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Complement and COVID-19: Three years on, what we know, what we don't know, and what we ought to know

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A B S T R A C T

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus was identified in China in 2019 as the causative agent of COVID-19, and quickly spread throughout the world, causing over 7 million deaths, of which 2 million occurred prior to the introduction of the first vaccine. In the following discussion, while recognising that complement is just one of many players in COVID-19, we focus on the relationship between complement and COVID-19 disease, with limited digression into directly-related areas such as the relationship between complement, kinin release, and coagulation. Prior to the 2019 COVID-19 outbreak, an important role for complement in coronavirus diseases had been established. Subsequently, multiple investigations of patients with COVID-19 confirmed that complement dysregulation is likely to be a major driver of disease pathology, in some, if not all, patients. These data fuelled evaluation of many complement-directed therapeutic agents in small patient cohorts, with claims of significant beneficial effect. As yet, these early results have not been reflected in larger clinical trials, posing questions such as who to treat, appropriate time to treat, duration of treatment, and optimal target for treatment. While significant control of the pandemic has been achieved through a global scientific and medical effort to comprehend the etiology of the disease, through extensive SARS-CoV-2 testing and quarantine measures, through vaccine development, and through improved therapy, possibly aided by attenuation of the dominant strains, it is not yet over. In this review, we summarise complement-relevant literature, emphasise its main conclusions, and formulate a hypothesis for complement involvement in COVID-19. Based on this we make suggestions as to how any future outbreak might be better managed in order to minimise impact on patients.

1. Introduction

In December 2019, a novel human coronavirus, SARS-CoV-2, was identified in Wuhan, China. Infection with SARS-CoV-2 frequently led to a severe acute respiratory illness, termed COVID-19 and, in severe cases, to multi-organ involvement and damage. Was this an unexpected or unpredictable event? Possibly not. Coronaviruses are widespread throughout the animal kingdom, with 4 distinct genera, alpha, beta, gamma and delta described (de Groot et al., 2013). SARS-CoV-2 is not the first coronavirus that has made a recent cross-species jump to become a new human pathogen; since the year 2000, two additional new human-adapted coronaviruses have been described. The first of these, SARS-CoV-1, was identified in 2002, and gave rise to a disease termed Severe Acute Respiratory Syndrome (SARS). By the end of 2004 the SARS epidemic had been contained (see https://www.cdc.gov/dotw/sars/index.html), with around 8,500cases reported worldwide, and an associated fatality of around 10%. The second of these, MERS-CoV, was first identified in 2012. While fewer cases of MERS have been described worldwide (around 2,500), associated fatality is higher, at around 35%, and the epidemic is not yet contained (see https://www.cd

Abbreviations: ACE-2, angiotensin-converting enzyme-related carboxypeptidase-2; AE, adverse event; ADE, antibody-dependent enhancement; ADCD, antibody-dependent complement deposition; ADCP, antibody-dependent cell-mediated phagocytosis; ADCC, antibody-dependent cell-mediated cytotoxicity; AKI, acute kidney injury; ALI, acute lung injury; AP/AL, Alternative pathway or amplification loop of complement; C1-INH, C1 inhibitor; CL-11, collectin-11; C3, Complement Component 3; C4, Complement Component 4; C5, Complement Component 5; CPE, chronic panencephalitis; CP, Classical pathway; *COLEC10*, the gene for collectin subfamily member 10; *COLEC11*, the gene for collectin subfamily member 11; COVID-19, Coronavirus Disease 2019; CRP, C-reactive protein; CVF, cobra venom factor; DPP4, dipeptidyl peptidase 4; FCN-2, ficolin-2; Fgl2, fibrinogen-like protein 2; H1N1, influenza A virus subtype H1N1; ICU, intensive care unit; LP, Lectin pathway; MAC, Membrane attack complex; MASP1, Mannan-binding lectin serine protease 1; MASP2, Mannan-binding lectin serine protease 2; MAP19, MBL-associated protein of 19 kDa; MBL, mannose-binding lectin; MERS-Cov, Middle East Respiratory Syndrome coronavirus; MHV, mouse hepatitis virus; MHV-JHM, Mouse hepatitis virus (MHV) strain JHM; Msr1, macrophage scavenger receptor 1; PRM, pattern recognition molecule; RBD, receptor binding domain; PTX3, pentraxin 3; SARS-Cov-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV-1 spike, S-protein, SARS-CoV-1 nucleocapsid N-protein; sC5b-9, soluble form of the MAC; SDE, subacute demyelinating encephalomyelitis; TGEV, transmissible gastroenteritis virus; TP, Terminal pathway.

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c.gov/coronavirus/mers/about/index.html). SARS-CoV-1, MERS-CoV and SARS CoV-2 all belong to the beta-coronavirus genus of coronaviruses.

Critically, unlike SARS-CoV-1 and MERS CoV, SARS-CoV-2 proved highly contagious, and, despite drastic measures to try to contain its spread, COVID-19 rapidly emerged as a global pandemic, with disastrous health and economic consequences. As of April 2023, over 670 million cases had been diagnosed worldwide, with nearly 7 million deaths attributed to COVID-19 disease (see https://gisanddata.maps.arcgis.com/apps/dashboards/bda7594740fd40299423467b48e9ecf6). These numbers are almost certainly an under-estimate of the true incidence and mortality associated with COVID-19 (see https://www.who.int/data/stories/the-true-death-toll-of-COVID-19-estimating-global-excess-mortality). Around 2 million of these COVID-19 deaths occurred before introduction of the first COVID-19 vaccines, and, while vaccine rollout has been rapid across much of the globe, and has been successful in reducing both the incidence and the severity of the disease, COVID-19 is far from contained, with around 1000 daily deaths still attributed globally to COVID-19 infection (see https://gisanddata.maps.arcgis.com/apps/dashboards/bda7594740fd40299423467b48e9ecf6). In addition to morbidity and mortality directly linked to COVID-19 infection, there has been a huge indirect impact on morbidity and mortality associated with other diseases as, worldwide, health resources have been overwhelmed the need to provide acute care of COVID-19 patients, resulting in delayed medical treatment for other conditions. These figures make it imperative that, together, the scientific, medical and pharmaceutical communities maximise their understanding of the COVID-19 in order to minimise the impact of any future emergence of a coronavirus-transmitted disease. While a coordinated effort is applicable across all disciplines, in this review we limit our discussion to the relevance of the complement system to coronavirus disease.

2. History of complement involvement in coronavirus infections

2.1. SARS-CoV-1 and MERS-CoV in man

Prior to the COVID-19 outbreak, little had been published regarding complement activation, and possible consequences of this, by coronavirus infection in man. In a small proteomic study comparing serum samples taken from normal individuals (n = 14), from patients with SARS both before and after treatment (with corticosteroids and ribovirin) (n = 13), and from patients with non-SARS pneumonia (n = 12), a 30 kDa activation fragment of C4 was detected. This was significantly elevated in untreated SARS when compared with the other groups (x4 vs. normal controls), remaining slightly elevated after treatment (x1.5), but below the level seen in non-SARS pneumonia (x2 vs. normal controls) (Ren et al., 2004). A separate proteomic study found C3c, a terminal inactivation product of C3, one of the most-sensitive markers for SARS infection, again indicative of significant complement activation early in SARS-CoV-1 disease (Pang et al., 2006).

In a considerably larger study (569 patients, 1188 controls), a possible role for mannose-binding lectin (MBL) and the lectin pathway (LP) in SARS-CoV-1 infection was evaluated. This found that polymorphisms associated with low MBL expression levels correlated with SARS infection. Expression levels were confirmed in measurements of the protein in serum; lower levels were seen in patients than in controls. These associations did not however extend to mortality. The authors also showed that MBL bound directly to the SARS virus, in a calcium-dependent fashion; this binding was inhibited by mannan. Binding of MBL to SARS-CoV-1 resulted in C4 deposition, indicative of LP activation, and a decrease in viral infectivity of fetal rhesus kidney cells (Ip et al., 2005). That said, it should also be noted that the correlation between low MBL and infection reported above was not confirmed in a separate but smaller and less comprehensive study (Yuan et al., 2005). Similarly, a lack of association of mannan-binding lectin serine protease 2 (MASP-2) polymorphisms and SARS-CoV-1 disease susceptibility has also been reported (Wang et al., 2009). These data are compatible with a protective, not damaging, role for the LP in SARS-CoV-1 infection. Supportive of a primary role for the LP in protection against infection is the (single) observation that MBL deficiency might predispose to repeat infection (Hayes et al., 2021).

Analysis of the interaction of the SARS-CoV-1 spike (S) protein with MBL demonstrated that MBL blocked viral infectivity. The interaction of MBL with the S-protein was critically-dependent on an N-linked glycosylation site (Asn330). However, despite this site being close to the ACE-2 receptor-binding motif, MBL did not block the receptor interaction, nor did it influence S-protein driven membrane fusion. The mechanism by which the MBL-S-protein influences infectivity this remains to be elucidated (Zhou et al., 2010).

The SARS-CoV-1 protein 3c is expressed on the infected cell surface, where it may play a role in virus release and/or down-regulation of the type 1 interferon receptor. However, it is also strongly immunogenic, and elicited antibodies kill infected cells in a complement-dependent manner (Zhong et al., 2006). It has also been claimed that the nucleocapsid (N) -protein of SARS-CoV-1 binds MAP19, an alternatively-spliced product of the MASP2 gene (Liu et al., 2009), but as the N-protein is contained within the intact virus membrane envelope, the relevance of this is unclear. (With swine transmissible gastroenteritis virus, an alphacoronavirus, it was shown that while antibodies to membrane proteins elicited complement-dependent neutralising activity, antibodies to the N-protein did not (Woods et al., 1988). Even if the N-protein is able to sequester MAP19, the relevance of this remains uncertain as the role of MAP19 within the lectin pathway itself remains controversial, with a claim that it plays a regulatory role within the pathway, competing with MASP2 for binding to MBL (Iwaki et al., 2006), disputed by others (Degn et al., 2011).

2.2. SARS-CoV-1 and MERS-CoV in animal models

MERS-CoV utilises the dipeptidyl peptidase 4 (DPP4) receptor to infect cells, and mice transgenic for the human DPP4 receptor (Agrawal et al., 2015), when challenged nasally with MERS-CoV, rapidly develop systemic inflammation, respiratory distress and pneumonia, multi-organ damage and mortality (Zhao et al., 2015), pathologies replicated in severe COVID-19 infection in man. Further studies with this mouse model demonstrated the importance of the C5a-C5a receptor (C5aR) axis in development of MERS-associated pathology. Following infection, systemic C5a levels initially rose significantly above baseline levels (day (d) 1) when compared with mock-infected mice, partially recovered (d3), then rose even further (d7). Increased deposition of C5b-9 was also seen in lung tissue (time point not stated). Interestingly, little, if any, increase in C3 deposition was observed. Mice treated with an anti-C5aR antibody at d3 showed decreased macrophage infiltration into the lung, a decrease in pro-inflammatory cytokines, both locally and systemically, decreased viral replication, and a reduction in lung damage. Beneficial effects of anti-C5aR treatment were also seen in the spleen and brain of infected animals (Jiang et al., 2018). Somewhat less clear are the consequences of anti-C5aR inhibition of pyroptosis of macrophages (Jiang et al., 2019), a programmed cell death associated with intracellular pathogens and thought to reduce their replication.

While SARS-CoV-1 is more closely related to SARS-CoV-2 than is MERS-CoV, it is also, with respect to complement, less well studied. While it is naturally infective in young mice, unadapted it does not produce disease. A mouse-pathogenic strain of SARS-CoV-1 was generated by repeated passage in the respiratory tract of young mice. Following 15 passages, a strain, SARS-CoV-1M15, which was lethal in mice following intranasal inoculation, was produced. Lethality was preceded by pathological changes in lung epithelial cells (Roberts et al., 2007). In a follow-up study the contribution of complement to pathology was investigated. Infection resulted both in weight loss and in elevated C4, C3 and Factor B levels in lung homogenates when compared with mock-treated animals. The elevation in complement protein levels was

significantly higher in lethal vs. non-lethal infection. More detailed western blotting analysis of C3 protein in lung homogenates demonstrated clear activation of the protein. In contrast, nasal inoculation of C3 deficient (C3 −/−) mice with SARS-CoV-1M15 showed protection against weight loss, a pronounced reduction in respiratory dysfunction, a decrease in neutrophil and monocyte infiltration into lung tissue, reduced lung pathology, and a reduction in both local and systemic chemokine and cytokine levels when compared with C3 sufficient controls. Less complete protection against weight loss was also seen in C4 −/− and FB −/− mice, but the additional parameters noted above were not reported, making conclusions as to pathways involved in the complement response to infection difficult to reach (Gralinski et al., 2018).

2.3. Non-Human adapted coronavirus infections in animals

In the non-human coronavirus literature there are a limited number of potentially informative complement-relevant studies.

2.3.1. Mouse

Many strains of the mouse hepatitis virus (MHV), a mouse-specific betacoronavirus, the same genus as SARS-CoV-1, SARS-CoV-2 and MERS-CoV, have been identified. Some show a primary tropism for the upper respiratory tract, with subsequent dissemination to the lung (as well as other organs and tissues) (Körner et al., 2020). Complement-relevant studies however appear restricted to different primary tropisms.

Many laboratory mice strains are deficient in the complement component C5 (Lynch and Kay, 1995). It had been noted that susceptibility of mice to the MHV-3 coronavirus strain correlated with the presence or absence of C5, mice with C5 deficiency being resistant to severe infection. A study based on this observation demonstrated that the C5a/C5aR pathway was essential for the pathogenesis of MHV-3 -induced fulminant hepatitis. Infected mice showed a rapid increase in serum C5a levels and development of fulminant hepatitis. Mice deficient in the C5aR and mice treated with a C5aR antagonist had significantly attenuated disease. Attenuation was accompanied by a marked reduction in hepatic fibrinogen-like protein 2 (Fgl2) levels, a protein that causes liver necrosis. Macrophage secretion of Fgl2 was stimulated, *in vitro*, by C5a administration. These data show that in mice infected with the MHV-3 strain of coronavirus, fulminant disease is consequent on potentiation of Fgl2/fibroleukin expression by C5a (Xu et al., 2014). In a study focussed primarily in elucidating the role of the macrophage scavenger receptor 1 (Msr1) in fulminant hepatitis caused by the A59 strain, it was shown that both Msr1 −/− mice and mice treated with a C5aR antagonist were protected against liver damage (Tang et al., 2018).

Coronavirus MHV-JHM infection of rodents can also result in demyelinating encephalomyelitis. Besides acute disease (AE), chronic panencephalitis (CPE) and subacute demyelinating encephalomyelitis (SDE) can be induced. In a study in Lewis rats, viral nucleocapsid protein (N protein) was demonstrated in the cytoplasm, and the spike protein (S protein) was displayed on the surface of infected neural cells, with expression of S protein relative to N protein severely impaired in SDE lesions. Active demyelinating SDE lesions displayed an enhanced IgG content and deposits of complement C9 (Zimprich et al., 1991). Infection of mice with the neurotropic JHM strain of MHV can result in demyelination of infected cells, in the absence of T cells, by anti-JHM antibodies. This T cell-independent demyelination was reduced by around 75% in cobra venom factor (CVF)-treated (complement-depleted) animals (Pollard and Bussell, 1957).

These data illustrate the potential for a double-edged role of the complement response to coronaviruses, a protective anti-virus role as well as a self-destructive role. As the virus buds the spike protein will be exposed on the cell surface, and the primary MBL binding response to this, as well as an antibody responses in primed animals, will precipitate complement attack on the infected cell. The consequences of this will be determined both by the strength of the complement activation trigger, and the homologous restriction competence of the infected cell.

2.3.2. Other species

As early as 1979 it was shown that porcine transmissible gastroenteritis virus (TGEV), an alphacoronavirus, was inactivated, *in vitro*, by complement, with lesions resembling those produced by the membrane attack complex (MAC) in its envelope membrane (Pike and Garwes, 1979). Subsequently it was shown that an antibody against the virus matrix protein had neutralising activity only in the presence of complement (Körner et al., 2020). Antibodies to the nucleocapsid protein were without neutralizing activity, with or without complement.

Infectious bronchitis virus (IBV) is a gammacoronavirus that causes acute respiratory disease in chickens. An early study indicated that susceptibility to disease correlated with serum mannose binding lectin (MBL) levels, with high MBL levels associated with protective complement activation in the trachea (Juul-Madsen et al., 2007). In a follow-up study it was demonstrated that elevated MBL levels were a part of the acute-phase response, and that MBL-dependent complement activation and virus neutralisation occurred prior to any humoral antibody response (Juul-Madsen et al., 2003). It has also recently been suggested that IBV budding and release might be facilitated by CD59, with the released virion incorporating CD59 into its membrane envelope, a strategy to evade MAC-dependent elimination (Wei et al., 2017).

