Editorial

COVID-19: disCOVering the role of complement system

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Received on June 16, 2020; accepted in revised form on July 13, 2020.

Clin Exp Rheumatol 2020; 38: 587-591.

Key words: complement system, COVID-19, cytokines, inflammation

Competing interests: none declared.

The outbreak of the SARS-CoV-2 virus emerged as a pandemic risk. The disease (COVID-19) is mainly characterised by fever, dry cough, fatigue, and lung involvement leading to pneumonia (1) and possible development of acute respiratory distress (ARDS). As of June 8th 2020, there were 7,049,649 confirmed cases and 409,886 deaths worldwide and, in Italy, there were 234,998 confirmed cases and 33,899 deaths (Johns Hopkins University, https://coronavirus.jhu.edu/map.html, accessed June 8, 2020). Evidence reports that SARS-CoV-2 binds toll-like receptors (TLRs) that recognise RNA, with inflammasome activation and production of interleukin (IL)-1β and IL-6, resulting in fever, lung inflammation and fibrosis, as well as thromboembolic events related to the involvement of the coagulation system (2-4). SARS-CoV-2 infects cells through binding with the angiotensin-converting enzyme (ACE)2 via its spike (S) protein allowing the virus to invade epithelial cells through intracellular assembly (5). The CoV-S protein thus seems to be crucial for receptor binding, membrane fusion via conformational changes, internalisation of the virus and host tissue tropism (5). As known, the complement system (CS) exerts a central role in host defence to infection. CS activation can be divided into 3 main phases: the first is the binding between different recognition molecules and foreign molecules on the pathogen surface; the second is the formation of convertase enzymes that cleave the key proteins C3 and C5; the third is the constitution of the membrane attack complex (MAC) leading to microorganism lysis (6). A large variety of microorganisms have been recognised as targets of mannan-binding lectin (MBL) of the lectin pathway, including viruses (7). Conversely, some organisms take advantage of the CS to increase their virulence (8). Data from the literature support the hypothesis that some viruses bind complement regulatory proteins and receptors as a means of escaping defence mechanisms (9). This likely supports a key role of CS activation in COVID-19. CS is one of the main actors of the innate immune system response and the activation of CS protects the host against pathogens. However, uncontrolled CS activation can lead to tissue damage and persistent inflammation (9, 10). The mechanisms of C3 regulation and the role of complement activation in SARS-CoV-2 pathogenesis can be explained by the close relationship with inflammatory cytokines including IL-1β, IL-6 and tumour necrosis factor (TNF)-α (Fig. 1). C3 and C5 synthesis can result up-regulated by exuberant pro-inflammatory cytokine production induced by SARS-CoV-2 (11, 12). The release of anaphylotoxins C3a and C5a promotes a chemotactic and pro-inflammatory *milieu* in the lungs as the main target tissue. Accordingly, the viral infection may turn on the complement "engine" with the formation of convertase enzymes that cleave the key proteins C3 and C5 with the formation of the MAC leading to microorganism lysis in a vicious circle (9, 11). In addition, MBL, which can be modulated by SARS-CoV S glycoprotein, takes part in the battle by activating the CS (5). Therefore, CS may have a role in both the early and late phase of the disease: the first immune defence-based phase, critical for pathogen clearance, and the second inflammation-driven damaging phase. Dysregulated CS activation is likely to play a crucial role in the pathogenesis of acute lung injury (ALI) induced by highly pathogenic viruses including influenza A virus, H1N1, H5N1, H7N9, SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-Cov). Previous studies report-

