong reaction with specific IgM and IgG can lead to severe inflammation due to the classical pathway, coagulation, and cytokine storm. Therefore, complement activation appears to be beneficial in the early stages of infection but may be severely harmful in later phases. Furthermore, excessive C5a production due to persistent complement activation can cause lung damage through the anaphylaxis and chemotaxis mechanisms. Uncontrolled activation of C5a, the most important and potent anaphylatoxin, leads to exacerbated primary responses and activation of neutrophils and macrophages through the PI3K/Akt and MAPK signaling pathways. The C5a/C5aR interaction disrupts the immune defense against the virus by T cell exhaustion[102]. Both T cell exhaustion and the induction of apoptosis in thymocytes are two main causatives of immune paralysis[103]. Interestingly, non-canonical decomposition of C3 and C5 most likely occurs inside the cell which mediates the opsonization of intracellular pathogens[104]. The binding of an intracellular virus with C3b/iC3b activates the cytoplasmic sensors and the antiviral mitochondrial signaling pathway, which ultimately results in the activation of NF-κB and IFNs mediators[75,105]. Thus, intracellular C3 acts as a DAMP to trigger the innate cellular response. Determining this pathway in connection with SARS-CoV-2 infection requires further investigation to better understand the modulation or exacerbation of inflammatory responses. The complement system is persistently activated in patients with severe

COVID-19 that could be considered a potential therapeutic target. The inhibition of the complement system particularly C3 and C5 in patients with COVID-19 is directly contributed to the reduction of inflammation, thrombosis, and severity of the COVID-19 infection.

6. Novel therapeutic targets through complement activation

Recent evidence has demonstrated an aberrant activation of the complement cascade in COVID-19 patients[92]. Therefore, the inhibition of the complement system could be considered an effective therapeutic modality in the clinical management of COVID-19 patients. Several experiments have reported a rapid clinical improvement in patients with COVID-19 that have been treated with complement system inhibitors[106,107]. To date, several ongoing clinical trials have evaluated the safety and efficacy of complement system inhibitors in the treatment of patients with COVID-19 (**Table 1**).

Table 1. The clinical trial phase of complement blockers in the management of COVID-19 patients

Drug name	Mechanism of action	Descriptive	Phase	Status	References
Zilucoplan	Complement C5 inhibitor	Zilucoplan has considerable effects on lung repair mechanisms. Zilucoplan improved lung oxygenation parameters, such as oxygen saturation, in COVID-19 patients with acute hypoxic respiratory failure.	II	Completed	NCT04382755
Ravulizumab	Humanized monoclonal antibody C5 inhibitor	Ravulizumab could inhibit terminal complement activation in renal endothelial cells and eventually thrombotic microangiopathy in COVID-19 patients.	III	Recruiting	NCT04570397
		Combination therapy with Baricitinib (anti-rheumatic drug). Ravulizumab and Baricitinib are two immunomudolatory aggent. Excessive immune system response in COVID-19 infection leads to multi-organ injury, especially lung injury. The efficiency of these immunomodulatory agents is compared with the Standard of Care during a 14-day treatment period.	IV	Recruiting	NCT04390464
		Investigation of the safety, effectiveness, pharmacokinetics, and pharmacodynamics of Ravulizumab in adult patients with COVID-19, acute lung injury, or ARDS.	III	Terminated	NCT04369469
Eculizumab	Humanized monoclonal antibody C5 inhibitor	Evaluation of membrane attack complex inhibition in regulation of the immune response, decrease in morbidity and mortality of COVID-19 infected patients.	I	Available	NCT04288713
		Determination of terminal complement blockage in improving the outcome of moderate or severe COVID-19 pneumonia patients.	II	Recruiting	NCT04346797
		Investigation of Eculizumab effectiveness in COVID-19 patients with severe pneumonia, acute lung injury, or ARDS who need emergency treatment.	-	No longer available	NCT04355494
		Adminestion of Soliris in COVID-19 patients with severe acute respiratory syndrome that exhibit organ injury.	-	No longer available	NCT04802083
AMY-101	Complement C3 inhibitor	Evaluation of AMY-101 potency and safety in COVID-19 ARDS patients through inhibition of complement 3.	II	Not yet recruiting	NCT04395456
APL-9	Complement C3 inhibitor	Evaluate the APL-9 effect in decreasing lung inflammation by complement inhibition.	I, II	Completed	NCT04402060
Conestat alfa (Ruconest)	Recombinant human C1 esterase inhibitor	Investigation of C1 esterase inhibitor in treating neurological symptoms after COVID-19 infection.	IV	Recruiting	NCT04705831
		Assessment of recombinant C1-inhibitor in the improvement of admitted COVID-19 patients.	II	Recruiting	NCT04530136
		72 hours scanning of recombinant human C1INH effect on alleviation of lung injury and capillary leakage through C1 inhibition and	II	Terminated	NCT04414631

Table 1. Continued.