In vitro studies with cells infected with the feline infectious peritonitis (FIP) coronavirus, an alphacoronavirus and often fatal despite high antibody levels, possibly one of the first animal coronaviruses described, suggest that it too has acquired a MAC-dependent lysis evasion strategy (Cornelissen et al., 2009).

Taken together, these pre-COVID-19 studies and data tell us that, in animals, coronaviruses are capable of activating the complement system, at least in some instances through activation of the lectin pathway. Prior to the COVID-19 outbreak it could be deduced that the complement response can be protective, as a virus neutralising agent, something expected as a primary role of complement is to provide an innate immune response to infection prior to acquisition of an adaptive immune response. This protective role can also be deduced by the adoption of complement evasion strategies by coronaviruses. However, in severe disease, it seems that a non-protective role dominates, with the C5a/C5aR axis being a primary effector arm for this damaging response. Excessive complement activation provokes pathological changes in the host, leading to pronounced morbidity and, in the most severe cases, mortality. Mouse models of SARS-CoV-1 and MERS-CoV reinforce the concept that the complement response can provoke a damaging in the host, again highlighting a key role for the C5a/C5aR axis, and identifying C3 as playing a critical role. While mice are not men, in particular having developed different sets of complement regulatory molecules, none of the above is contradicted by the limited complement-relevant data obtained from clinical observations of SARS-CoV-1 and MERS-CoV infection in man. Certainly these data provided sufficient rationale for a number of complement-directed therapeutic strategies to be applied in COVID-19 disease.

3. Complement activation in COVID-19 infection in man

3.1. Systemic complement activation

Several plasma biomarkers studies have shown increased levels of complement activation products (C3a, iC3b, C4d, Ba, Bb, C5a, sC5b-9) and a decrease in levels of specific complement proteins (C1q, C4, C3 and C5) and regulators (FH, FI, Properdin) in critically ill patients, demonstrating ongoing complement consumption in COVID-19. In some of these studies, longitudinal sampling showing significant changes in levels of the complement biomarkers during disease progression (Zelek et al., 2020; Holter et al., 2020; Boussier et al., 2022; Alosaimi et al., 2021; Leatherdale et al., 2022; Siggins et al., 2023; De Nooijer et al.,

2021; Ma et al., 2021; Henry et al., 2021).

3.2. Lectin pathway involvement

One of the earliest publications relating to the Lectin Pathway (LP) and respiratory infection came from the observation that low levels of MBL correlated with an opsonisation defect in children (Super et al., 1989). The role of the pathway in respiratory infections has recently been reviewed (Świerzko and Cedzyński, 2020). MBL is a primary pattern recognition molecule of the LP and activates complement via the mannan-associated serine proteases, MASP-1 and MASP-2, in an antibody-independent manner. Of note, these proteases have also been claimed to possess coagulation factor-like substrate specificities. MASP-1 activates factor XIII (FXIII), the enzyme that cross-links fibrin in the clotting cascade, albeit at a far slower rate than thrombin (Krarup et al., 2008; Hess et al., 2012), and MASP-2 activates prothombin to thrombin (Krarup et al., 2007). While the physiological significance of these activities is unclear, co-localisation of the LP and coagulation enzymes might enhance these interactions, providing an early link between the coagulation and complement systems. Observations in COVID-19 patients show that the LP is associated with inflammation-induced thrombosis, a common feature of the disease. D-dimer concentrations correlated strongly with MBL levels, but no other complement markers (Eriksson et al., 2020).

It has been reported that the BB genotype of the *MBL2* gene, which leads to MBL deficiency, is associated with severe disease, but not with mortality (Medetalibeyoglu et al., 2021; Stravalaci et al., 2022). This is consistent with an initial role of the LP in fighting infection, but with a limited role in subsequent complement-driven events. In a more extensive study of *MBL2* polymorphisms, COVID-19 patients were compared with both H1N1 influenza patients and with normal controls. Although subject numbers were small, nasopharyngeal tissue from COVID-19 patients (post-death biopsy), when compared with H1N1 patients (hospitalised patients) showed increased expression of MBL (Malaquias et al., 2021).

Complement can be directly activated by the SARS-Cov-2 S- and N-proteins, through binding of the LP recognition molecules MBL, ficolin-2 (FCN-2) and collectin-11 (CL-11), and recruitment of the MASP-1 and MASP-2 proteases. An additional route of LP activation was suggested by the direct binding of MASP-2 to the SARS-CoV-2 N-protein. These interactions result in LP-dependent C4b and C3b generation and deposition. Robust LP activation and C3b deposition *in vitro* was shown using HEK 293 cells expressing SARS-CoV-2 S protein; C3b deposition was inhibited by an anti-MASP-2 antibody, confirming the LP-dependence of the C3 activation (Ali et al., 2021). MASP-2 binding, and thus direct activation of the LP has been extended to the N-proteins of SARS-CoV-1 and MERS (Gao et al., 2022). In addition to MBL, pentraxin-3 has also been reported to bind the N-protein. *In vitro* evaluation of MBL binding was extended to show that, in addition to the initial isolate, the S-protein from several variants of concern also interacted with MBL, in a glycan-dependent manner, activating the LP. Modelling studies also predicted that the Omicron S-protein would be recognised by MBL (Stravalaci et al., 2022).

In a small (5 subjects) study, lung and skin tissue from severe COVID-19 patients with respiratory failure and skin rash was examined for the presence of complement proteins. Lung tissue contained significant deposits of C5b-9, C4d and MASP-2 in the microvasculature, possibly reflecting systemic activation of complement. Similarly, skin lesions showed C5b-9 and C4d deposition. In 2 of the 5 patients examined SARS-CoV-2 S protein was co-localised with the C4d and C5b-9 deposition, suggesting a degree of local as well as systemic activation (Magro et al., 2020). In a separate study, in which lung and kidney autopsy samples were examined for complement proteins, and compared with non-COVID controls (cardiovascular event-related deaths), MASP-2, as well as C3d and C5b-9, deposition was increased in the COVID patients. Interestingly, in these COVID patients, SARS-CoV-2 S-protein was observed in lung, but not kidney, tissue. The authors interpreted the pattern of complement protein deposition as indicative of LP activation in both tissues, but with greater involvement of the alternative pathway/amplification loop (AP/AL) in the lung (Niederreiter et al., 2022). It is also possible that these differences simply reflect differences in systemic activation at the time of death. In contrast, others have reported that while autopsy samples from lung, kidney and liver of COVID-19 patients, whose cause of death was respiratory failure, contain deposited CP and AP/AL components, evidence of significant systemic complement activation, MASP-2 and MBL were barely detectable (Macor et al., 2021; Macor et al., 2021). In yet another study, skin biopsies taken from severe (15 patients) and mild disease (6 patients) were examined for MASP-2 and C5b-9 deposition. MASP-2 (and C5b-9) deposition was restricted to severe cases. Sepsis-related ARDS and non-COVID AKI patients were used as controls in this study, defining these observations as COVID-specific, and confirmatory of the systemic nature of complement activation in severe disease (Laurence et al., 2022). The authors suggested that, if confirmed in a longitudinal study, MASP-2 (and/or C5b-9) deposition in the skin microvasculature could be used as a prognostic marker of severe disease.

A study of 65 intensive care unit (ICU) patients reported that a subset had highly elevated MBL levels, and that elevated MBL was associated both with higher D-dimer levels and with pulmonary thromboembolic events. These associations were independent of other markers of complement activation (e.g. C3d), or of other organ involvement, suggesting a specific lung-LP mechanism (Eriksson et al., 2020). In a study designed specifically to look at ethnic association of LP pattern recognition molecules, and their impact on chronic kidney disease, LP activation early in COVID-19 infection was confirmed, with indications of ethnicity- and protein-specific associations. While this study was limited to patients with chronic kidney disease, the authors speculated that differences seen in LP protein levels could be consequent on primary events localised to the lung (Medjeral-Thomas et al., 2021). Whether LP involvement can be indicative of, or prognostic for, disease severity is less clear. In the above study in chronic kidney disease, while clear associations between lectin pathway proteins and disease pathology were evident, for example with Map19 lower in non-severe COVID-19, and higher in severe disease, there were no correlations with disease prognosis, including death (Medjeral-Thomas et al., 2021). In a study of Chinese patients (180 subjects, 132 moderate disease, 26 in ICU, 22 deaths) the association between MBL genes and disease severity was analysed, with the conclusion that, in this population, there was no link between MBL polymorphisms and disease course or severity (Yuan et al., 2005). A similar lack of association with MASP-2 polymorphisms and disease susceptibility, again in a Chinese population, has also been reported by others (Wang et al., 2009). In contrast, in a study of ICU-admitted COVID-19 patients (123), when compared with sepsis patients and healthy controls, an association between both MBL and pentraxin 3 (PTX3) and 28-day mortality was seen (Gutmann et al., 2021). (Interestingly, in the same study, an association of mortality with C1-INH was also observed.) MASP-2 is elevated in active disease, but not in convalescent patients; the elevation in MASP-2 correlated not just with the PRMs ficolin-2 and ficolin-3, but also with sC5b-9 levels (Götz et al., 2022). Elevated pentraxin 3 (PTX3), but not ficolin, was reported by others in COVID-19, highest in critical disease (Moulana et al., 2022). Looking specifically at rare genetic variants of complement system proteins, and comparing asymptomatic elderly subjects with those that were hospitalised, an association of *MASP1*, *COLEC11*, and *COLEC10* genes with asymptomatic infection was noted. Decreased LP activity, and decreased MASP-1, Collectin-11 and Collectin-10 expression in the lung, further implicated LP activation with symptomatic disease (D'Alterio et al., 2022). A longitudinal study of hospitalised patients (n = 89) found that low MBL levels were associated with mortality. The same study found that while all complement pathways were activated in these patients, alternative pathway activation was predominant early in disease (Devalaraja-Narashimha et al., 2023). In contrast, a Scandinavian

study of hospitalised patients found no association between MBL levels and disease severity (respiratory failure), leading the authors to conclude that while the LP might play an initial role in activation, it did not play a predominant role in maintaining systemic complement activation (Holter et al., 2020). A lack of correlation between LP activity, or with MBL or FBN-3 levels and COVID-19 severity, has been reported by others (Charitos et al., 2021). In one single-centre study, a hierarchical clustering analysis of samples taken on admission from hospitalised patients was performed; of the four clusters identified, two exhibited highly distinctive complement activation profiles. In one, there was evidence of significant LP and AP/AL activation, with low MBL (as well as low C3, C4, C5 and FB) when compared to other clusters. This cluster was associated with higher ICU admission, higher levels of oxygen support, and higher mortality. In the second profile, while high levels of components and CP activity were found, ICU admission was low, and no mortalities were observed (Defendi et al., 2021). These data are consistent with complement activation reaching a tipping point, before which it is potentially beneficial to the patient, and beyond which it becomes damaging (see Fig. 1).

It should be noted that in most studies of hospitalised (i.e. severe) COVID-19 infection, there are no observations relevant to early events in infection; observations are limited to what is likely to be dysregulation of appropriate defence mechanisms following initial infection. For example, patients will likely already have mounted an antibody response (Holter et al., 2020). It is also worth noting that patients will be heavily medicated. Medication will have been determined by the state of

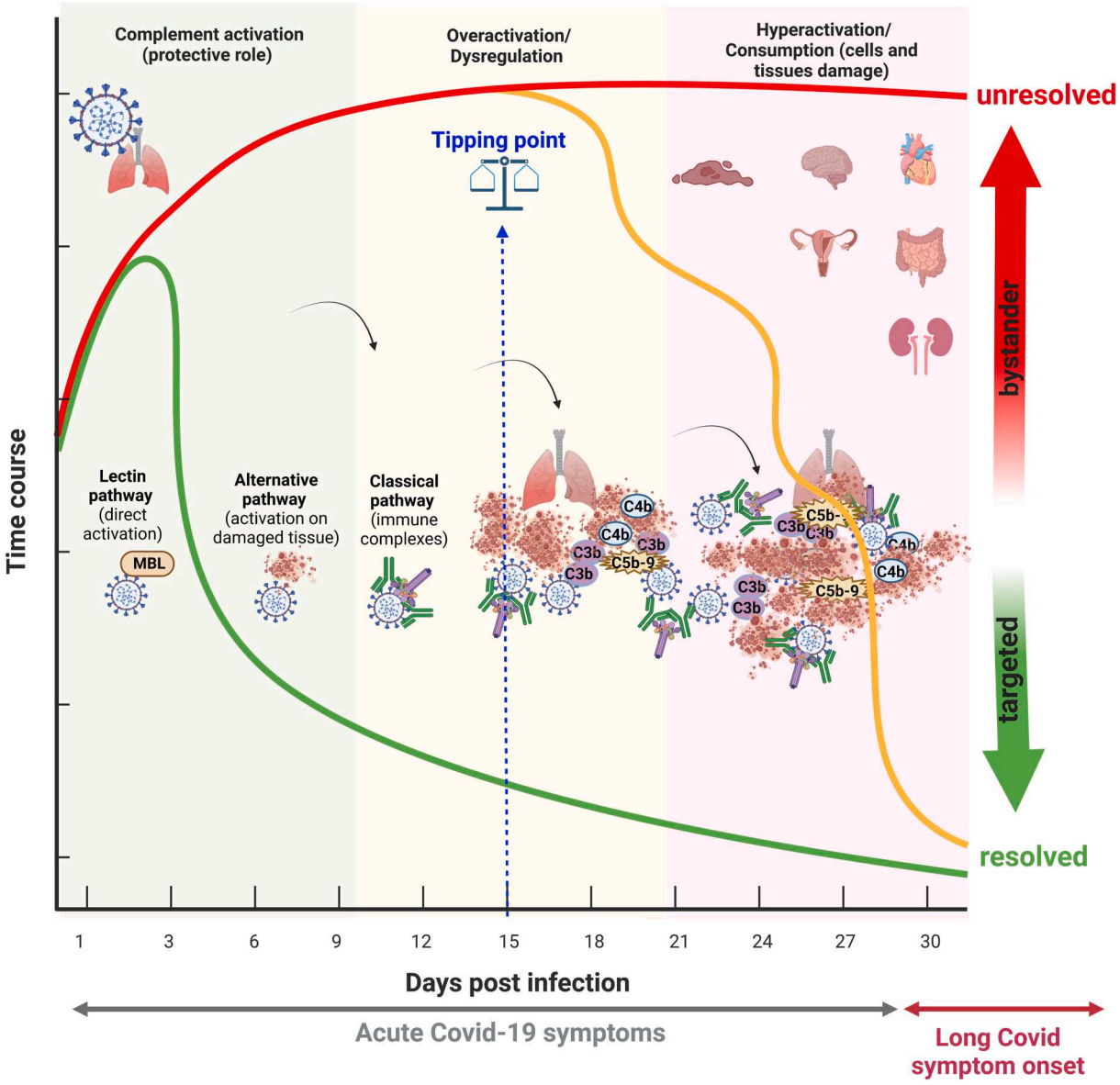

Fig. 1. A hypothesis for complement involvement in COVID-19 infection. In early stages of infection complement, primarily following LP activation, is protective, and infection is overcome. This represents the vast majority of cases, for whom we have no actual data as they are not hospitalised. In a minority of cases, those requiring hospital admission, complement activation has become dysregulated and a damaging role overcomes its protective role. This is probably consequent on a number of factors, including AP/AL activation by damaged cells, enhanced CP activation consequent on a newly acquired antibody response, and complotype, and involves bystander attack on otherwise healthy, mainly endothelial, cells, and represents a "tipping point" in disease progression. Effective therapeutic support gives the individual time to resolve dysregulated systems, including that of complement, and the patient recovers, albeit often with relapses. In a significant minority of cases, therapeutic support is unable to allow resolution, and the patient's condition worsens, leading to death caused by one or more organ failures. The situation with respect to LONG COVID is unclear, but our hypothesis is that latent infection of endothelial cells by the SARS-CoV-2 virus results in low level expression and budding of COVID-19 proteins on the cell surface, with continued low-level complement activation.

knowledge at that time in the pandemic, and will have varied from centre to centre. Possible confounding effects of medication on complement activation cannot be discounted. One attractive feature of the last-mentioned study above (Defendi et al., 2021) is that it was a single centre study, with all sampling taken within a 30 day window, so all patients are likely to have received highly similar treatment regimens.

In a slightly different approach, Hurler et al looked at levels of C1-inh complexes in COVID-19. As discussed below, C1-inh is an inhibitor of both the CP proteases, C1r and C1s, and the LP proteases, MASP-1 and MASP-2. Both C1s-C1-inh and MASP-1-C1-inh complexes were elevated in disease, a not unexpected finding (Hurler et al., 2022). Although not analysed in this study, it would be interesting to know at what stage in disease progression C1s-C1-inh complexes, indicative of CP activation, are first detected. Is this consistent with anti-COVID antibody generation, or does it occur earlier, as might be expected if there were non-antibody-dependent CP activation mechanisms at play in COVID-19?