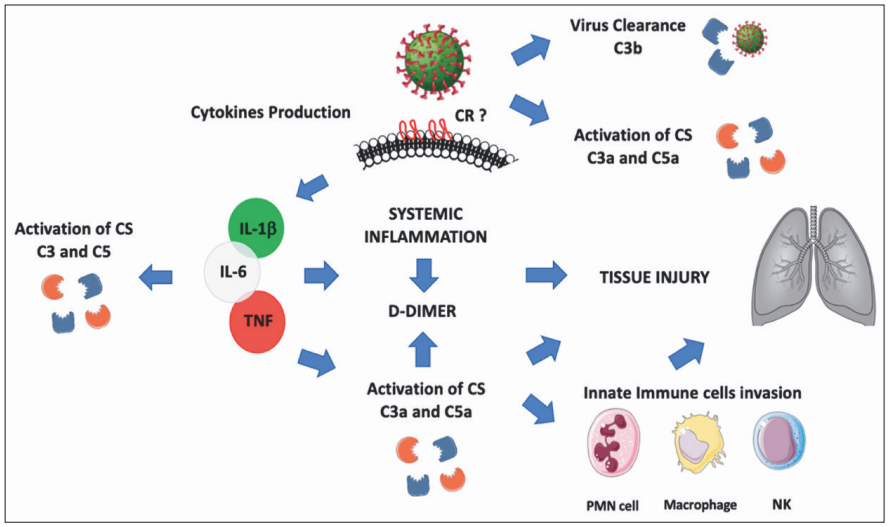

Fig. 1. Hypothesis of cross-talk between COVI-19 and complement system and systemic consequences. C5aR promotes COVI-19 infection in a ligand (C5a) dependent manner. COVI-19 induced cytokines such as interleukin (IL)-1β, IL-6, thus inducing C3 and C5 synthesis. Anaphylatoxins C3a and C5a binds to C3aR and C5aR to induce the synthesis of cytokines such as IL-1β, IL-6, tumour necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1. IL-1β, IL-6; TNF-α can further promote viral replication while MCP-1 promotes monocyte/macrophage invasion.

ed that in H5N1-infected mice the ALI was related with an abnormal activation of CS in lung tissue. In H5N1-infected mice, the complement inhibition with C3aR antagonist or anti-C5a antibody led to significantly reduction of the inflammation in the lungs, alleviating ALI (13-15). Overproduced C5a has been described as a part of the cytokine storm in virus-induced ALI. Therefore, the blockade of C5a signalling has been implicated in the treatment of ALI induced by highly pathogenic viruses (14, 15). Animal models to study the influenza virus-induced ALI recently confirmed that blockade of the C5a-C5aR1 axis inhibits viral replication in lung tissue and alleviates ALI by inhibiting endothelial cell activation and pro-inflammatory cytokines (*i.e.* TNF-α, IL-1β, IL-6) (14, 15). Furthermore, complement C3 activation has been implicated as an initial effector mechanism that exacerbates ALI in SARS-CoV infection (16). Previous studies on C3-/- mice infected with SARS-CoV documented that without complement, SARS-CoV is unable to induce a severe inflammatory response as it does in wild-type mice (16) Additional studies reported that an excessive CS is triggered by MERS-CoV infection promoting a severe immune dysregulation in the host. The blockade of the C5a-C5aR axis provided the reduction of MERS-CoV-induced tissue damage, suggesting a new strategy for clinical intervention in MERS-CoV cases (17, 18). However, the specific contribution of CS to lung diseases based on innate and adaptive immunity are just beginning to emerge. Elucidating the role of CS-mediated immune regulation in virus-induced severe pneumonia could help identify new targets for therapeutic interventions (12, 16).

As reported, smell dysfunction and anosmia have been described in subjects affected by COVID-19 (19). However, the authors suggest that, from the evidence available, anosmia whilst associated with COVID-19 should not yet be considered pathognomonic for the disease (20). The pathogenesis is postulated to be due to invasion of the olfactory neuro-epithilium and olfactory bulb seen previously in other coronaviruses, due to the high expression of ACE – receptor which allows virus cellular entry – present in the respiratory system (21). Interestingly, smell impairments have been described in patients affected with systemic autoimmune diseases characterised by the hyper-activation of CS, such as systemic lupus erythematosus and hereditary angioedema (22). A close link between neuro-immunological disorders and olfactory dysfunction has been reported in patients and animal models of autoimmune diseases (23). As in these conditions, it can be speculated that also in COVID-19 the CS dysregulation might play a role in the pathogenesis of smell dysfunction together with the damage to the olfactory pathways via ACE2 receptors.