Drug name	Mechanism of action	Descriptive	Phase	Status	References
		kinin-kallikrein system respectively, in COVID-19 pneumonia patients.			
Avdoralimab	C5aR inhibitor	Assessment of Avdoralimab in the improvement of mechanical ventilators requirements in severe COVID-19 pneumonia patients.	II	Completed	NCT04371367
		Investigating anti-C5aR along with anti-NKG2A and autophagy inhibitors in patients with metastatic cancer infected with COVID-19.	II	Active, not recruiting	NCT04333914
Vilobelimab	C5a inhibitor	Evaluation of Vilobelimab effectiveness in patients with severe COVID-19 pneumonia.	II, III	Recruiting	NCT04333420
Narsoplimab	MASP-2 inhibitor	Combination therapy with Remdesivir, Pulmozyme, IC14, Celecoxib Famotidine, Narsoplimab, Aviptadil Acetate, and Cyclosporine to decrease mortality and ventilator requirements.	II	Recruiting	NCT04488081
Danicopan	Complement factor D inhibitor	Combination of Remdesivir with Danicopan to the treatment of adults hospitalized with COVID-19.	II	Recruiting	NCT04988035
C1 inhibitor human	(C1r), C1s, coagulation factor XIIa, and kallikrein	Evaluate of C1 inhibitor potency, alone or in combination with icatibant, on COVID-19 patients.	II	Completed	NCT05010876

6.1 C1 inhibition

6.1.1 Conestat alfa

The classical complement pathway is initiated upon the activation of the C1 complex. Inhibition of complement cascade has been applied in the treatment of COVID-19. The C1 inhibitor is the largest member of the serpin superfamily protein that inhibits serine protease and inactivates the C1 complex[108]. The C1 esterase inhibitor, conestat alfa with the trade name of Ruconest, is being studied in patients hospitalized with non-critical SARS-CoV-2 pneumonia[109,110].

6.2 C3 inhibition

6.2.1 Compstatin

Compstatin is the first complement-specific drug that inhibits component C3 through selective neutralization. This cyclic synthetic peptide prevents C3 convertase formation and C3 cleavage[111]. C3 activation initiates the thrombo-inflammatory innate immune process that confers to lung injury and organ damage in COVID-19. The prohibition of the C3 component can be considered as a novel approach to COVID-19 management.

6.2.2 AMY-101

AMY-101 is a compstatin-based C3 inhibitor designed to modulate the complement-mediated diseases. It has been investigated that AMY-101 could improve clinical outcomes in COVID-19 patients with ARDS[106]. Besides, Mastaglio S *et al*. have reported the successful treatment of a 71-year-old patient with COVID-19-mediated ARDS and severe pneumonia with systemic hyper inflammation by intravenous (IV) complement C3 inhibitor of AMY-101[106].

6.2.3 APL-9

APL-9, a PEGylated synthetic cyclic peptide is designed as a potent C3 inhibitor. APL-9 binds to C3 and controls complement cascade by regulating the cleavage of C3 which is in the experimental phase (NCT04402060).

6.2.4 Danicopan

Danicopan is a small-molecule inhibitor of complement alternative pathway Factor D (FD)[112]. Danicopan is considered a therapeutic administer in Intraventricular Hemorrhage (IVH) and prevents C3-mediated Extravascular Hemolysis (EVH)[112]. Inhibition of factor D leads to prevention of upstream activation in complement alternative pathway and subsequently classical or lectin pathway[112,113]. In ongoing phase II of the clinical trial study, the efficacy of Danicopane with Ramsivar in adults hospitalized with COVID-19 was evaluated (NCT04988035).

6.3 C5 inhibition

6.3.1 Pexelizumab

Pexelizumab, a recombinant humanized single-chain monoclonal antibody directed against C5, prevents the cleavage of C5 to C5a or C5b-9. Pexelizumab is a monoclonal antibody fragment developed to prevent complement-mediated tissue damage and inflammation associated with reperfusion injury[114,115]. No pre-clinical and clinical studies are looking at pexelizumab's efficacy on patients' recovery from COVID-19, suggesting a need to validate this approach in COVID-19 patients via human clinical trials.

6.3.2 IFX-1

IFX-1 (Vilobelimab) is a chimeric monoclonal IgG4antibody directed against C5a, with no effect on MAC formation[116]. IFX-1 has been proven to be effective in toxin and virus-induced acute lung damage in nonhuman primates[117,118]. A recruiting clinical trial will investigate the efficacy of IFX-1 on patients with severe COVID-19 pneumonia (NCT04333420).

6.3.3 Avdoralimab

Avdoralimab (IPH5401) is an IgG1-κ fully human Fc-silent monoclonal antibody that blocks C5aR1 from binding to C5. Carvelli *et al*. have revealed that the C5a-C5aR1 axis may have a role in the pathogenesis of ARDS, as shown by the elevated amounts of soluble C5a molecule and C5aR1 in the blood and pulmonary myeloid cells in COVID-19 patients[119]. Therefore, it seems that the blockade of C5aR with avdoralimab would be effective in COVID-19 patients[120]. In addition, a phase 2 clinical trial investigating the effect of avdoralimab in patients with COVID-19 severe pneumonia has been completed (NCT04371367).