Possibly related to lectin pathway involvement in disease, changes in IgG glycoslyation have been associated with disease severity. These may be associated with an increase in LP-initiated AP/AL, activity (Hou et al., 2021).

4. Classical pathway involvement

The Classical Pathway (CP) is the complement pathway that links complement-dependent responses to the humoral adaptive (antibody) response. Canonical CP activation is a direct consequence of C1q binding to multiple Fc domains contained in aggregated Ig, for example in immune complexes (ICs). In the absence of natural antibodies or of direct binding of C1q to the virus it would play no part in the initial response to coronavirus infection in the naive individual. Furthermore, as both LP activation and CP activation merge at the point of C4 activation, it is only analysis of C1q and of the C1 proteases and their complexes with C1-INH that will give definitive insight into CP involvement.

It has been proposed that natural IgA and IgM antibodies, through recruitment of C1q, augment the LP in initiating complement activation and clearance of the virus, particularly in the upper airway. It is only when virus overwhelms this first response and reaches the alveoli, which have no mucosal complement-dependent protection, and when viral replication and a strong adaptive antibody response has been elicited, that severe disease is seen, and the potential for a CP-initiated damaging complement response occurs (Matricardi et al., 2020). In support of this, strong C1q staining was seen in histological analysis of lung, kidney and liver autopsy material taken from deceased patients. In the lung C1q staining was localised to the interalveolar septa capillaries and alveolar cells, with IgG showing a similar even distribution. (Interestingly, the S-protein did not show a similar even distribution, suggesting that, in severe illness, other viral molecules are also being targeted by IgG.) Similar patterns were seen in liver and kidney samples. Downstream activation products, C4, C3 and C5b-9, as well as the AP/AL protein, Factor B, were also visualised, demonstrating amplification of CP activation (Macor et al., 2021; Vuitton et al., 2020). C1q deposition in the lung, and C1q-dependent (interpreted as CP-dependent) deposition of C4 and C3 has also been reported by others (Satyam et al., 2021), although, in this latter case, LP involvement cannot be ruled out. While a similar increase in Ig and C1q deposition in kidney biopsies has been reported by others (Jamaly et al., 2021), this has not been a universal finding. In one study weak or absent staining for C1q was seen in both kidney and lung tissue (Niederreiter et al., 2022); others, looking specifically in kidney biopsies, while confirming deposition of downstream activation products, have failed to detect C1q (Pfister et al., 2021). A strong correlation between levels of anti-nucleocapsid (N) or other SARS-Cov-2 IgG or IgM antibodies and complement activation fragments in severe disease has also been reported, again implying significant CP involvement. (Kim et al., 2021). Evidence for systemic CP activation in COVID-19 also comes from observations on circulating blood cells. Monocytes stained strongly for C1q. This deposition was associated with increased deposition of C3 (and, interestingly, up-regulation of the membrane-bound inhibitor of both CP and AP/AL activation, CD55) and with an increase in circulating C-reactive protein (CRP) (Lage et al., 2022).

A powerful IgG1 and IgG3 response to infection would also suggest significant CP involvement; IgG1 and IgG3 are the most potent CP-activating antibody subclasses. IgG1 and IgG3 were the most abundant antibody subclasses observed in COVID-19 convalescent plasma, with IgG1 showed better virus neutralisation properties than IgG3 *in vitro* (demonstrating a protective function for the host). Deposition of C3, C4 and C5b-9 in tissue was anti-COVID-19 antibody-dependent, demonstrating a CP requirement, and was virtually abolished in the absence of the anti-RBD mAb. However, the strength of IgG3 response to the S1 and RBD regions of the spike protein was associated with COVID-19 severity. Excessive generation of IgG3 may thus drive complement hyper-activation, inflammation and tissue damage. (Jarlhelt et al., 2021). While not specifically evaluating the role of the CP in complement hyper-activation in COVID-19, one progressive study of COVID-19-specific antibodies in hospitalised patients found that, on admission, IgG and IgM against the RBD domain were present respectively in 60% (IgG, rising over time to 100%) and 70% (IgM, rising to 95%) of all patients. In contrast, antibody levels against the N-protein were seen in 70% (rising to 100%) and 17% (rising to 40%) of all patients. C4d levels correlated to both spike protein RBD and N-protein IgG antibody levels, consistent with a strong CP component to their generation. IgG and IgM anti N-protein levels at admission were associated with respiratory failure, both on admission and throughout hospital stay (Boussier et al., 2022).

In this context, antibody-dependent enhancement (ADE) of viral infection has been noted for many viruses, including COVID-19. ADE is, in part, driven by C1q, supporting the concept of excessive CP activation being damaging to the host. In the absence of C1q (or the Fc□R) these same antibodies exhibited virus-neutralising activity. From this the authors argued that the balance of neutralising and ADE activity was critical, especially in respiratory tissue, in determining the course of the disease (Okuya et al., 2022). Developing this theme, others have also argued that ADE is dependent on non-neutralising antibodies, or antibodies at non-neutralising levels. Failure to clear virus or viral particles potentially results in enhanced Fc□R-enhanced entry of virus into cells, and enhanced C1q/CP-mediated complement activation and complement-driven inflammation (Thomas et al., 2022). As low plasma C1q has been suggested as an independent predictor of mortality in high-risk patients (Wu et al., 2020), it seems probable that there is a critical balance between C1q levels and antibody levels, the balance determining the fate of C1q-coated virus, between direct C1q-mediate clearance, or C1q-mediated CP activation, which may or may not be protective for the host. Support for CP activation being associated with COVID-19 disease severity and driving complement mediated hyper-inflammation comes from a study by Castanha et al. In this study, plasma levels of SARS-CoV-2 RNA correlated with the C3a/C3 ratio and levels of the pro-inflammatory cytokines IL-6 and IL-8. Anti-SARS-Cov-2 antibody titres, C1q, and circulating IC levels were significantly higher in the hospitalised group, correlating with disease severity. Levels of IgG against the S- and N-proteins correlated positively with IC-C1q. Comparison with other complement activation pathway markers suggested this to be the major driver of severe disease, with early non-neutralising antibodies playing a key role is disease exacerbation (Castanha et al., 2022). A subsequent study by the same group extended these findings to suggest that a rapid anamnestic, non-neutralising, response to the N-protein of coronaviruses causative of common colds, provoked by exposure to a previously encountered antigen, was associated with increased circulating IC levels, complement activation, and disease severity, with the conclusion that a similar response in COVID-19 might play a key role in complement hyper-activation in severe disease (Castanha et al., 2022). In a more detailed analysis of the consequences of CP-initiated complement activation, anti-S1 and anti-RBD antibodies

from hospitalized COVID-19 patients were evaluated for antibody-dependent complement deposition (ADCD; measured as C3 deposition from guinea pig complement), antibody-dependent cell-mediated phagocytosis (ADCP), and antibody-dependent cell-mediated cytotoxicity (ADCC). Anti-S1 and anti-RBD antibodies from hospitalized COVID-19 patients elicited higher ADCD, correlating with higher systemic inflammation, but lower ADCP, correlating with lower systemic inflammation, when compared to antibodies from non-hospitalized COVID-19 patients (Adeniji et al., 2021). One possible interpretation of this is that the severity of COVID-19 disease is, in part, a consequence of defective opsonisation and phagocytosis of circulating virus. A second key message that comes from this study is that, at the time of hospital admission, patients have already mounted an antibody response. One highly informative study looked at the consequences of CP activation by antibodies to either the S-protein or the N-protein (only seen following natural infection as all vaccines currently licensed target just the S-protein). As expected, C1q binding correlated strongly with antibody, especially IgG1, levels. Differences in downstream complement profiles were however seen in different patient groups. Patients convalescing from ICU showed consistent C3 and C5b-9 deposition with anti S-protein antibodies, something not observed in non-hospitalised convalescent patients. In contrast, C3 and C5b-9 deposition with anti-N-protein antibodies did not differ between the two groups. Anti S-protein and anti N-protein antibodies also differed in the antibody levels required to drive complement deposition; higher IgG1 anti S-protein levels were required to for equivalent complement deposition (Lamerton et al., 2022). While these findings may reflect the different protein oligomeric structures, and the influence these have on "productive" C1q binding (the S-protein is trimeric), it nevertheless has implications for future vaccine strategy.

A further supportive argument for CP involvement in severe disease could come from CH50 measurements. While a reduced CH50 titre will not be definitive for CP involvement (C4 and C2 will also be consumed in strong LP activation, and significant depletion of downstream components such as C3 and C5 might also affect CH50 titres), minimal changes in CH50 titres would not be compatible with strong CP activation. This, reduced CH50, has been reported in a number of studies (Keshavarz et al., 2021; Ali et al., 2022; Henry et al., 2022), with partial recovery in convalescent patients (Ali et al., 2022), and with correlation to progression to AKI (Henry et al., 2022). CP activity has also been reported as only slightly decreased in severe disease (Charitos et al., 2021), and no different to that seen in patients in ICU for non-COVID-19 respiratory disease (Gauchel et al., 2022). These differences may reflect different methodologies used in determining CH50 titres. A decrease in plasma C1q levels, through consumption, would also be indicative of strong CP activation. This was reported in an early study of 71 COVID-19 patients, with greater reduction in C1q being associated with more severe disease (and elevation of mediators/markers of inflammation) (Wu et al., 2020), but this finding appears contradictory to the elevated C1q levels associated with severe disease reported by Castanha et al (Castanha et al., 2022).

In vitro studies with recombinant viral proteins has also suggested that the spike S-protein, the N-protein, the membrane (M) protein, and the envelope (E) protein, are all capable of binding C1q and initiating CP activation in the absence of anti-COVID-19 antibodies, and of binding gC1qR, a possible second mechanism for recruiting C1q (Savitt et al., 2021). If these interactions occur in physiological responses to the virus, they provide yet another mechanism whereby complement could be activated. In the same vein, and possibly also relevant to severe disease, it is worth noting that, in addition to antibody-dependent recruitment and activation, C1q can be recruited and activated by apoptotic cells, through DNA and phosphatidylserine binding, and by binding to CRP (Reid, 2018). Although not regarded as a major player in CP activation, CRP might, in severe respiratory infection, and in COVID-19 in particular, play a significant role, especially in the lung, the primary organ affected. CRP destabilised as a consequence of interaction with phosphocholine-enriched membranes bound C1q and activated the CP (Dix et al., 2022). Ischemia induced by intra-alveolar edema and hemorrhage results in a dramatic increase in CRP, with potential for concomitant recruitment of C1q and CP activation (Sheriff et al., 2021).

During clinical evaluation of an anti-MASP-2 mAb (narsoplimab) in severe COVID-19, it was noted that C1s-C1inh complex levels remained elevated throughout the course of the disease, correlating with antibody levels, a finding best interpreted as indicative of continued CP activation (Lynch et al., 2022). In the absence of narsoplimab both C1s-C1inh and MASP-2-C1inh complexes are generated in COVID-19 (Hurler et al., 2022). Furthermore, treatment with narsoplimab restored serum haemolytic and bactericidal activity, identifying that a common LP/CP component had been significantly depleted in a LP-dependent process. While C2 is the most probable component to have been depleted, depletion of any component of the CP or TP downstream of (and including) C4 would have the same impact.

Investigation of differentially-expressed genes in COVID-19 has further highlighted a probable role for C1q in COVID-19 pathogenesis, with the genes for all three chains of C1q (*C1QA*, *C1QB* and *C1QC*) showing the highest degree of connectivity (Zhang and Zhang, 2022). This elevated expression may be stage of disease-dependent. Elevated *C1QA* and *C1QC* RNA expression appears limited to moderate disease, tapering off in severe and/or critical disease, where elevated expression of AP/AL genes predominates (Boussier et al., 2022).

5. Alternative pathway/amplification loop involvement

The alternative pathway, or amplification loop (AP/AL), of complement provides a mechanism whereby the active C3 and C5 convertase form of C3, C3b, can amplify its own production, irrespective of how the initial C3b is generated. To function as a true activation pathway, direct AP-dependent activation of C3, independent of CP and/or LP activation must occur. The relevance of such activation remains debated, but it is clear that the amplification role, particularly in disease, is a major function of AP/AL components (Harrison, 2018). Components of the AP/AL are C3, Factors B and D, and properdin. Of these, C3 is also a component of the CP and the LP, and study of C3 in isolation is relatively uninformative as to activation processes. Amplification requires tight regulation. This is provided systemically by Factor I in association with members of the Factor H (FH)/Factor H-related (FHR) protein family (Lucientes-Continente et al., 2023). Regulation at the cell surface is provided by additional members of the regulator of complement activation (RCA) gene family as well as by soluble regulators (such as FH) recruited to the cell surface (Rodríguez de Córdoba, 2023).

Evidence of escape from tight regulation is provided by elevated Ba and Bb levels in plasma. This has been reported in several studies, with Bb and/or Ba elevation corresponding to disease severity (Yu et al., 2022; Devalaraja-Narashimha et al., 2023; Leatherdale et al., 2022; Siggins et al., 2023). In one longitudinal plasma complement protein study of hospitalised patients (admission to d7), while all complement pathways were activated, AP/AL activation appeared prominent earlier in disease. In this study high Bb, C3a and sC5b-9, and low MBL, levels were associated with increased mortality. A modest inhibitory effect on complement activity was also claimed with anti-IL6 treatment, illustrating the possible confounding effect of treatment regimens on measurements of complement activity (Devalaraja-Narashimha et al., 2023). Consistent with this, increased Ba and Bb levels (and a reduction in FH) were associated with increased hypoxia and respiratory failure, with Ba (and FD) levels at admission being strongly predictive of mortality. In contrast to other markers of inflammation, such as IL-6, ferritin and CRP, whose levels fluctuated during ICU occupancy, all complement activation markers remained elevated throughout ICU stay (Leatherdale et al., 2022). In one of the most comprehensive studies reported, elevation in plasma Ba at hospital admission was correlated with elevated iC3b and sC5b-9, in all severity groups, with elevated Ba and iC3b, together with lower properdin, correlating with increased disease severity. Of these

markers, Ba was the strongest predictor of severity and death, leading the authors to argue that sustained and progressive amplification of C3 activation is a common feature in COVID-19 disease (Siggins et al., 2023). The C3a/C3 ratio, a measure of C3 activation, has also been proposed as an indicator of AP/AL hyper-activation, correlating both with disease severity and mortality and levels of pro-inflammatory markers such as IL-6, CRP, and ferritin (Sinkovits et al., 2021). In yet another study, measurement of a number of complement and coagulation parameters, including FD, led to the conclusion that elevated AP/AL activity, with a corresponding increase in sC5b-9 and C5a levels, correlated with disease severity and with markers of endothelial injury and hypercoagulability (Ma et al., 2021). In a single-centre comprehensive evaluation of hospitalised patients early in the pandemic, complement profiles allowed grouping into 4 distinct clusters. One (15 patients) had low FB, low AP activity (as well as low C3, C4, C5 and MBL), and was associated with high mortality (27%), high ICU occupancy (53%) and a high need for oxygen support (80%). A second cluster (19 patients), with high CP activity and high antigenic levels of complement proteins, was associated with a low ICU requirement (26%) and zero mortality (Defendi et al., 2021). These data suggest that, early in the pandemic, complement-driven pathology was primarily a consequence of AP/AL amplification of initial LP activation events. One seeming paradox with markers of AP/AL activation concerns measurement of C3bBbP. While association of complement activation with disease severity, especially respiratory failure, at the time of hospital admission, was provided a small study, the elevation in C3bBbP (regarded as a specific marker of AP/AL activation) levels was not significant, except in one patient, whose data collection was only partial, and for whom an extremely high level was recorded (along with other complement activation parameters), and who subsequently died (Holter et al., 2020). Others, looking at C5a levels in severe COVID-19 convalescent patients, which remain persistently high, found no association with C3bBbP (or C4d, a marker for both CP and LP activation), concluding that a non-canonical mechanism for C5 activation was at play (Kowalska et al., 2022). While this remains a possibility, C3bBbP is primarily generated on C3b covalently bound to a surface, and non-properdin-stabilised fluid-phase AP/AL activity cannot be discounted. High properdin (stabilisation of the AP/AL C3 convertase) levels, and low FI (inactivation of the convertase) have also been recorded in severe disease (Alosaimi et al., 2021). Consistent with the low FI association, low levels of FH, its cofactor in C3b inactivation, have also been associated with mortality (Laudanski et al., 2022). While their precise mechanisms of action are incompletely understood, the Factor H-related proteins (FHRs) are believed to compete with FH in their interactions with C3b, with an opposite effect on C3b function. Elevated levels of FHRs, especially FHR2, are associated with severe disease and mortality (Tierney et al., 2022), yet another argument strengthening the case for dysregulation of the AP/AL as a major causative factor in poor outcome in a significant proportion of COVID-19 patients.