As already known, COVID-19 has aslo been related with cardiovascular complications including arrhythmia, heart injury, and disseminated intravascular coagulation (24). Markedly elevated D-dimer levels are revealed in severe COVID-19 patients and have been related to a poor prognosis (25). In this context, there is evidence from the literature showing CS activation in several thrombotic microangiopathies and, accordingly, it can be suggested that, in COVID-19, CS may contribute to haemostatic activation leading to microvascular injury and coagulopathy (26). Indeed, the pathological characteristics of COVID-19-infected pneumonia, investigated in a case series from Northern Italy, showed damage in the lung structures with capillary congestion, necrosis of pneumocytes, hyaline membrane formation, interstitial oedema, pneumocyte atypical hyperplasia and platelet-fibrin thrombi detected in the small arterial vessels (27). The damaged cells induce innate inflammation in the lungs, activation of the CS and anaphylatoxin, causing a cytokine storm and ARDS (3, 11, 12).

Taken together, these findings support the hypothesis that CS activation may certainly contribute to the inflammatory response seen in some patients with severe COVID-19, and the inhibition of C3 or C5 together with the use of immunomodulatory/immunosuppressants, such as hydroxychloroquine (HCQ) and anti-IL-6, may have therapeutic potential (2, 28).

Nowadays, C5- and C3-targeted agents are under investigation as a potential treatment in COVID-19 (29, 30): ongoing clinical trials are available to explore how the modulation of CS could act on the clinical course and mortality of COVID-19 patients (Table I). It is noteworthy to observe that many of these CS-targeted therapies may be

Table I. Ongoing Clinical Trials on complement system modulation in cohorts of patients with COVID-19 (updated on 8th of June, 2020).

Title	Study type (identifier)	Arms	Primary outcome measures	CS target (drug)
mulTi-Arm Therapeutic Study in pre-ICU patients admitted with Covid-19 - repurposed drugs (TACTIC-R)	Interventional, RCT (NCT04390464)	Ravulizumab plus SOC *vs*. Baricitinib plus SOC *vs*. SOC	Time to incidence of a composite endpoint	C5 (Ravulizumab)
Soliris to stop immune mediated death in Covid 19 infected patients. A trial of distal complement inhibition.	Individual patients (NCT04288713)	Eculizumab	Mortality Time in the ICU / on a ventilator	C5 (Eculizumab)
CORIMUNO19-ECU: Trial evaluating efficacy and safety of Eculizumab (Soliris) in patients with COVID-19	Interventional, RCT (NCT04346797)	Eculizumab *vs*. SOC	Survival without needs of intubation	
SOLIRIS® (Eculizumab) for the treatment of participants with COVID-19 – an expanded access program for hospital-based emergency treatment	Open label, multicentre (NCT04355494)	Eculizumab	4-week treatment period and safety follow-up	
Zilucoplan® in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure (ZILU-COV)	Interventional, RCT (NCT04382755)	Zilucoplan plus SOC *vs*. Placebo Comparator plus SOC	Changes in oxygenation	C5 (Zilucoplan)
A double-blind, randomised study *versus* placebo of Avdoralimab (IPH5401), an anti-C5aR antibody, in patients with severe COVID-19	Interventional, RCT (NCT04371367)	Avdoralimab *vs*. Placebo Comparator	Patients alive and no longer hospitalised; ventilator-free days	C5a receptors (Avdoralimab / IPH5401)
A phase 2 clinical trial to assess the safety and efficacy of complement 3 inhibitor, AMY-101, in patients with ARDS due to COVID-19	Interventional, RCT (NCT04395456)	AMY-101	Patients alive without ARDS and any oxygen support	C3 (AMY-101)
A randomised, double-blinded, vehicle-controlled, multicentre, parallel-group study of APL-9 in mild to moderate COVID-19 ARDS	Interventional, RCT (NCT04402060)	APL-9 plus SOC *vs*. Placebo Comparator	Treatment-emergent serious adverse events	C3 (APL-9)
Recombinant human C1 Esterase inhibitor (Conestat Alfa) in the prevention of severe SARS-CoV-2 infection in hospitalised patients with COVID-19: a randomised, parallel-group, open-label, multicentre pilot trial (PROTECT-COVID-19)	Interventional, RCT (NCT04414631)	Conestat Alfa plus SOC *vs*. SOC	Disease severity on the 7-point Ordinal World Health Organization scale	C1 inhibitor (recombinant human, Conestat Alfa)