6.3.4 Ravulizumab and eculizumab

Ravulizumab (ALXN1210) and eculizumab are humanized monoclonal antibodies directed against C5 thus preventing its cleavage to C5a[121]. The presence of high levels of baseline C5 in patients with severe COVID-19 indicated that COVID-19 is characterized by amplification of terminal complement activation[122]. It has been proposed that Ravulizumab is used to treat COVID-19-induced microvascular and endothelial dysfunction, which causes thrombotic microangiopathy and acute renal disease. In this regard, a phase 3 clinical trial is ongoing (NCT04570397). Ravulizumab has been shown to reduce complement-mediated inflammatory damage associated with COVID-19 infection in patients with paroxysmal nocturnal hemoglobinuria (PNH), according to a clinical case report study[123]. Annane *et al*. suggested that Eculizumab leads to improved survival and reduced hypoxia in patients with severe COVID-19[124]. Diurno *et al*. have reported that after being treated with Eculizumab, all patients could completely recover, and inflammatory indicators were reduced[107]. A clinical trial investigating the efficacy of Eculizumab in COVID-19 patients is ongoing (NCT04288713).

6.3.5 PMX-53

The cyclic hexapeptide PMX-53 is a noncompetitive inhibitor of complement C5a receptor 1 (C5aR1)[125]. PMX-53 is a cyclic hexapeptide that inhibits complement C5a receptor 1 (C5aR1). PMX-53 is a noncompetitive inhibitor that has beneficial effects on human neurological disorders, such as neurodegeneration, amyotrophic lateral sclerosis, spinal cord injury, and Alzheimer's disease[125–128]. Despite the good results of this drug in inhibition of C5, so far, no study has been done on its effect on reducing the severity of COVID-19 disease. However, it seems that PMX-53 could be considered as a therapeutic candidate for this disease.

6.3.6 Zilucoplan

Zilucoplan is a small macrocyclic peptide that binds C5 and blocks the downstream formation of the membrane attack complex through the prevention of C5 cleavage and interaction between C5b and C6[129]. Zilucoplan has shown an improving effect on oxygen saturation and the outcome of COVID-19 patients with acute hypoxic respiratory failure[130].

6.4 Lectin pathway inhibition

6.4.1 Narsoplimab

Narsoplimab (OMS721) is a completely human immunoglobulin gamma 4 (IgG4) monoclonal antibody that binds to MASP-2 and inhibits the activation of the lectin pathway. Rambaldi *et al*. have indicated that Narsoplimab can be an effective COVID-19 therapy by reducing COVID-19-related endothelial cell damage, inflammation, and thrombotic risk[131]. Ali *et al*. have suggested that the inhibition of MASP-2 by Narsoplimab provides a novel therapeutic approach in patients with COVID-19[132].

7. COVID-19 vaccination and complement system

The activation of the complement cascade is an immunological response that leads to protection against infection with SARS-CoV-2. From another angle, if it is engaged in an abnormal manner, it will contribute to the worsening of patients who are already experiencing severe symptoms. The antibodies that are generated as a result of COVID-19 vaccination are referred to as neutralizing antibodies (nAbs). These antibodies bind to the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein[133–136]. The portion of nAbs known as Fc is capable of interacting with FcRs that are located on immune cells as well as recognition proteins of the complement system, such as C1q, which are responsible for stimulating the classical complement pathway. On the other hand, the extent to which different nAbs bind to C1q and activate complement can be quite variable[137]. A study investigated the complement component 1q (C1q) binding and subsequent complement stimulation by nAbs in participants receiving BNT162b2 vaccine. The study demonstrated that after receiving the Pfizer BioNTech vaccine, anti-SARS-CoV-2 S-RBD IgG develops, which has the ability to bind C1q and activate complement. This study also indicated that C5b-9 had a significant and strong correlation to the amount of bound C1q, which had a significant correlation to the amount of bound anti-RBD-IgG. Additionally, the data from the study indicated that anti-RBD IgG, and not IgM, was more important in complement activation following vaccination[138].

8. Concluding remarks

Studies in both clinical and fundamental research reveal that unregulated complement system activation may play a major role in the development of COVID-19. The results of clinical trials are now starting to confirm this assumption. This is undoubtedly a promising development, but caution should still be exercised in interpreting it because clinical trials are likely to show that the advantages of complement-targeting therapies depend on a number of confounders, including the severity of the disease, the timing of treatment initiation, and the use of co-administered medications. Because of this, there is not enough data at this time to recommend complement-targeting therapies for all patients who come with severe COVID-19.

To sum up, it appears that complement activation-induced inflammation plays a role in the severity of COVID-19. Despite the widespread vaccination worldwide, still, some affected patients are hospitalized and need intensive care to decrease immune system and inflammation responses. Thus, the complement inhibition-based therapeutical approaches would modulate the exacerbated immune responses in COVID-19 patients. In this regard, the efficacy of several drugs targeting complement components is investigated in clinical trials. However, there are concerns about these approaches. For example, patients are predisposed to infection by encapsulated organisms due to complement inhibition. It is noteworthy that the interventions based on complement inhibition should be considered at an appropriate point of the disease period.

Conflict of interest

The authors declare that they have no conflict of interest.

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