In one single-centre study overall pathway activity was evaluated in the serum of hospitalised patients (154). In this predominantly male cohort the strongest association that was observed was that of a decreased AP/AL activity in patients requiring mechanical ventilation and in those who died (Charitos et al., 2021), implying exhaustive consumption of one of the components of the AP/AL, most likely FB. Similarly, a decrease in AP/AL activity has been described in COVID-19-associated AKI (Henry et al., 2022).

Examination of lung, kidney, and liver autopsy tissue has shown FB, as well as C4, C3 and C5b-9, deposition, but not MBL or MASP-2, suggesting that, at the time of death, AP/AL and CP activity was ongoing, but LP activation was insignificant, in this patient cohort (Macor et al., 2021; Macor et al., 2021). In contrast, in a smaller study of 3 patients, kidney autopsy tissue showed Collectin-11 and MASP-2, but not C1q staining (Pfister et al., 2021). It is possible that this difference can be attributed to activation on the plasma absorber material used in extracorporeal therapy of these patients. FD deposition in lung autopsy tissue has also been reported (Niederreiter et al., 2022).

Direct activation of the AP/AL by the S-protein of SARS-CoV-2, but not by the N-protein has been reported. C3 and C5 fragment, as well as Bb, generation, was blocked by a specific FD inhibitor. Activation appeared dependent on S-protein inhibition of FH decay and cofactor functions (Yu et al., 2020). In a follow-up study it was reported that the S-protein also blocked FH binding to heparin, and thus FH-augmented regulation at the cell surface (Yu et al., 2022). Complement activation following a direct interaction between the intact SARS-CoV-2 virus and heparin sulphate is also blocked by a FB inhibitor, confirming AP/AL involvement (Lo et al., 2022). A potential role for the SARS-CoV-2 ORF8 protein is harder to understand. It binds to C3b, blocking both C3b interactions with both FH and FB. Thus, while formation of the amplification loop convertase is inhibited, so is the irreversible proteolytic action of FI, leaving C3b available for future convertase formation (Kumar et al., 2023). This might be of benefit to the virus if AP/AL activation is critical to its elimination.

In a comprehensive genetic study of patients with severe disease, two complement risk factors for AP/AL-driven TMA, in *C3* (21 patients) and in *CFH* (34 patients), were associated with ICU admission (32% of patients carried both risk factors). In the absence of rs800292 in *CFH*, ICU admission was not required. (While no direct association of *ADAMTS13*, also a risk factor for TMA, with the AP/AL has been proved, a genetic association of *ADAMTS13* with COVID-19 severity was also found.) Interestingly, in the same study gender-specific differences were seen in complement variants, not just in *C3* and *CFH*, but also in *CFI*, *CD46*, and *CFHR3*, all regulatory components of the AP/AL (Gavriilaki et al., 2021; Asteris et al., 2022). Two of the polymorphisms in C3 described above, variants that confer increased stability on the AP C3 convertase, have been independently verified by others (Tsiftsoglou, 2021). Multisystem inflammatory syndrome in children (MIS-C) is a rare disorder caused by COVID-19 infection. Genotypic evaluation of components of the LP and the AP/AL highlighted that MIS-C patients had increased frequencies of specific SNPs in the *CFB* and *CFH* genes associated with reduced efficiency of inactivation of the C3 convertase (C3bBb) as well as promoting slower and weaker assembly of the convertase on virions. The authors suggested that this would lead to a decreased opsonisation capacity and hence compromised immune clearance and systemic inflammation (Gavriilaki et al., 2022). While the condition is primarily driven by immune cells, a complement component to pathology is suggested by its response to IVIg therapy, and a decrease in complement activation markers, including AP/AL markers concomitant with therapy (Sinkovits et al., 2022). AP/AL involvement in COVID-19 can also be inferred, even in mild disease, from relapses seen in aHUS patients and other TMA-associated conditions (Pinte et al., 2022; Ville et al., 2021; Khandelwal et al., 2022; Raina et al., 2021; Korotchaeva et al., 2022; Kurian et al., 2021). Evaluation of complement gene expression in COVID-19 showed an association of elevated expression of CP genes with moderate disease, but in severe disease it was LP and AP/AL genes that were elevated. Parallel evaluation of serum protein levels was notable in that it showed that in severe disease, while properdin RNA levels were high, circulating protein levels were low, indicating a high level of AP/AL activity and properdin sequestration. Low properdin levels were also significantly associated with mechanical ventilation (Boussier et al., 2022). This latter observation should be treated with caution as properdin is an unstable protein, and low levels it might be reflective of ventilation, rather than causative for the need for ventilation.

Possibly one of the most compelling arguments for AP/AL involvement in severe COVID-19 disease comes from the observations with age-related macular degeneration (AMD) patients. AMD mainly affects the elderly, and is strongly associated with dysregulation of the AP/AL. One might therefore expect an increased incidence of infection/severe disease among AMD patients than among an age-matched non-AMD population. This has been observed both in patient studies (Tuuminen et al., 2021) and in very large genomic studies (Ramlall et al., 2020; Ramlall et al., 2020; Chung et al., 2022), with a suggestion that PDGFB might be

a driver of severe disease in the AMD population (Chung et al., 2022). A second finding that came out of these studies was identification of a link with specific coagulation disorders (Ramlall et al., 2020; Ramlall et al., 2020), confirmation that more than one genetically-determined dysregulated effector mechanism probably plays a part in severe disease, and hence that, while COVID-19 is caused by the SARS-CoV-2 virus, it is unlikely that subsequent life-threatening pathology is caused by one common effector mechanism. This has implications for therapy.

One aspect of potential complement AP/AL involvement that has received little attention is that of the influence of N-glycosylation. Severe disease has been associated with low IgG sialylation (Hou et al., 2021), potentially lowering the efficacy of FH-mediated down-regulation of the AP/AL C3 convertase. In this context it should be noted that early characterisation of the N-glycome of C3 nephritic factors (autoantibodies that stabilise the AP/AL C3 convertase and cause kidney disease) also identified abnormal glycosylation of these IgGs (Rodríguez de Córdoba, 2023).

Crosstalk between the complement and coagulation systems occurs at many levels. One possible route whereby AP/AL activity might be increased is through VWF released from vascular endothelial cells following IL-8 and/or TNF□ stimulation. Retention large multimers of VWF at the cell surface would act as a template for C3b deposition and C3 convertase assembly (Fujimura and Holland, 2022).

6. C3 activation and downstream effector molecules

The primary role of the CP and LP is to activate C3 to C3b, and that of the AP/AL to amplify this activation. C3b is an essential component of both the CP and the AP/AL C5 convertase enzymes. The protective function of complement is dependent of appropriate regulation of these C3b-generating activities. Effector functions can be broadly divided into those that are C3-driven (by C3a, C3b, iC3b or C3dg (often designated C3d)) and those that are C5-driven (by C5a or C5b-9). C3a plays a major role in the modulation of immune cells; surface-bound C3b and its inactivation product iC3b are powerful opsonins, enhancing immune adherence and phagocytic clearance of ICs and invasive organisms. But possibly the major effector mechanism consequent on C3 activation is C5 activation, generating C5b-9, the MAC, with endothelial cell activating properties, and C5a, a powerful inflammatory agent, acting both as a chemo-attractant for immune cells and as an endothelial cell activator. Early in the pandemic, excessive C5a generation was flagged as a potentially harmful mediator of COVID-19 pathology [see Chauhan et al., 2020].

Many reports of elevated C3a levels in COVID-19 patients have been made. In most of these, a positive association of increased C3a levels with severity of disease and/or mortality and a prothrombotic state has been made (Alosaimi et al., 2021; De Nooijer et al., 2021; Devalaraja-Narashimha et al., 2023; Detsika et al., 2022; Hassan et al., 2022). This replicates what has been reported for the earlier coronavirus, MERS, epidemic (Hamed et al., 2021). The increase in C3a has been associated with the AP/AL genotype/phenotype of the patient, with less potent down-regulation leading to higher C3a (Asteris et al., 2022). In some studies an elevated C3a/C3 ratio has also been reported, indicating increased C3 consumption, again associated with severity of disease and/or mortality (Sinkovits et al., 2021). Elevated C3a levels and an elevated C3a/C3 ratio are also strongly associated with increased levels of inflammatory markers/mediators such as IL-6, CRP and ferritin (Sinkovits et al., 2021). Even within these however there are some inconsistencies. For example, in one study, while elevated C3a and C3a/C3 (as well as elevated sC5b-9/C3) was reported in moderate disease, these complement activation products, despite paralleling many other pro-inflamatory and pro-thrombotic markers, failed to predict progression to severe disease (Henry et al., 2021). Elevated C3a and an elevated C3a/C3 ratio, along with elevated pro-inflammatory and pre-thrombotic markers, is also seen in COVID-19-associated AKI (Henry et al., 2022), and elevated C3a in patients on maintenance hemodialysis (Prendecki et al., 2020), again with an association with inflammatory markers and a prothrombotic state and disease severity.

C3a has often been regarded as a poor indicator of C3 activation because of its rapid clearance from the circulation, either through receptor (C3aR1) engagement or by secretion. The fact that such elevated levels in COVID-19 are seen speaks much to the rate at which it is being generated, and it probably reflects accurately ongoing events, possibly underestimating their magnitude. It is therefore noteworthy that high C3a levels correlate with high SARS-CoV-2 antibody titres, themselves associated with severe disease progression (Lafon et al., 2021). The authors attribute this systemic hyperactivation to the formation of aberrant IC formation. Consistent with systemic hyperactivation is the observation that sera from hospitalised COVID-19 patients spontaneously deposit C5b-9 onto endothelial cells (Devalaraja-Narashimha et al., 2023). For this to occur, C5b67 must be continuously generated and deposited onto the membrane, followed by recruitment of C8 and C9. Preformed sC5b-9 does not bind membranes. It has been suggested that local elevated C3a levels, consequent on intracellular activation and release from infected lung epithelial cells, may also contribute to COVID-19 pathology (Posch et al., 2021).

Measurement of C3 and/or its major activation/inactivation products (iC3b, C3c, C3d(g) and equivalent products generated from C3 (H_2O)) in plasma is also fraught with difficulty, and in many cases it is not clear what is being measured. That said, assays that distinguish between native C3 and the major inactivation product seen in plasma, iC3b, can give valuable information about C3 activation. (Only a small proportion of activated C3, C3b, will bind to a surface; fluid-phase C3b is rapidly inactivated to form iC3b, which persists in the circulation.) A surrogate for a specific iC3b assay is the measurement of "C3bc". "C3bc" is not a single molecular entity, but a collection of molecules that express a neo-epitope not seen in native C3, but present on C3b, iC3b, and C3c (and presumably C3(H_2O) and iC3(H_2O)) (Garred et al., 1988).

With these provisos in mind, C3 (and C4) protein levels were reported as higher in mild and moderate disease, when compared to healthy individuals, but not in severe patients (Marcos-Jiménez et al., 2021). Low C3 levels have been associated with clinical worsening (Xie et al., 2020; Li and Chen, 2020; Fan et al., 2020; Xiao et al., 2020) but, interestingly, both associated with death (Zhang et al., 2021; Zhao et al., 2020) and not associated with death (Jiang et al., 2022). Low C3 at admission has also been reported as predictive for development of respiratory distress (Zhang et al., 2020). All these findings are consistent with increased consumption in severe disease. Data from a study restricted to hospitalised patients with severe disease is supportive of this conclusion, survivors having significantly higher C3 levels than non-survivors, and correspondingly lower C3a levels (Hassan et al., 2022), and from an investigation of the susceptibility of severe COVID-19 patients to secondary infections, with low C3 levels linked to compromised defense against bacteria such as Klebsiella and Staphylococcus (Ali et al., 2022), A review of 19 studies (3764 patients) published between January 2020, and February 2021, is broadly supportive of all of the above, the authors reporting that C3 (and C4) levels were lower in severe disease and in non-survivors than in moderate disease, and concluding that this was consequent on hyperactivation and consumption (Zinellu and Mangoni, 2021). Further insight into decreased C3 levels consequent on consumption, and also of systemic activation, comes from the observation that CD35 (CR1, the C3b/C4b receptor) expression on erythrocytes is considerably reduced in ICU patients (Kisserli et al., 2021). A primary role for CD35 is that of IC handling, with CD35 being turned over in the process. Capture of ICs, with ongoing CP activation, would account for the increased C4d and C3d deposition on erythrocytes. It is also interesting to note that while low C3 is associated with severe disease, levels recover in survivors, an indication that, in these patients, regulatory control of the complement system is re-established (Fang et al., 2020).

Decreased C3 levels are, however, not a universal finding. Some studies have reported that C3 levels are highly elevated in disease,

particularly in severe cases (Bagherimoghaddam et al., 2022), with the level continuing to rise throughout disease progression (Zhang et al., 2021). There are also reports that, in contrast to C4, for which an association was found, the C3 concentration has no associative relationship with disease severity (Stjepanovic et al., 2022), and that, despite a significantly reduced CH50, C3 (and C4) levels remained normal in hospitalised patients (Keshavarz et al., 2021).

There are many possible explanations for these discrepancies, for example, different disease severity classification, different treatment regimens, different sampling and assay procedures, different antibody profiles, differing ethnicities of study subjects, different COVID-19 strain predominance, and age. The latter is particularly interesting: In a Chinese study, elevated C3 levels were only associated with severe disease in the young (Cheng et al., 2021). It is also worth noting that in a more detailed analysis of serum proteins, low C3 levels (as well as low MBL, C4, Factor B, and C5) were only associated with one of four distinct profile clusters (Defendi et al., 2021), albeit that with the worst outcome (disease severity, mortality), reflecting heterogeneity in disease. A different balance of clusters in any one disease population will impact on the ability to detect associations. Heterogeneity is also evident from an early study of infected health care workers in Wuhan, China, with only just over 50% showing a reduced C3 level (Wei et al., 2021).

The C3b inactivation products, iC3b, "C3bc", C3c and C3dg, with prolonged half-lives over C3a, are probably more reliable markers of C3 activation, but tend to be used only in "specialist" complement laboratories. In one large and comprehensive study of complement-related biomarkers, significantly elevated iC3b, along with Ba and sC5b-9, at the time of hospital admission, was seen in all groups, with the degree of elevation correlating with subsequent severity and risk of death (Siggins et al., 2023). This same study noted that the levels of most complement markers reduced with time in severe disease, consistent with consumption. In a prospective longitudinal study, circulating C3c levels have been positively correlated with disease severity, thrombotic complications, and death (De Nooijer et al., 2021). A comparison of the levels of the complement activation markers, C3bc and C4bc, as well as other markers of inflammation and of coagulation, between those seen in plasma and those seen in the bronchoalveolar lavage fluid of critically ill patients with prolonged ARDS, in this small number of mechanically-ventilated patients, led the authors to suggest that a local pulmonary rather than systemic procoagulant and inflammatory "storm" predominated in these patients (Nossent et al., 2021). However, elevated C3bc (and sC5b-9) is also seen in non-hospitalised young adults and adolescents, demonstrating that increased complement activation alone is not sufficient, in this particular population, to cause severe disease (Lund Berven et al., 2022). The suitability of fluid-phase C3bc as a marker of severe disease can also be questioned by the findings of Holter et al, who observed elevated sC5b-9 and C4d, but not C3bc (nor C3bBbP or C5a), in a prospective study of COVID-19 patients with respiratory failure, when compared with patients without respiratory failure (Holter et al., 2020). Circulating C3dg has been measured in COVID-19 patients, both on hospital admission and, in a subset of patients, longitudinally over a period of up to one month, with the conclusion that, along with other inflammatory and coagulation markers, elevation was associated with severity, organ damage, and survival (Lipcsey et al., 2021). Elevated levels of C3dg have also been correlated with viral load (Brasen et al., 2021). Low C3c levels, a marker of C3 activation, were specifically associated with infants under 1 year old, a population with an increased severity of disease and prolonged recovery time. The authors attributed this to low antibody levels, and hence a low level of CP activation (Ji et al., 2021).

Finally, in some studies plasma levels of C3bBbP, the properdin-stabilised AP/AL C3 convertase, have been measured. These have either failed to record an increase in circulating C3bBbP levels (Kowalska et al., 2022), or have shown only a modest increase (Holter et al., 2020), but as C3bBbP is primarily a surface-bound complex, the significance of this is unclear.

With this in mind, deposited C3 (defined as C3c or C3d) was seen in kidney biopsies of COVID-19 patients, with the association pattern with other complement proteins indicating compartment-specific activation pathway predominance (Pfister et al., 2021). C3 deposition (measured as C3c and C3d) has also been assessed in the lung and kidney biopsies from deceased patients, with the conclusion that complement activation played a prominent role in damage in both organs (Niederreiter et al., 2022). *Ex vivo* evaluation of plasma taken from convalescent patients has been used to investigate antibody-complement activation relationships, with C3 deposition, measured as "C3bc" (and also C5b-9 depostion) correlating strongly with antibodies against the receptor-binding domain of the S protein. C3 and C5b-9 deposition (and C4 deposition) levels correlated with disease severity (Jarlhelt et al., 2021). C3 deposition is also seen on circulating monocytes, whatever the severity of disease and, interestingly, associated with up-regulation of CD55 expression, C3 deposition levels remaining elevated even in recovering patients, indicating continuing complement activation (Jarlhelt et al., 2021).