ARDS: acute respiratory distress syndrome; ICU: intensive care unit; RCT: randomised clinical trial; SOC: standard of care.

associated with elevated infectious risk, mainly eculizumab which is related with a markedly increased risk of infection due to encapsulated bacteria. Interestingly, C3-targeted intervention with compstatin-based inhibitors (such as AMY-101) has been successfully administered in a patient with severe ARDS due to COVID-19 pneumonia (12, 30). Data on HCQ well document its ability to inhibit CS activation *in vivo* and *in vitro*. Specifically, *in vitro* studies support that fact that HCQ inhibits CS activation by inhibiting the binding of C1q, and thus the generation of the C3 convertase and cleavage of C3 (28, 31). CS, including C5aR, plays an inflammatory role following tissue injury by stimulating the release of cytokines such as IL-6. The role of IL-6 production induced by injury or infection has been largely provided by studies on knock out animals showing its ability to coordinate important signals that regulate immune reactions and acute phase response (32). Both CS and IL-6 are associated with detrimental effects in autoinflammatory and autoimmune diseases as well as in cytokine release syndrome (CRS) and sepsis (33-36). The evidence from the literature supports the interplay between IL-6 and CS activation, providing results on CS-modulation exerted by tocilizumab (33-36). Another therapeutic approach in COVID-19 may be Janus Kinase inhibitors (JAKi): inhibiting the JAK/STAT pathway, JAKi interfere with cytokine storm and ALI (28, 37). The JAK-STAT pathway plays a critical role in the regulation of the immune system, especially the fate of T helper cells. Therefore, the possible action of JAKi on the CS might be indirectly related to both the cytokine network and the adaptive immunity. Intravenous immunoglobulins (IVIG) have been identified as potential treatment able to possibly ameliorate the lung damage in SARS-CoV disease. In this context, as well documented, IVIG act by neutralising the activated

complement components, thus preventing complement-mediated damage (28, 38, 39). Similarly, convalescent plasma (CP) might act in COVID-19 by direct neutralisation of the virus, by controlling the overactive immune system (including CS) and the immunomodulation of a hypercoagulable state (40). The available data indicate that administration of CP is a safe treatment option for those with severe COVID-19 (41). However, the small case series with no control group represents the main limitations to adequately evaluate the effectiveness of CP in COVID-19 (42).

Reliable measurement of CS activation is not an easy challenge but it could be an ideal tool in the clinical assessment of severe conditions characterised by a wide pro-inflammatory status. The measurement of markers appearing only after CS activation [uncleaved C3 and C4, activated C3 fragment (C3a), activated C5 fragment (C5a), active fragment of Complement Factor B (Bb)] seems to be more reliable than the measurement of native complement protein consumption and could be used for disease monitoring and to predict the development of complications (43). However, levels of CS proteins are affected by both the consumption and the changing rate of synthesis because many complement factors are acute-phase response proteins. Given the well-established networking between CS and the coagulation system and their close link with inflammation, plasma D-dimers can be a useful laboratory marker for the assessment of CS activation status (44). Nonetheless, the value of measuring plasma D-dimers in the decision-making process needs to be further investigated (25, 44).

To date, there is no approved targeted treatment in COVID-19. New approaches modulating the CS might represent the key challenge for a potential treat-to-target treatment of COVID-19. Further investigations should concentrate their efforts on a more reliable measurement of complement factors and their activation markers, as well as on the safety and effectiveness of CS-targeted drugs in COVID-19 patients, also in the context of multi-target integrated therapeutic protocols (45).

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