The C3F/S polymorphism, consequent on a single amino acid change in the native protein, is relatively common in Caucasians (but less so in Asian and African populations). The C3S variant appears to be negatively associated with COVID-19 infection and mortality (Delanghe et al., 2021).

Elevated plasma levels of C5a and/or sC5b-9 have been reported in many studies of COVID-19 patients, in most instances paralleling disease severity (Holter et al., 2020; Leatherdale et al., 2022; De Nooijer et al., 2021; Detsika et al., 2022; Hassan et al., 2022; Lafon et al., 2021; Cugno et al., 2021; Huber et al., 2021; Cyprian et al., 2021; Diorio et al., 2020). As an example, in one single-centre study, C5a and sC5b-9 levels at the time of hospital admission and 30 days later were correlated with disease severity and with markers of coagulation and endothelial cell damage. At the time of admission, C5a and sC5b-9 levels were elevated in all patient groups, with sC5b-9 levels in particular closely paralleling disease severity and von Willebrand Factor (vWF) levels. Tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) (markers of coagulation) were also elevated at admission. In contrast, soluble E-selectin levels were only elevated in patients with severe disease. At 30 days C5a, sC5b-9 and vWF levels were reduced in all evaluated patients, decreasing to normal levels in a many of patients. From these data the authors concluded that complement activation closely paralleled disease status, playing a role in disease pathology (Cugno et al., 2021). While consistent with this, additional findings reported by others have noted that C5a is elevated during acute disease, but returns to normal levels in recovering patients (Patra and Ray, 2022), that higher sC5b-9 levels are seen in non-survivors vs. survivors (Huber et al., 2021), that C5a levels correlate closely with vWF (again, a single centre study) (Gauchel et al., 2022), that C5a (but not sC5b-9) levels correlate with hypoxemia (Leatherdale et al., 2022), and that elevated C5a correlates with disease severity and mortality (Cyprian et al., 2021; Alosaimi et al., 2021). Elevated C5a and sC5b-9 have also been correlated with length of hospitalisation (Laudanski et al., 2022). In a seemingly contradictory finding to that reported in Leatherdale et al. (2022), a link between sC5b-9, not C5a, and respiratory failure has been reported (Holter et al., 2020). One interesting observation from early in the spread of the pandemic to Europe, in North Italy, is that in some patients, C5a and sC5b-9 were elevated and predictive of severe disease/mortality in the absence of elevated pro-inflammatory cytokine levels (Meroni et al., 2023). Looking into possible links between pre-existing co-morbidities, in COVID-19 patients on maintenance hemodialysis, already elevated C5a levels rise yet further prior to clinical worsening, leading the authors to suggest that clinical worsening was a complement-driven event (Prendecki et al., 2020).

In contrast, others have reported that C5a levels increase in all patients during disease progression, regardless of severity (Marcos-Jiménez et al., 2021). It has also been reported that C5a levels remain high in recovering severely ill patients, still elevated 90 days after

hospital discharge (Kowalska et al., 2022; Senent et al., 2021). One seeming anomaly in the first of these studies is that this is not associated with C4d or C3bBbP levels, leading the authors to suggest non-canonical activation of C5 (Kowalska et al., 2022). An alternative explanation is that C3bBbP, a fluid-phase complex, does not reflect surface-bound AP/AL C3bBbP convertase activity, and that surface-dependent C5 activation by the C3bBbP complex remains ongoing. The suggestion that abnormal IC formation might drive C5a generation (Lafon et al., 2021) could also be considered here. The second study made a further association between persistently high C5a levels and continuing lung complications (Cristiano et al., 2021). A whole blood model of virus-plasma-cell interactions has demonstrated that direct activation of C5 by the SARS-CoV-2 virus is possible. Use of specific inhibitors demonstrated that this activation was AP/AL driven, and dependent on a virus-cell interaction (Lo et al., 2022). However, the observation that C4d, sC5b-9, and C5a levels correlate with antiviral antibodies, but not with viral load (Holter et al., 2020), suggests that, while it may play a role early in infection, direct viral activation is relatively unimportant in severe disease. As with C3a and the larger inactivation products of C3/C3b, discussed above, C5a may be a less reliable marker of C5 activation than sC5b-9. Supportive of this is the observation that, in tracheal fluid, reflecting upper respiratory tract events, sC5b-9, but not C5a, is elevated in patients (Huber et al., 2021), and that, in some patients, sC5b-9 levels remain high after C5a levels have fallen (Cugno et al., 2020).

One interesting observation that came out of a comparative study of tissue biopsies of COVID-19 patients who died from respiratory failure was that C5b-9 deposition in the lung mirrored deposition in the kidney and liver, illustrating multi-organ involvement in the disease (Macor et al., 2021). Confirmation of C5b-9 deposition in the kidney (along with C3) has been provided by others (Jamaly et al., 2021; Diao et al., 2021). C5b-9 deposition has also been correlated with disease severity (Jarlhelt et al., 2021). Analysis of heart biopsy material from COVID-19 patients who died following major cardiac injury, and comparison of this with heart biopsy material from patients who suffered non-COVID-19-related cardiac injury, showed that the COVID-19 material was unique in that microthrombi contained significantly greater amounts of C5b-9 (Pellegrini et al., 2021). In a small study of 5 subjects with severe COVID-19, C5b-9 deposition in lung tissue was accompanied by C5b-9 deposition in both purpuric skin lesions and in normal skin, yet another indication of uncontrolled systemic complement activation, at least in some patients (Magro et al., 2020). Microthrombi with deposited C5b-9 were seen in skin biopsies of severe, but not mild or moderate COVID-19 patients, nor in patients with non-COVID sepsis-related ARDS or acute kidney injury. This deposition paralleled that of MASP-2 (Laurence et al., 2022).

Although not understood, genetic associations between COVID-19 and C5a and sC5b-9 levels have been made. The rs11385942 G > GA variant in chromosome 3 is linked to severe disease, and is associated with elevated C5a and sC5b-9; non-O blood group patients, again associated with severe disease, have elevated C5a (Valenti et al., 2021).

Possibly the most critical property of C5a and C5b-9 in COVID-19 disease is that of endothelial cell (EC) activation. This may be augmented by the SARS-CoV-2 spike protein, which can itself activate the endothelium and lead to enhanced leukocyte recruitment following increased adhesive molecule expression and thrombomodulin loss. Once a proinflammatory environment is established at the cell surface, C3a and C5a generation, and C3b and C5b-9 deposition are enhanced, amplifying the activation stimulus (Perico et al., 2022). In a prospective study, suspected COVID-19 patients attending a hospital ED were evaluated for markers of complement and EC activation. Patients subsequently diagnosed with COVID-19 had higher C5a and VCAM-1 and decreased E-selectin levels, with C5a and sC5b-9 higher in COVID-19 males vs. females. While elevated C5a and EC activation markers were seen in all ICU admissions and mortality, there was a greater association with COVID than with non-COVID patients, leading the authors to suggest that, in COVID-19, hyperactivation and consequent EC activation was more pronounced than in non-COVID ARDS (Bruni et al., 2022). That said, there appears to be an as yet identified, non-C5-derived, factor present in COVID-19 plasma that disrupts EC membrane integrity (Kovacs-Kasa et al., 2022).

Neutrophil extracellular traps (NETs) can provide a defence mechanism against infective organisms, and their formation (NETosis) can be triggered by C5a (Garcia et al., 2013). NET formation, along with sC5b-9, is increased in COVID-19 patients. These NETs are rich in active tissue factor (TF). Furthermore, incubation of platelet-rich COVID-19 plasma with human aortic endothelial cells (HAECs) and neutrophils generated NETS rich in TF, and induced thrombotic activity in the HAECs. This was inhibited by C5aR1 blockade and by an inhibitor of C3 activation (Skendros et al., 2020). Independent verification of C5a- (and C3a-) dependent induction of NETosis in HUVEC cells by COVID-19 sera has also been demonstrated, with NETosis blocked by anti-C3a and anti-C5a antibodies, and by carboxypeptidase B (C3a and C5a activities are dependent on their C-terminal arginine residues) (Zhang et al., 2021). An association of NETs with organ damage was provided in a study of COVID-19 patients who progressed to AKI. Cell-free DNA (a surrogate for NETs) was measured in the plasma of patients, and was significantly higher in those who developed AKI (Henry et al., 2022). In contrast, while neutrphils were implicated in ischemic stroke victims with COVID-19 disease, there was no correlation with either sC5b-9 or NETs (Genchi et al., 2022). Systemic NETosis has been correlated with systemic, but not with local (tracheal) C5a and sC5b-9 levels in COVID-19 disease (Huber et al., 2021). Direct C5b-9 induction of NETosis has also been reported; this can be blocked by exogenous (mesenchymal stem cell exosomal) CD59 (Loh et al., 2022).

There is much cross-talk between the complement and coagulation systems (see Li and Liu, 2021), most outside of the scope of the current review. However, some aspects are directly relevant to excessive C3a and C5a generation. One consequence of systemic complement hyperactivation is that C4b, C3b, and C5b-9 will be deposited in a bystander fashion on adjacent endothelial or blood cell surfaces at a sufficient rate to overwhelm self-defense provided by the membrane proteins CD35, CD46, CD55 and CD59. This has been discussed specifically with respect to CD59, C5b-9 deposition and the release of unusually-large vWF multimers from Weibel-Palade bodies. In the absence of ADAMTS13 cleavage, these multimers will recruit platelets to ECs and trigger localised thrombosis (Hang, 2021). Consistent with this, a decreased ADAMTS13/vWF ratio (possibly exacerbated by raised IL-6 levels was observed. IL-6 both inhibits ADAMTS13 production and its interaction with vWF), together with elevated C3a and sC5b-9, is a hallmark of severe COVID disease. In addition to promoting localised thrombosis, the EC-tethered ULVWF multimers may provide a platform for the AP/AL C3 and C5 convertases, amplifying C5a-mediated EC activation and NET formation (Fujimura and Holland, 2022). This sequence of events leading to a prothrombotic environment is highly similar to that of atypical haemolytic uremic sysndrome (aHUS), a genetic disease predominantly consequent on defective AP/AL regulation, and, in both cases, appears driven by the C5a/C5aR1 axis (Aiello et al., 2022). Platelets can also be activated by C5a; platelet activation by COVID-19 sera was inhibited C5aR1 blockade, demonstrating the importance of C5a in this disease setting (Apostolidis et al., 2022). Insufficient surface regulation could also account for the reported activation of monocytes, promoting further C5a release, generation of an autocrine signal through the C5aR1, NET formation, release of pro-inflammatory cytokines, and a pro-thrombotic stimulus (Patra and Ray, 2022).

Much of our understanding of the role of complement activation in COVID-19 comes from comparative dissection between COVID-19 and other viral infections leading to ARDS. One such study has suggested a link between excessive C3a production and induction of cytotoxic CD16 + T cells, with both being linked to mortality (Georg et al., 2022). The importance of the C3a/C3aR and C5a/C5aR1 axis in viral infection and inflammation was further highlighted in a study of the H5N1 influenza virus in mice. Acute lung inflammation was reduced to similar levels by both a C3aR antagonist and by an anti-C5a antibody (Sun et al., 2013). A

key role of the C5a/C5aR1 (CD88) interaction is the recruitment and activation of neutrophils and monocytes. Along with highly elevated levels of C5a in severe COVID-19, C5aR1 becomes highly expressed in myeloid cells, both systemically and locally, in the lung. Anti-C5aR1 antibodies blocked myeloid cell activation and acute lung injury in human C5aR1 knock-in mice, lending further support for a role of excessive C5a production in COVID-19-related ARDS (Carvelli et al., 2020).

Multisystem Inflammatory Syndrome (MIS), a condition with close similarities to Kawasaki disease, can occur in children several weeks after COVID-19 infection. In addition to a well-defined immune cell signature, sC5b-9 is highly elevated, indicative of aggressive complement activation, and pointing to complement as a component of disease pathology (Syrimi et al., 2021). Comparison of the complement genetic signature of pediatric MIS patients with hospitalised and non-hospitalised COVID-19 pediatric patients has identified associated SNPs in *CFB* and *CFH* in MIS, but, interestingly, not in membrane-bound regulators, indicative of defective fluid phase AP/AL regulation. The authors suggested that this might lead to defective opsonisation and virion clearance (Gavriilaki et al., 2022). Others have defined MIS-C as a unique manifestation of SARS-CoV-2 infection in children, distinct from COVID-19. This distinction is however defined by blood cells, and not by complement activation profile. sC5b-9 is highly elevated in pediatric COVID-19, but less so in MIS-C when compared to mild disease (Diorio et al., 2020).

"Long COVID" or the "post COVID-19 condition" affects approximately 65 million people worldwide. It is characterised by multiple symptoms including fatigue, dyspnea, joint or chest pain, cognitive difficulties such as headaches, and "brain fog", with impacts on multiple organ systems. Since the underlying mechanism is not defined, and patients display heterogeneous symptoms, there are no established diagnostic criteria other than symptoms persisting for more than 12 weeks (https://www.who.int/europe/news-room/fact-sheets/item/post-COVID-19-condition#:~:text=no%20other%20explanation.-,Symptoms,an%20impact%20on%20everyday%20functioning). While very little is understood about the mechanisms that lie behind the condition, it has been suggested that the SARS-CoV-2 S protein might be released from infected cells and be transported to uninfected tissues and organs, where it can provoke damaging responses. In support of this, infusion of the S protein into the mouse brain initiates complement-dependent engulfment of synapses and a delayed impairment of cognitive function (Fontes-Dantas et al., 2023). A different hypothesis for the pathogenic mechanism of long COVID is that virus persistence leads to generation of autoantibodies that target the endothelium and lead to endothelial dysfunction (Castanares-Zapatero et al., 2022; Wang et al., 2021; Davis et al., 2023; Chen et al., 2021). However, a recent comprehensive study failed to identify autoantibodies as the main contributor to long COVID. In this study, plasma complement C4d levels were significantly higher in long COVID cases (on average, more than a year after the initial infection) compared to convalescent and healthy controls, suggesting ongoing complement activation (Klein et al., 2022). Although the complement system plays a key role in immunity, and its hyper-activation is well characterised in COVID-19 pathogenesis, it is yet to be established whether a role for complement will extend to long COVID (Siggins et al., 2023). Patients with pulmonary fibrosis associated with long COVID have elevated sC5b-9 (also CRP), irrespective of the severity of their condition, but, interestingly, no elevation in IL-6 levels (Colarusso et al., 2021). Although not defined as "long COVID", a longitudinal study of recovered patients showed that persistent (up to 1 year) lung abnormalities were also associated with elevated C3 levels (Tsiftsoglou, 2021). Possibly relevant to Long COVID, it has also been noted that anti-S protein antibodies in convalescent subjects differ in their continued activation of C4, C3 and C5. Activation is seen in hospitalised, but not in non-hospitalised, convalescent patients. The association with IgG1 levels implicates the CP (Lamerton et al., 2022).

7. Complement and COVID-19 vaccination

There can be no doubt that the advent of COVID-19 vaccines marked the turning point in control of the pandemic, with an immediate impact on patient numbers, disease severity, and mortality. That said, there have been a number of adverse events associated with SARS-CoV-2 vaccination, many of which appear to have a complement-driven etiology (Mastellos et al., 2021). While association does not necessarily align with causation, it is nevertheless appropriate to examine the data, if only to be better positioned to mitigate against potentially damaging side effects.

Perhaps the most informative data (from a complement viewpoint) comes from Gerber and colleagues, who reported severe breakthrough hemolysis in 4 PNH patients following vaccination (Pfizer/Biontech, BNT162b2, and Moderna, mRNA-1273; both mRNA vaccines expressing the S-protein) despite all receiving anti-C5 therapy. One of the patients was participating in a clinical trial of danicopan, an oral Factor D inhibitor, and did not experience breakthrough hemolysis after the first vaccine dose while still taking danicopan, but did after the second vaccine dose, when danicopan treatment had been temporarily suspended (Gerber et al., 2021). A similar report of breakthrough hemolysis following vaccination (with the same mRNA vaccines) came from Japan. Interestingly, breakthrough hemolysis was only seen in untreated (for PNH) patients, although one patient, on anti-C5 therapy, experienced increased hemolysis that failed to meet breakthrough criteria (Kamura et al., 2022). Other reports of breakthrough hemolysis following vaccination come from the University of Washington (Portuguese et al., 2021) and from Italy (Cavallaro et al., 2022), the latter report noting also that breakthrough hemolysis is also seen in some cases of natural COVID-19 infection in anti-C5-treated PNH patients.

Observations in vaccinated atypical haemolytic uremic syndrome patients, another relapsing disease with a strong AP/AL dysregulation link, are also informative. As with PNH, COVID-19 infection alone can be sufficient to provoke a relapse (Boldig et al., 2022). However, a report of 3 patients vaccinated either with Pfizer/BioNTech (BNT162b2, mRNA) or AstraZeneca (ChAdOx1 nCoV-19, adenoviral vector), vaccines, both against the S protein, states that the TMA and AKI that they developed (2 cases of relapse in known aHUS patients, 1 de novo case) occurred in the absence of any other know precipitating factors. Two possible additional cases, less clear-cut in their symptomology, were noted (Bouwmeester et al., 2022). Additional reports of vaccine-associated aHUS include a patient with a homozygous CFHR3/CFHR1 gene deletion (ChAdOx1 nCoV-19) (Ferrer et al., 2022), and a *de novo* case in a patient with a pre-disposing C3 variant (mRNA-1273, booster dose) (Claes et al., 2023).

Insight into complement involvement in vaccine-related AEs also comes from observations of patients with hereditary angioedeme (HAE). HAE is a disease consequent, in most cases, on reduced C1-INH levels. Analysis of a Dutch cohort of adult HAR patients, most on long-term prophylaxis with C1inh and/or danazol, recorded that 10 (of 63) patients experienced breakthrough HAE attacks following vaccination (vaccines used were BNT162b2, mRNA-1273, both mRNA vaccines, and Ad26.COV2.S, an adenoviral vector vaccine) (Fijen et al., 2021). The most plausible explanation of this is that vaccination induced increased consumption of C1inh, possible through MASP or C1 protease activation.

Evaluation of a single patient who suffered a post-vaccine (ChAdOx1) immune thrombotic thrombocytopenia stroke, and comparison with 13 other non-vaccine-induced stroke victims, showed that she alone had C1q and C3d deposition, indicative of CP activation (de Buhr et al., 2022). Others have suggested that vaccine-induced complement activation, particularly in cases of TMA, is secondary to formation of anti-platelet factor 4 antibodies (Pitkänen et al., 2021). While this might explain late vaccine-induced TMAs, it cannot account for early onset events. Biopsies of subjects experiencing peripheral neuropathies following vaccination showed C4d deposition on the vascular

endothelium. While not necessarily indicative of complement involvement in pathology, it does again demonstrate that there has been vaccine-induced CP and/or LP activation (Safavi et al., 2022). Additional diseases with a possible complement-driven component in their pathology have also been linked to vaccination. Disease activity flares (TMA) following COVID-19 vaccination have been reported for immune thrombocytopenia (ITP) and acquired von Willebrand Disease (mRNA-1273, mRNA; ChAdOx1 nCoV-19, adenoviral; Ad26.COV2.S, adenoviral, Johnson and Johnson) (Portuguese et al., 2021; Shazley and Alshazley, 2021; Pai et al., 2021; Tiede et al., 2021), for autoimmune haemolytic anemia (ChAdOx1 nCoV-19) (Simon et al., 2022), for adult paroxysmal cold hemoglobinuria (BNT162b2), with C3 deposition on erythrocytes (Misawa et al., 2022), for IgA vasculitis (BNT162b2), with C3 deposition evident in skin biopsies (Rista et al., 2023), in tubulointerstitial nephritis with dense deposit disease (BNT162b2), again with glomerular C3 deposition (Nakamura et al., 2022), and in optic neuromyelitis (AZD1222) (Hernandez-Vega et al., 2022). As the authors of the last cited example correctly state, coincidental occurrence of these disease flares with vaccination cannot be ruled out. However, the temporal association of vaccination with such a wide range of conditions with known complement etiology, together with demonstration of complement involvement, is strongly indicative of vaccine-induced complement activation, of sufficient strength, to induce disease flares.

Allergic or intolerance reactions to vaccines are not new, but what is potentially different about these is that they appear to represent a complement-activation-related pseudoallergy (CAPRA) (Altrichter et al., 2021), and possibly driven not by a reaction to the polyethylene glycol or polysorbate stabiliser contained in the vaccine, as suggested by others (Altrichter et al., 2021; Klimek et al., 2021; Ibrahim et al., 2022), but directly by the expressed S protein antigen. Little is known about the complement profile in the above presumed CAPRA events, but in one report (BNT162b2 vaccine), very high C3a levels were measured in all 3 patients who experienced this AE (Lim et al., 2021). This finding, of high C3a (and also sC5b-9) could be recapitulated, using the same BNT162b2 vaccine, in the pig (Dézsi et al., 2022). In a more detailed analysis of an AE in a single patient following a ChAdOx1 nCov-19 vaccination, CP and LP activity were both absent, and sC5b-9 levels were significantly elevated. While AP/AL activity was slightly reduced, native C2 (detected by immunoblotting) was totally absent (Cugno et al., 2021), indicating that the initial activating principle triggered either the LP, or the CP, or both. IVIg treatment restored a normal complement profile. Rhabdomyolysis as the first symptom of TMA has also been reported as a very rare AE of vaccination {mRNA-1273 vaccine). Complement involvement was suspected because of a depleted CH50 and C3 deposition in the kidney (Kamura et al., 2022). A second case (SARS-CoV-2 ChAdOx1 vaccine) presented similarly, but complement parameters were not assessed (Cirillo et al., 2022). Treatment of both patients included eculizumab, but neither recovered.

It is possible that all of these reactions are simply a reflection of a generalised inflammatory response, something seen in recipients of many vaccines. The argument against this is the degree of complement-involvement, against all COVID-19 vaccines, in response to S protein expression. Instead, the data point to poorly-controlled systemic C3 activation by the vaccine-induced expression of the S protein. This is in keeping with data discussed above that point both to direct activation of the LP by the S protein, a possibility also suggested by others when looking at post-vaccination skin biopsies in subjects who had experienced adverse cutaneous reactions to mRNA vaccines (BNT162b2 and mRNA-1273) (Magro et al., 2021), and potent CP activation. Interestingly, vaccine responses to the S protein appear to elicit antibodies with greater potency to activate complement than those elicted through natural infection, with higher levels of IgG1 and IgG3 (Klingler et al., 2021). This may change with second and subsequent booster vaccinations, where the proportion of poorly-complement-activating non-inflammatory IgG4 antibodies increases significantly (Irrgang et al., 2023), possibly indicative of a decreased risk of complement-mediated AEs. The SARS-CoV-2 spike protein is highly glycosylated (Wang et al., 2020), and antibodies elicited following vaccination with mRNA vaccines appear to have altered glycosylation patterns to those elicited by natural infection, with enhanced C1q binding (and, presumably, CP activation) (Farkash et al., 2021).

There is no doubt that the introduction of vaccines had a massive beneficial impact on both on virus spread and on disease severity. However, the vaccine humoral response appear to be relatively short-lived As the strongest correlate of protection is with the humoral antibody response (Lai et al., 2022), one consequence is that frequent booster vaccinations are likely to be required, particularly amongst the most vulnerable populations. As the virus continues to evolve rapidly, it is likely that, as with the influenza virus, the vaccine "composition" will need regular revision. With this scenario, one possible way to reduce unwelcome complement-mediated side effects might be to engineer out sites responsible for LP activation, for example, specific N-linked glycosylation sites, or to modify them in such a way as to minimise complement-activating potential, without affecting overall immunogenicity. The suggestion of including a complement inhibitor with vaccination (Chang and Hawley, 2021) seems less feasible, unless targeted at specific "at risk" populations such as those with PNH or aHUS.

8. Clinical observations with complement therapeutic intervention

Taken together, the above data derived from coronavirus studies and observations, pre-COVID-19 pandemic, and from early observational studies in patients hospitalised with COVID-19 infection, a strong case for evaluation of complement-directed therapeutic intervention is made. This is reflected in the number of clinical trials with complement inhibitors that have been initiated These, together with their status, are listed in Table 1. They can be grouped as trials directed at inhibition of key effector molecules of complement in COVID-19 (anti-C5, anti-C5a), those inhibiting amplification of C3 activation, and those blocking activation pathways. Additionally, a number of case reports of "compassionate use" of complement-directed agents have been reported. These generally comprise low patient numbers, often with loosely-defined entry and/or outcome criteria, making interpretation difficult. Nevertheless, it is worthwhile noting what has been learned from these.

Early in the pandemic, it appeared that significantly elevated C5 activation, with release of the highly pro-inflammatory C5a peptide, might provide the initial effector trigger behind damaging complement-dependent responses to COVID-19 infection, and a number of reports describing efficacy of C5-directed molecules have been published. One of the earliest reports came from the ASL Napoli Nord study, in which the outcome of 4 patients who were treated with up to 4 infusions of eculizumab, an anti-C5 mAb, was detailed. These patients, with a confirmed diagnosis of SARS-CoV-2 infection, were admitted to ICU suffering from severe pneumonia or ARDS, and were on non-invasive ventilation at the time of treatment. All recovered, with a fall in inflammatory markers (for example, CRP levels falling by around 75% from 14.6 mg/dl to 3.5 mg/dl). In addition to anti-C5, these patients were treated with multiple additional agents, including an anticoagulant, an antibiotic and anti-virals, making the contribution of eculizumab to recovery difficult to define (Diurno et al., 2020). This study was closely followed by a report of the treatment of a small number of subjects with COVID-19-related ARDS with either a combination of eculizumab and ruxolitinib, a JAK1/2 inhibitor (n = 7), or with best available therapy (n = 10). Patients treated with the novel combination, when compared with the best available therapy group, had significantly improved respiratory function, reduced pulmonary lesions, and a decrease in circulating D-dimer levels (a fibrin degradation product and marker of coagulation) (Giudice et al., 2020). Other have used eculizumab to treat critically ill COVID-19 patients, non-responsive to standard of care therapy, but with documented lectin pathway

Table 1
Clinical Trials in COVID-19 with Complement-Directed or Complement-Related Inhibitors.

Trial Number	Title	Drug	Target	Type	Status
NCT04355494	SOLIRIS® (Eculizumab) Treatment of Participants With COVID-19	Eculizumab	C5	mAb	No longer available
NCT04802083	COVID-19 Soliris Expanded Access Protocol	Eculizumab	C5	mAb	No longer available
NCT04369469	Efficacy and Safety Study of IV Ravulizumab in Patients With COVID-19 Severe Pneumonia	Ravulizumab	C5	mAb	Terminated
NCT04288713	Eculizumab (Soliris) in Covid-19 Infected Patients	Eculizumab	C5	mAb	Available
NCT04346797	CORIMUNO19-ECU: Trial Evaluating Efficacy and Safety of Eculizumab (Soliris) in Patients With COVID-19 Infection, Nested in the CORIMUNO-19 Cohort	Eculizumab	C5	mAb	Unknown status
NCT04570397	Ravulizumab and COVID-19	Ravulizumab	C5	mAb	Recruiting
NCT04390464 /EudraCT 2020–001354-22	mulTi-Arm Therapeutic Study in Pre-ICu Patients Admitted With Covid-19 - Repurposed Drugs (TACTIC-R)	Ravulizumab	C5	mAb	Ongoing/phase 4
NCT04382755/ EudraCT 2020–002130-33	Zilucoplan® in Improving Oxygenation and Short- and Long-term Outcome of COVID-19 Patients With Acute Hypoxic Respiratory Failure	Zilucoplan	C5	peptide	Completed
NCT04590586	Industry Alliance Platform Trial to Assess the Efficacy and Safety of Multiple Candidate Agents for the Treatment of COVID-19 in Hospitalized Patients	Zilucoplan	C5	peptide	Completed
EudraCT 2020–001736-95	ACCORD 2: A Multicentre, Seamless, Phase 2 Adaptive Randomisation Platform Study to Assess the Efficacy and Safety of Multiple Candidate Agents for the Treatment of COVID 19 in Hospitalised Patients	Zilucoplan	C5	peptide	Not EU - Status not listed
NCT04369820	C5a Receptor Expression - COVID-19 (C5-COV) (C5-COV)	C5a receptor expression on myeloid cells	C5aR	Gene therapy	Unknown
NCT04333420	Randomized, Controlled Study of IFX-1 in Patients With Severe COVID-19 Pneumonia (PANAMO).	IFX-1	C5a	mAb	completed
NCT04395456	A Study of the C3 Inhibitor AMY-101 in Patients With ARDS Due to COVID-19 (SAVE)	AMY-101	C3	peptide	Not yet recruiting
EudraCT 2020–001550-22	A Phase 2 Clinical Trial to Assess the Safety and Efficacy of Complement 3 Inhibitor, AMY-101, in patients with Acute Respiratory Distress Syndrome (ARDS) due to Covid-19.	AMY-101	C3	peptide	Ongoing
EudraCT 2020–004408-32	ITHACA: A phase 2, randomized, single-blind, placebo-controlled clinical trial to evaluate the safety and efficacy of the complement C3 inhibitor, AMY-101, in COVID-19 patients with acute respirato…	AMY-101	C3	peptide	Prematurely Ended
NCT04402060	A Study of APL-9 in Adults With Mild to Moderate ARDS Due to COVID-19	APL-9	C3	peptide	Completed
NCT04988035	ACTIV-5/Big Effect Trial (BET-C) for the Treatment of COVID-19	Danicopan	FD	Small molecule	Completed
NCT05298787	A Phase 1 SAD/MAD Study of RLS-0071 in Healthy Volunteers in Support of a COVID-19 Development Program	RLS-0071	C1 NETs	peptide	Completed
NCT04705831	Study to Evaluate the Benefit of RUCONEST in Improving Neurological Symptoms in Post COVID-19 Infection	Ruconest	C1/MASP/ kallikrein/Factor XII	protein	Recruiting
NCT04530136	Prevention of Severe SARS-CoV-2 Infection in Hospitalized Patients With COVID-19	Ruconest	C1/MASP/ kallikrein/Factor XII	protein	Completed
EudraCT 2021–001655-13	SARS-CoV2 vaccination and activation of the coagulation system (C1inh)	C1 inhibitor	C1/MASP/ kallikrein/Factor XII	protein	Ongoing
NCT04414631	Conestat Alfa in the Prevention of Severe SARS-CoV-2 Infection in Hospitalized Patients With COVID-19	Conestat Alfa	C1/MASP/ kallikrein/Factor XII	peptide	Terminated
EudraCT 2020–002225-29	Evaluation of the effects of bradykinin antagonists on pulmonary manifestations of COVID-19 infections. (C1inh; Icatibant)	Icatibant C1 inhibitor	Bradykinin receptor C1/MASP/ kallikrein/Factor XII	Small molecule	Completed
NCT05010876	Evaluation of the Effects of Bradykinin Antagonists on Pulmonary Manifestations of COVID-19 Infections (AntagoBrad-Cov Study).	Icatibant C1 inhibitor	Bradykinin receptor C1/MASP/ kallikrein/Factor XII	Small molecule	Completed
NCT04978051	Investigating the Efficacy and Safety ICATIBANT For The Treatment of Patients With SARS-CoV-2 (COVID-19) Infection	Icatibant	Bradykinin receptor	Small molecule	Completed
NCT05407597	Inhibition of Bradykinin in COVID-19 Infection With Icatibant	Icatibant	Bradykinin receptor	Small molecule	Recruiting
NCT04871646	Phase 3 Clinical Trial to Evaluate the Efficacy and Safety of CKD-314	Nafamostat	Serine proteases/ FD	Small molecule	Recruiting
NCT04628143	A Study Evaluating the Efficacy and Safety of CKD-314 in Hospitalized Adult Patients Diagnosed With COVID-19 Pneumonia	Nafamostat	Serine proteases/ FD	Small molecule	Completed

(*continued on next page*)

Table 1 (*continued*)

Trial Number	Title	Drug	Target	Type	Status
NCT04390594	Efficacy and Safety Evaluation of Treatment Regimens in Adult COVID-19 Patients in Senegal	Nafamostat	Serine proteases/ FD	Small molecule	Recruiting
NCT04623021	A Study Evaluating the Efficacy and Safety of CKD-314 (Nafabelltan) in Hospitalized Adult Patients Diagnosed With COVID-19 Pneumonia	Nafamostat	Serine proteases/ FD	Small molecule	Completed
NCT04483960	Australasian COVID-19 Trial (ASCOT) ADAptive Platform Trial	Nafamostat	Serine proteases/ FD	Small molecule	Recruiting
NCT04352400	Efficacy of Nafamostat in Covid-19 Patients (RACONA Study)	Nafamostat	Serine proteases/ FD	Small molecule	Recruiting
NCT04473053	DEFINE - Evaluating Therapies for COVID-19	Nafamostat	Serine proteases/ FD	Small molecule	Active, not recruiting
NCT04418128	Clinical Efficacy of Nafamostat Mesylate for COVID-19 Pneumonia	Nafamostat	Serine proteases/ FD	Small molecule	Unknown status
EudraCT 2020–002570-27	A Randomized Clinical Trial of Nafamostat: A Potent Transmembrane Protease Serine 2 (TMPRSS2) Inhibitor for the Treatment of Covid-19	Nafamostat	Serine proteases/ FD	Small molecule	Ongoing
EudraCT 2020–002230-32	Rapid Experimental Medicine for COVID-19	Nafamostat	Serine proteases/ FD	Small molecule	Not EU- Status not listed

Studies with Nafamostat have been included as, while a relatively non-specific serine protease inhibitor, it does have inhibitory activity against many of the early proteases of the complement system as well as against some coagulation proteases.
Data from the NCT04333420 Trial with IFX-1 have recently been published (Carvelli et al., 2022; Lim et al., 2022).

activation, and MASP-2, C4d and C5b-9 deposition in the endothelial microvasculature. All patients experienced a fall in circulating D-dimer levels and in neutrophil counts, but in only 1 (of 3) was a complete remission seen. Despite eculizumab therapy, one died with respiratory failure and one, though experiencing improved respiratory function, had continued renal failure (Laurence et al., 2020). Comparison of 10 eculizumab-treated patients with 65 contemporary controls, with similar complement and coagulation baseline parameters, demonstrated improvement in respiratory performance, an improvement that correlated with a drop in sC5b-9, a marker of C5 activation. In the eculizumab-treated group, death or chronic complications were seen in 4/10 patients, whereas in the untreated group this rose to 52/65 patients (Ruggenenti et al., 2021). A larger proof-of-concept study, evaluating the impact of eculizumab therapy on COVID-19 patients in ICU, found that patients treated with eculizumab plus standard of care (n = 35) had enhanced survival at day 15 (82.9% vs. 62.2%) when compared with patients receiving standard of care only (n = 45). In general, patients receiving eculizumab showed greater improvement in oxygenation and reduction in inflammation (Annane et al., 2020). Compassionate use of a different anti-C5 mAb, LFG316, in critically-ill mechanically-ventilated COVID-19 patients has also been reported. In this, and unlike the above reports, patients, selected for high circulating sC5b-9 levels, were treated with a single infusion of the mAb only, calculated to be sufficient to provide inhibition of C5 activation for around 4 days. One patient, with multiple co-morbidities, clinically non-responsive to LFG316, and who subsequently died, was found to have an occult Klebsiella infection. The remaining 4 patients showed marked clinical improvement, and significant reduction in CRP levels. The significance of this report is that it suggests that it might be possible to break the damaging complement-dependent hyper-inflammatory cycle in severe COVID-19 disease with minimal intervention, minimising any inhibition-related infection risk and other long-term sequelae of complement inhibition (Zelek et al., 2020). Other case reports of successful intervention or rescue therapy with eculizumab have been reported (Pitts, 2021), and include pediatric cases of ARDS (Raghunandan et al., 2020) and renal failure (Mahajan et al., 2020), transplantation (Gill et al., 2022), cardiac failure associated with thrombotic microangiopathy (TMA) (Utebay et al., 2021), antiphospholipid syndrome (APS) (Chidharla et al., 2021), pregnancy (Burwick et al., 2022), COVID-19-triggered aHUS (Leone et al., 2022; Dawudi et al., 2022) and myasthenia gravis (Chidharla et al., 2021). One possible confounder in treatment of COVID-19 patients with eculizumab is that of plasma C5 concentration. Preliminary pharmacokinetic and pharmacodynamic data from an ongoing study with ravulizumab, a second-generation anti-C5 mAb, a follow up to eculizumab with an increased plasma half-life, has suggested elevated plasma levels of circulating C5 in severe COVID-19 (McEneny-King et al., 2021). Blockade of C5a-dependent effects while leaving the lytic pathway intact is also possible with an anti-C5a mAb, IFX-1 (vilobelimab). Preliminary data from an ongoing study with this has been published; analysis of an initial 30 patients treated with the antibody showed that it was safe, that it successfully neutralised C5a (Vlaar et al., 2022), and that there was a trend in favour of improved oxygenation and reduced mortality when compared with the control group (Vlaar et al., 2020). What is notable in these reports, however, is that, while improvement was seen in many patients, inhibition of C5 activation alone was not sufficient for effective management of COVID-19 disease in all.

While excessive C5 activation and C5a generation appear to be critical mediators of a damaging complement response, and hence a logical point for therapeutic intervention, successful intervention with upstream inhibitors of C5 activation has also been described. As all activation pathways merge at the level of C3, and C3 activation is a prerequisite for C5 activation, inhibition of C3 activation might be an effective therapeutic approach. Early in the pandemic, a case report of successful intervention in severe COVID-19-associated ARDS with AMY-101, a compstatin-based inhibitor of C3 activation, was reported (Mastaglio et al., 2020). A rapid decrease in inflammatory markers such as CRP was paralleled by an improvement in lung function. A follow-up report confirmed and extended observations made in this single patient, and also compared AMY-101 with eculizumab. In this, the authors claimed broader benefit for patients with inhibition of C3 vs. C5, particularly with respect to normalisation of neutrophil and lymphocyte parameters (Mastellos et al., 2020). Far larger numbers of patients would, however, be required for this claim to be verified.

As discussed above, there is considerable evidence that the lectin pathway provides a key complement activation pathway following SARS-CoV-2 infection, particularly in the naive host. This prompted the evaluation of narsoplimab, an anti-MASP-2 mAb, in COVID-19 patients receiving either continuous positive airway pressure or intubation therapy. 6 patients were treated, with a reduction in inflammatory markers such as CRP and IL-6. All treated patients survived whereas mortality was seen in parallel control groups (Rambaldi et al., 2020). Narsoplimab also has anti-coagulant properties, suggesting that it might have a dual role in suppressing both dysregulated complement activation and dysregulated coagulation, a second hallmark of severe COVID-19 disease (Rambaldi et al., 2020). Consistent with this, in the above study a decrease in circulating endothelial cells (a marker of vascular endothelium damage) was also seen.

The dual role that MASP-2 plays in both lectin pathway activation, activating both C4 and C2 to form the classical pathway C3 convertase (Héja et al., 2012), but also in coagulation, activating prothrombin to

thrombin (Gulla et al., 2010) is one of many examples of crosstalk between the complement and coagulation systems, a feature epitomised by C1-INH (Kaplan and Ghebrehiwet, 2021; Bekassy et al., 2022). C1-INH is not only an inhibitor of C1r, and C1s, the proteolytic components of C1, but also of the MASP proteases MASP-1 and MASP-2, of plasma kallikrein, and of coagulation Factor XII. Given this profile, and of the apparent dysregulation of not just the complement system, but also the kallikrein/kinin and coagulation systems in COVID-19 (Schüller et al., 2021), upstream intervention with C1-INH is also a logical therapeutic option (Nilsson et al., 2022; Urwyler et al., 2022). A large online self-reporting study of the incidence of COVID-19 in patients with hereditary angioedema (HAE) and non-HAE household members, conducted in the USA, is supportive of a role for C1-INH in combating COVID-19 infection. In addition, it provides insight into the relative susceptibility to infection of C1-INH-deficient HAE patients, and HAE patients with normal C1-INH levels (primarily C1-INH-resistant Factor XII subjects), the latter being at greater risk of infection. While COVID-19 severity was similar in all 3 groups, HAE subjects taking prophylactic medication (sub-cutaneous C1-INH or Icatibant, a bradykinin B2 receptor antagonist) had a reduced risk of infection over those taking no medication (Veronez et al., 2021). One possible interpretation of these data is that possession of sufficient C1-INH activity at the time of infection resulted in asymptomatic (and hence unreported) disease.

Intravenous administration of Conestat alpha, a C1-INH preparation, licensed for use in hereditary angioedema (HAE), in severe COVID-19 patients, resulted in an improvement in inflammatory markers, and stabilisation or improvement in respiratory symptoms in 4 of 5 patients. The fifth required ventilation, but all recovered (Urwyler et al., 2020). These observation triggered a larger multi-centre study with the same agent (Urwyler et al., 2021). A separate study, of 30 severe patients, compared a short treatment with C1-INH (plasma-purified, Behrinert, 10 patients) with a bradykinin antagonist (Icatibant, 10 patients) and with standard care (no kinin-directed therapeutic, 10 patients). Both treatments were well-tolerated and judged safe, and, while not achieving significance in time to clinical recovery, did bring improvement in lung tomography and blood eosinophils, markers for disease recovery (Sipka et al., 2020). Concomitant targeting of 3 different systems in COVID-19 disease (complement, coagulation and kinin) was well tolerated.

While encouraging, these data with C1-INH suggest that it alone might not be sufficient to control the complement-driven aspects of COVID-19. One possible reason for this could be that C1-INH was underdosed – C1-INH is a relatively abundant complement protein. More intriguing is the possibility that dysregulated coagulation, with concomitant activation of plasminogen, leads to C1-INH consumption. Plasmin, previously thought to be inhibited by C1-INH, actively consumes C1-INH without itself being inhibited (Wallace et al., 1997). The plasmin-cleaved inhibitor persists in the circulation, and many evaluations of C1-INH levels in COVID-19 disease will fail to differentiate between active and inactive protein. The high D-dimer levels seen in severe COVID-19 (Lipcsey et al., 2021) are indicative of significant plasminogen activation, highlighting the possibility that, in COVID-19, plasmin is poorly regulated, and contributes to defecient regulation of both the CP and the LP. Lung transcriptomic data lend further support for a crucial plasmin/complement interaction in COVID-19 (Mukund et al., 2020). The possibility that C1-INH consumption, albeit through unspecified mechanisms, lies behind many of the systemic abnormalities seen in COVID-19 has also been proposed by others (Thomson et al., 2020).

Similar considerations provide a case for intervention with inhibitors specific for the kallikrein/kinin system. While these will not address complement-driven pathology directly, they may ameliorate common pathological features consequent on increased vascular permeability. The impact of Factor XII on both plasminogen activation and on kinin generation may be exacerbated by the binding of Factor XII and high molecular weight kininogen (Savitt et al., 2021).Clinical trials with Icatibant, (Sipka et al., 2020; Mansour et al., 2021; Malchair et al., 2022) will provide insight into this hypothesis. Lanadelumab, a monoclonal antibody inhibitor of plasma kallikrein, has also been proposed as a therapeutic agent, with a clinical trial ongoing (Xu et al., 2020).

While the above small case studies and clinical trials involved very small numbers, no safety concerns arose, and the data was sufficiently promising to encourage further evaluation of complement-directed agents in larger controlled clinical studies. However, it should be noted that in many of these the standard of care was that practised in early stages of the pandemic, and that treatment options undoubtedly improved with increased knowledge of the disease. There was also likely wide variation in local practices early on, clouding comparative evaluation. What is apparent from these studies is that complement inhibition does not provide a magic bullet therapy. An open question that remains is whether there are subsets of patients that will, and others that won't, respond to complement inhibition, whatever the complement target might be.

While many of these studies involve the use of exploratory therapeutics, two of the drugs in question are already registered for clinical use. While these registrations are in rare diseases (anti-C5 (eculizumab/ravulizumab) in PNH, aHUS, NMO and MG; C1-INH (Conestat alpha, Berinert, Cinryze) in HAE), the nature of these diseases means that patients are maintained on the complement inhibitors. This, coupled with the widespread penetration of COVID into the population, means that significant numbers of patients on chronic complement inhibitor therapy will have been exposed to SARS-C0V-2. From a number of case reports it is clear that despite already being on complement-inhibitory therapeutic, disease can still develop, in some cases proceeding to death. For example, relatively early in the pandemic, the UK National PNH service in Leeds reported 4 cases of COVID-19, one of which proceeded to death (Pike et al., 2020). A similar report came from analysis of COVID-19 in Italian PNH patients. Three of the 156 patients surveyed contracted COVID-19 while receiving anti-C5 therapy, illustrating once again that C5 blockade does not prevent disease. While PNH patients did not appear to be at greater risk of contracting COVID-19, there was a suggestion that the disease might be more benign in PNH patients than in parallel non-complement inhibitor-treated individuals (Barcellini et al., 2021). A confounder in drawing this conclusion is that, because of their chronic therapy and the risk of meningococcal disease that this brings, PNH patients tend to be much more alert to infection risk, and its prevention, than the general population. Single case study reports with PNH patients on long-term anti-C5 therapy are mostly in accord with the above PNH registry studies (Schüller et al., 2021; Shikdar et al., 2021), but again, not in every case (Genthon et al., 2021). Similar findings have been published with respect to patients with aHUS-related kidney transplant recipients (Trimarchi et al., 2020; Bašić-Jukić and Atić, 2022; Cognard et al., 2021), notably in one case without resolution of endothelial cell damage, NMO (Cabal-Herrera and Mateen, 2021) and MG (Mimori et al., 2022; Alis et al., 2022), the latter particularly relevant as MG patients are at greater risk of poor outcomes in COVID-19. The situation with respect to HAE is more complex. In a survey of 1162 subjects, those with C1-INH deficiency-dependent HAE, had a similar incidence of COVID-19 to normal controls. However, when the HAE subjects were sub-divided into those treated prophylactically with a C1-INH preparation and those that were untreated, those receiving medication had a significantly-reduced infection rate compared to normals, and those not receiving medication had a significantly higher incidence of disease. Interestingly, HAE patients receiving icatibant (a bradykinin B2 receptor antagonist, bradykinin is released from high-molecular-weight kininogen by the enzymatic action of kallikrein), were similarly significantly protected against disease (Veronez et al., 2021).

Perhaps one of the most compelling arguments for a direct complement involvement, particularly for the AP/AL, in severe COVID-19 disease comes from analysis of the incidence of COVID-19 in AMD. AMD is a highly prevalent disease of the elderly, with over 50% of the genetic risk attributed to AP/AL proteins, particularly regulators of

amplification. At its most basic level, risk of developing AMD is strongly linked to a reduced ability to regulate alternative pathway activation. In an elegant retrospective analysis of COVID-19 patients, Ramlall and colleagues showed that morbidity and mortality in COVID-19 showed an association with macular degeneration (Ramlall et al., 2020; Ramlall et al., 2020). SNPs associated with decreased levels of C4bp and CD55, respectively key soluble and membrane-bound regulators of the classical pathway C3 convertase and, in the case of CD55, of the alternative pathway C3 convertase correlated with hospitalisation. Interestingly, the same study also found an association between a history of coagulation disorders and COVID-19 related morbidity and mortality.

9. Wider implications

Initial hospitalisation of patients with COVID-19 is predominantly because of respiratory distress. In severe cases this can develop into a COVID-19 induced pneumonia and a need for oxygen supplementation and/or ventilation. Acute respiratory distress can be consequent on a number of different respiratory insults, and whatever the etiology, the condition has become known collectively as Acute Respiratory Distress Syndrome, or ARDS. While recovery from pneumonia can be complete, recovery from ARDS is frequently associated with long-term injury to, and scarring of, the lungs.

Complement has long been implicated in ARDS pathology. For example, in 1980 it was shown that rabbits infused with complement-activated sera developed ARDS-like symptoms, symptoms not seen in rabbits infused with unactivated plasma (Craddock et al., 1977). In a guinea pig model of sepsis-induced ARDS it was demonstrated that development of the ARDS-like condition required systemic C5 activation (Hosea et al., 1980). In a small study of *E. Coli*-induced ARDS in non-human primates, an anti-C5a antibody was fully protective (Stevens et al., 1986). Later studies demonstrated that a LMW C5aR antagonist protected rats against ARDS in a CVF-induction model (Proctor et al., 2006). Finally, again in a small study, inhibition of C3 activation was protective against *E. Coli*-induced ARDS in baboons (Silasi-Mansat et al., 2015).

In a prospective study of patients at risk of ARDS, elevated C5a was described as a useful prognostic indicator of development of the syndrome (Hammerschmidt et al., 1980). Others described an elevation of sC5b-9 levels in septic patients before development of ARDS, and also, interestingly, also immediately prior to its resolution (Langlois and Gawryl, 1988). In 1989 a correlation between both AP/AL and CP activation and development of ARDS was described (Langlois and Gawryl, 1988). (Note: at this time the LP had not been discovered.) Other studies also implicated AP/AL activation in the development of ARDS (Mayes et al., 1984; Langlois and Gawryl, 1988) and, in the later study, again in its resolution. The degree of complement activation (measured by C3a and C5a levels) in patients at risk of ARDS has been correlated with severity of the subsequent insult (Weigelt et al., 1988). Possibly relevant to clinical observations with COVID-19, a prospective observational study of severe infection with H1N1 influenza virus showed that high MBL levels were associated with poor outcome, particularly mortality (Liang et al., 2021).

More recently, a number of animal studies have focussed on virally-induced ARDS, specifically influenza virus-induced disease. Mice infected with the highly pathogenic (in man) avian influenza virus H5N1 suffer an acute lung injury with C3, C5b-9 and MBL deposition, as well as up-regulation of MASP-2 and the C3a and C5a receptors, in affected lung tissue. Decomplementation with CVF, treatment with a C3a receptor antagonist, or treatment with an anti-C5a antibody, were all equally protective against lung injury (Sun et al., 2013). Infection of mice with an Influenza A virus resulted in apparently dysregulated complement activation and an induced acute lung injury. This was alleviated by blockade of the C5a-C5aR1 axis by an anti-C5aR1 antibody. Viral replication in lung tissue was also inhibited, and levels of proinflammatory cytokines such as IL-6 reduced (Sun et al., 2015). Infection of African Green Monkeys with the H7N9 influenza virus leads to both systemic inflammation and acute lung injury, both of which were significantly attenuated by treatment with a human/non-human primate cross-reactive anti-C5a monoclonal antibody (IFX-1). Attenuation of lung injury was accompanied by a reduction of viral titre in infected tissue (Sun et al., 2015). The role of C5a in ALI induced by highly pathogenic virus infection, including SARS-CoV-1 and MERS-CoV, is reviewed in depth by Wang et al. (2015).

Taken together, these data suggest that, in addition to in COVID-19, excessive complement activation in virus-dependent ARDS can cause disease pathology. However, amidst seemingly strong evidence for this, and particularly for C5 activation, as damaging in ALI and ARDS, one cautionary note needs to be aired. This comes from studies of H5N1 influenza infections in C3 sufficient and C3 deficient mice. While H5N1 influenza virus infected C3-sufficient mice showed increased C3 and C5 activation when compared with mice infected with either seasonal or pandemic 2009 H1N1 influenza viruses, parallel analysis of infection in C3-deficient mice showed a clear protective effect for C3 (O'Brien et al., 2009). As with COVID-19, it is possible that an initial protective complement response can be triggered into a damaging response, a response that is likely dependent on multiple, as yet unknown, factors.

10. Preparedness for next emerging viral pandemic

History suggests that COVID-19 will not be the last newly-emerging coronavirus with pandemic potential. COVID-19 continues to mutate, so far to more virulent but attenuated forms (de Groot et al., 2013; Agrawal et al., 2015; Matricardi et al., 2020) – nevertheless, mutations causing increased morbidity remain possible. While SARS-CoV-1 appears to have been eliminated in man (Wang et al., 2015), MERS infection remains significant in the Middle East (de Groot et al., 2013; Agrawal et al., 2015). Is this just one mutation away from generating a MERS-CoV virus with unimpaired morbidity but higher virulence, with global pandemic potential? In this context it is worth noting that it required only 6 coding mutations in SARS-CoV-1 to produce a mouse-adapted virus causing significant disease and mortality in BALB/c Mice (Roberts et al., 2007).

There is also increasing speculation, backed by some intriguing data, that the 1889–90 pandemic commonly known as the Russian (or Asiatic) 'flu was in fact not caused by an influenza virus, but by a coronavirus. During this pandemic – before the days of widespread and rapid international travel – around 1 million people are estimated to have died. As with COVID-19, the initial phase of the pandemic was followed by successive waves, these petering out in early 1895 (O'Brien et al., 2009). Symptoms described for the 1889 pandemic were highly similar to those seen in COVID-19 disease and, as with COVID-19 (but not with 'flu pandemics such as that of 1918–1919, which disproportionately affected the 25–40 year old age group (Liang et al., 2021; Berche, 2022), those most severely affected were the elderly. Detailed genetic analysis of coronaviruses from multiple host species has indicated that the HCoV-OC43 strain, a strain now a frequent causative agent of the common cold, likely diverged from a bovine coronavirus, becoming human-adapted, in the late 19th century (Brüssow and Brüssow, 2021). While far from conclusive, it is plausible that this 1890 pandemic was consequent on a species jump of CoV-OC43 from cattle into humans, and therefore that commonalities between COVID-19 and the 1890 pandemics are strong indicators of what one might expect to see in any future jump of a coronavirus from its "natural" host into man, an initial high morbidity and mortality, followed by attenuation to more virulent but less severe strains that provide sufficient immunoprotection against less virulent but more lethal strains (Vijgen et al., 2005). That said, it appears that HCoV-OC43 is still capable of causing severe disease in man. In a study reported in 1980, sera from 14,000 patients with acute respiratory infection of suspected viral origin were screened against a panel of viral antigens, including OC43. Significant elevations of anti-OC43 antibodies were recorded 45 patients, with the conclusion that in 30 cases disease could be directly associated with OC43 infection.

While the majority of patients had fever and respiratory distress, 8 (4 adults, 4 children) developed pneumonia, and 4 had neurological symptoms. In an additional 14 cases (5 with pneumonia) a suggestive association with OC43 was found (Brüssow, 2021).

Significant control of the COVID-19 pandemic has been achieved through vaccination. The first regulatory approval for a COVID-19 vaccine came in late-2020, and vaccine programs were rolled out rapidly through much of the world such that the most vulnerable, at least in the developed world, had been offered vaccination by late-spring, 2021. This rapid development came about because of the readiness of Governments and Pharmaceutical Companies to make significant at risk commitments so that successive phases of vaccine development could be initiated as soon as supportive data was available.. Nevertheless, it was approximately 12 months from first identification of COVID-19 as a novel disease and recognition of its severity and pandemic potential before the first vaccines became available (https://www.england.nhs.uk/2020/12/landmark-moment-as-first-nhs-patient-receives-covid-19-vaccination/#:~:text=Landmark%20moment%20as%20first%20NHS%20patient%20receives%20COVID%2D19%20vaccination,-8%20December%202020&text=The%20biggest%20vaccine%20campaign%20in,jab%20following%20its%20clinical%20approval), and up to a further 6 months before vaccination was widely available to any other than the most vulnerable. Accurate estimates of COVID-19 deaths during this pre-vaccination period are difficult to come by, but it seems probable that up to 6 million people died as a direct consequence of COVID-19 infection prior to widespread vaccine availability. It is difficult to envisage a faster vaccine development scenario that complies fully with all safety and regulatory requirements.

Success in vaccine development is also not guaranteed. Attempts to control the spread of coronavirus infections in domesticated animals through vaccination have been largely unsuccessful. And, possibly reflecting experiences now being seen with COVID-19-infected or COVID-19-vaccinated individuals, people infected with HCoV-OC43 do not develop a long-lasting immunity (Riski and Hovi, 1980).

At the outset of the COVID-19 pandemic, intensive care specialists were overwhelmed with the sheer volume of patients requiring acute life-saving interventions. There was little strong supportive data for many of the therapies tried and, understandably, tightly-controlled clinical trials were difficult to organise and run. Both claims of "success" and "failure" have, in many cases, been based on limited patient numbers, and are often anecdotal, communicated via press release. With this background, and while the tragic consequences of the COVID-19 pandemic are fresh in our minds, it is incumbent on the scientific and medical and pharmaceutical communities to work together, with urgency, to identify rescue therapies, and a therapeutic strategy, to apply, if required, in any future pandemic, and to consider in acute severe respiratory disease of viral origin.

11. What we don't know, and what we ought to know

In the preceding sections, what we know about complement involvement in COVID-19 is summarised. However, despite considerable knowledge being gained, there remain key questions, to which we ought to have answers in order to be able to provide best treatment options for seriously ill patients in the current pandemic, and to be best prepared for any future newly-emergent (coronavirus) pandemic.

Most importantly, is complement a key driver of disease severity? If it is not, complement-directed therapeutics will, at best, only be of benefit to a small sub-group of patients. In the initial phase of SARS-CoV-2 infection, in the naive host, complement activation, primarily through the LP, is likely to be protective. Protection may be augmented by AP/AL involvement and, once an antibody response has been mounted, through CP involvement. What is incontrovertible is that, in severe disease, at the time of hospital admission (primarily because of respiratory distress), hyper-activation of complement is occurring, through both early activation pathways (LP and CP) and the AP/AL amplification loop. The degree of activation correlates well with parameters of disease severity such as ICU admission, a need for mechanical ventilation, and mortality. While this in itself does not prove that complement hyper-activation drives disease pathology, it is highly suggestive of this. There appears to be two key components of this hyper-activation. The first relates to the degree of coordinated inhibition of the complement CP (C1r, C1s), LP (MASP-1, MASP-2), coagulation and kinin (kallikrein, factor XII) pathways by C1-INH. Factors that deplete functional C1-INH, such as excessive plasmin activity, demonstrated by high levels of D-dimer production, likely predispose to severe disease. Loss of C1-INH-mediated control of the LP and the CP will result in high levels of C3b production, providing a continual high input trigger to the AP/AL, and excessive kallikrein and factor XII activation, feeding kinin release and coagulation. The second key component to severe disease appears to be that of poor regulation of C3b-dependent amplification of C3 activation by the AP/AL, hinted at by increased susceptibility to severe disease in AP/AL-driven diseases such as AMD (Tuuminen et al., 2021), and substantiated by the high level of AP/AL-specific activation fragments such as Ba and Bb. The complexity of AP/AL regulation, with genetic variability in many different components, has led to the concept of an AP/AL complotype (Harris et al., 2012) and associated disease susceptibility. Combined with genetic variability in the coagulation and kinin pathways, is it possible that a complement/coagulation/kinin COVID-19 susceptibility genotype (a "covitype") can be defined? And, if it can, does it extend to other coronavirus diseases such as MERS and SARS? The answers to these questions ought to come from ongoing and/or retrospective genetic analysis of COVID-19 (and MERS and SARS) patients, and could be valuable in informing treatment options for hospitalised patients.

While there is no definitive proof that treatment with a complement-directed therapeutic could be beneficial in COVID-19, the number of case reports of success, outlined above, with agents directed at different complement targets, is highly suggestive that a complement-directed therapeutic can be protective in severe disease. It is therefore disappointing that larger studies have largely failed to substantiate these early findings, and it is pertinent to identify reasons why this might be so. First, it is possible that, by the time of hospitalisation, complement-triggered events will have progressed in many patients beyond the point at which they can be treated successfully with a complement inhibitor. Against this, in many of the case reports that we have discussed, intervention with a complement inhibitor was applied as a last resort treatment, and some apparent efficacy was still seen. Case reports tend to focus on successes, and it would be informative to know more about unsuccessful interventions. It is also possible that only a sub-group of COVID-19 patients, possibly genotypically-defined, would benefit from such an inhibitor. Most discussion of complement inhibition in COVID-19 is focused on severe disease in naive patients, but vaccinated subjects can also develop disease. In these patients the CP component will be far more prominent early in disease – might these patients respond differently to complement inhibition?

All of these aspects ought to be addressed and answered in order for us to be better prepared with effective treatment options in any future pandemic. It is an open question however as to whether or not the opportunity to gain this information from the current pandemic has been lost, as natural attenuation appears to have resulted in less severe disease (Stålcrantz et al., 2022; Harrigan et al., 2023). In the absence of any rapid assessment of complement parameters in an ER/ICU setting, one option would be to initiate complement therapy immediately while complement involvement is assessed. While, depending on the target chosen, this could impact any ongoing protective role of complement, all of the available clinical data suggests that there are minimal associated risks. Rapid assessment of genetic susceptibility would also be beneficial, but probably not feasible in the timeframe of initial decision-making, unless the patient has already been diagnosed with an AP/AL-driven disease such as AMD or aHUS, where the rationale for complement therapy pre-exists. The use of complement biomarkers to identify

patients most at risk of complement-driven exacerbation of disease is currently severely restricted in two ways. First, while complement is hyper-activated in these patients, it is not clear what the most predictive marker of degree of complement involvement might be. Based on our understanding of complement and COVID-19, markers of AP/AL involvement, such as Ba or Bb, or of C5 activation, such as C5a or sC5b-9, would be logical choices. Secondly, measurement of these outside of "specialist" complement laboratories is not straightforward. Because of the lability of many complement components, sample preparation and storage prior to analysis is critical. This is likely difficult to manage in an ER/ICU setting, and a rapid and simple bedside diagnostic test is required.

Assuming that, at least in some patients, complement inhibition is mandated, it remains an open question as to how long treatment should be continued. Given that treated patients will be closely monitored, a logical period would be until complement activation markers had returned to within the normal range, probably, if not already in place, under cover of antibiotics to combat any increased infection risk. Away from the acute ER/ICU admission environment it ought to be possible for appropriate blood samples to be taken and processed such that reliable results can be obtained. A second open question is concerns the most appropriate target. There are now in clinical development complement-directed inhibitors specific for the LP (anti-MASP-2), for the AP/AL (orally-available low molecular weight FD and FB inhibitors), for the TP (anti-C5 and anti-C5a) and C3 inhibitors that block C3 activation and hence both amplification of C3 activation and C5 activation. Identification of the most appropriate target(s) in COVID-19 will only come from clinical trials. While the efficacy of complement inhibition will inevitably be measured primarily against parameters such as need for ventilation, mortality, and length of ICU stay/length of hospitalisation, possible long-term effects ought also to be monitored. Among these is the impact on Long COVID: Does complement inhibition impact, either positively or negatively, the incidence of Long COVID? One possible mechanism for Long COVID is that virus is not completely eliminated, and that low level expression of viral proteins, especially the S-protein, on, for example, endothelial cells, or on blood cells, gives a continued low level complement-driven inflammatory stimulus. Might complement inhibition in the acute stage of infection enhance latency? Might complement inhibition in Long COVID be an effective therapy against Long COVID symptoms? Long COVID is clearly a post-acute COVID-19 complication with a possible complement component, but are there others? For example, does complement hyper-activation trigger onset of dementia, or acceleration of dementia symptoms? Finally, complement activation has long been known to play a role in the development of adaptive immunity, both antibody and T cell (Carroll, 2004), and a report implicating anti-C5 in failure to respond to vaccination has recently appeared (O'Brien et al., 2009). Might complement inhibition during acute infection, or prolonged inhibition, adversely affect development of protective humoral and/or cellular immunity against SARS-CoV-2?

12. Summary and hypothesis

In our review we have presented a highly complement-centric view of COVID-19, with limited reference to other effector pathways and mechanisms operative in severe disease. The literature is vast, some of it contradictory, and it is inevitable that we will have overlooked many contributions. Nevertheless, we believe that we have presented a balanced summary of current knowledge. Our working hypothesis is that during the initial phase of infection in a naive individual the SARS-CoV-2 virus is recognised by pattern recognition molecules of the LP. In the fully-competent host, possibly augmented by AP/AL involvement, this is sufficient for viral elimination, and is protective. The SARS-CoV-2 virus may well have evolved evasion mechanisms such to defend against initial complement attack, and the effectiveness of these will influence infectivity. If rapid viral elimination is unsuccessful the host's adaptive response, with antibody formation, kicks in. This will increase complement activation in the host, potentially still protective, but with an increased need for appropriate regulation, particularly of the AP/AL. As the virus escapes the respiratory tract and infection becomes systemic, with multi-organ involvement, intense complement activation results in bystander deposition of activation products such as C4b, C3b, and C5b-9 onto the endothelium, with consequent endothelial cell activation and generation of a pro-coagulant state. In addition, bystander attack on the endothelium and on blood cells, initiates host inflammasome activation (Suresh et al., 2016). Increased involvement of the coagulation system, with plasminogen activation, will, through C1-INH depletion, augment both CP and LP activation as MASP and C1 protease inhibition is lost. (Concomitantly, kinin generation will also be augmented as kallikrein and Factor XII inhibition is lost.) At around 14 days a critical period, a "tipping point", in infection is reached. If infection is not controlled, hyper-activation of the complement system, with consequent pathological involvement of many immune processes, occurs. Multi-organ involvement, particularly of the lung and kidney, results in life-threatening complications. At this point, while complement hyper-activation will continue, it may well become a minor driver of severe disease pathology, and the window of opportunity for effective complement inhibition may well be narrow. That said, if disease is successfully brought under control, a degree of complement inhibition may well be beneficial.

A key lesson that has emerged from the COVID-19 pandemic is that it will be necessary for the scientific, medical and pharmaceutical communities to work closely together, across national boundaries, in order to minimise the impact of any future newly-emergent pandemic. It is equally clear that effective treatments to treat critically ill patients will be required t; vaccine development takes time, and over 2 million deaths were recorded before the first introduction of a vaccine against COVID-19. With our current knowledge of the COVID-19 disease course, and in the absence of timely effective diagnosis of complement involvement, we believe that immediate introduction of a complement inhibitor addressing amplification of C3 or C5 activation is warranted on hospital admission. Our hypothesis of plasmin/C1-INH involvement also suggests that steps to enhance the efficacy of C1-INH-dependent regulation of multiple systems be considered. While plasma-purified or recombinant protein might be sufficient to address this short-term, another option might be to consider the use of danazol, a drug that increases C1-INH synthesis and which has been used successfully to treat HAE for many decades (Bork et al., 2008).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: WMZ has no conflict of interest. RAH is owner and director of RAH Pharma Consulting Ltd., but has no current consultancy contracts that would constitute a CoI. WMZ is supported by Race Against Dementia Alzheimer's Research UK Fellowship.

Data availability

No data was used for the research described in the article.

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