



Make your **mark.**

Discover reagents that make
your research stand out.

DISCOVER HOW



COVID-19: Complement, Coagulation, and Collateral Damage

Martin W. Lo, Claudia Kemper and Trent M. Woodruff

This information is current as
of August 9, 2022.

J Immunol 2020; 205:1488-1495; Prepublished online 22
July 2020;
doi: 10.4049/jimmunol.2000644
<http://www.jimmunol.org/content/205/6/1488>

References This article **cites 112 articles**, 27 of which you can access for free at:
<http://www.jimmunol.org/content/205/6/1488.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
<http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at:
<http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
<http://jimmunol.org/alerts>

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2020 by The American Association of
Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



COVID-19: Complement, Coagulation, and Collateral Damage

Martin W. Lo,* Claudia Kemper,[†] and Trent M. Woodruff*

Coronavirus disease of 2019 (COVID-19) is a highly contagious respiratory infection that is caused by the severe acute respiratory syndrome coronavirus 2. Although most people are immunocompetent to the virus, a small group fail to mount an effective antiviral response and develop chronic infections that trigger hyperinflammation. This results in major complications, including acute respiratory distress syndrome, disseminated intravascular coagulation, and multiorgan failure, which all carry poor prognoses. Emerging evidence suggests that the complement system plays a key role in this inflammatory reaction. Indeed, patients with severe COVID-19 show prominent complement activation in their lung, skin, and sera, and those individuals who were treated with complement inhibitors all recovered with no adverse reactions. These and other studies hint at complement's therapeutic potential in these sequelae, and thus, to support drug development, in this review, we provide a summary of COVID-19 and review complement's role in COVID-19 acute respiratory distress syndrome and coagulopathy. *The Journal of Immunology*, 2020, 205: 1488–1495.

Coronavirus disease of 2019 (COVID-19) has become a worldwide viral pandemic that has, at the time of writing, infected over 10 million individuals and caused over 500,000 deaths (1). In response, governments have taken unprecedented measures including instituting nationwide shutdowns and border closures that have impacted economies to an estimated cost of US \$2.5 trillion (3% of global gross domestic product) (2). This double bind may seem like an insoluble problem, but only a small percentage of patients present with the lethal complications of acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation (DIC), and multiorgan failure (MOF) (3). Thus, better treatments for severe COVID-19 would allow countries to operate with substantially fewer restrictions and fatalities. Emerging evidence suggests that complement plays a key role

in these critical patients, and thus, in this article, we review COVID-19's key features and make a case for the use of complement therapeutics in COVID-19 ARDS and coagulopathy.

COVID-19 etiology and pathogenesis

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first emerged in December 2019 in Wuhan in Hubei Province in China and is believed to have originated from a bat reservoir and spread to an intermediate host where it developed the capacity for human transmission (4). During the infection process, SARS-CoV-2 targets specific cell types through its spike glycoprotein (5). Canonically, this protein is proteolytically activated by transmembrane serine protease 2/4 and binds to the angiotensin converting enzyme II receptor on pneumocytes, endothelial cells, enterocytes, myocardium, and kidney cells (6, 7). In addition, the spike glycoprotein can bind to CD147 on T lymphocytes, which has been linked to the defective immune response seen in severe COVID-19 (K. Wang, W. Chen, Y.-S. Zhou, J.-Q. Lian, Z. Zhang, P. Du, L. Gong, Y. Zhang, H.-Y. Cui, J.-J. Geng, B. Wang, X.-X. Sun, C.-F. Wang, X. Yang, P. Lin, Y.-Q. Deng, D. Wei, X.-M. Yang, Y.-M. Zhu, K. Zhang, Z.-H. Zheng, J.-L. Miao, T. Guo, Y. Shi, J. Zhang, L. Fu, Q.-Y. Wang, H. Bian, P. Zhu, and Z.-N. Chen, manuscript posted on bioRxiv). Once inside the host cell, the virus uncoats and hijacks its genetic machinery to undergo genomic replication and translation. This produces copies of its genome and various proteins (i.e., spike, envelope, membrane, and nucleocapsid), which are then assembled at the cell membrane to form a mature viral particle that can bud and propagate infection (8). These processes underpin the pathogenicity of SARS-CoV-2 and are key targets for future antiviral therapies.

COVID-19 clinical presentations

The clinical presentation of COVID-19 varies widely in nature and severity. Currently, there is an unknown number of asymptomatic carriers in the community (9, 10), whereas those who become symptomatic have a triphasic clinical progression. Initially, these patients experience an incubation

*School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, St Lucia, Queensland 4072, Australia; and [†]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

ORCID: 0000-0002-2736-5507 (M.W.L.); 0000-0003-4196-1417 (C.K.); 0000-0003-1382-911X (T.M.W.).

Received for publication June 1, 2020. Accepted for publication July 6, 2020.

This work was supported by National Health and Medical Research Council Grant APP1082271 (to T.M.W.) and by the National Heart, Lung, and Blood Institute.

Address correspondence and reprint requests to Prof. Trent M. Woodruff, School of Biomedical Sciences, Skerman Building (65), The University of Queensland, St Lucia, Brisbane, QLD 4072, Australia. E-mail address: t.woodruff@uq.edu.au

Abbreviations used in this article: ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease of 2019; DAMP, damage-associated molecular pattern; DIC, disseminated intravascular coagulation; MAC, membrane attack complex; MASP2, MBL-associated serine protease 2; MBL, mannose-binding lectin; MERS-CoV, Middle Eastern respiratory syndrome coronavirus; MOF, multiorgan failure; N, nucleocapsid; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TF, tissue factor.

Copyright © 2020 by The American Association of Immunologists, Inc. 0022-1767/20/\$37.50

period with a median duration of 5.1 d (11), after which they present with the archetypal features of fever, cough, fatigue, myalgia, anorexia, and diarrhea (12). These symptoms persist for ~1 wk, at which point most patients recover (~80%), whereas a smaller percentage rapidly deteriorate and develop ARDS (~20%) (13), DIC, and/or MOF (14). The latter is heralded by a profound lymphopenia and tends to occur in those over the age of 65 y and those with comorbidities (e.g., cardiovascular disease, diabetes mellitus, and obesity) (15, 16). Unfortunately, this cohort has a poor prognosis. Compared with ARDS from other causes, COVID-19 ARDS has an elevated mortality rate of 26–62% that rises to 66–94% once patients are placed on mechanical ventilation (17). Additionally, COVID-19 has a number of atypical presentations, including large vessel thromboses (18, 19) and a Kawasaki-like disease in children (20) that are likely due to an insidious form of chronic inflammation. These complications of COVID-19 have no currently available disease modifying treatments, and thus, there is an urgent need for therapeutics in this space.

COVID-19 immunophenotypes

The diverse clinical presentations of COVID-19 reflect the range of immune responses that occur in individuals. In the majority of cases, patients with mild to moderate disease experience a self-limiting course of symptoms and develop an effective immune response that involves all of the so far known aspects of innate and adaptive immunity. In this scenario, viral Ags (e.g., RNA) activate endosomal and cytoplasmic pathogen recognition receptors (e.g., TLR3/7/8/9) that initiate an effective viral response (21). This includes the activation and recruitment of innate leukocytes to the sites of viral sensing, a robust IFN response (22), and effective Ag presentation and stimulation of the adaptive immune system, which is critical for the production of cytotoxic lymphocytes and antiviral Abs (23). By contrast, those with severe disease are uniquely susceptible to the virus' ability to undermine this response. Specifically, SARS-CoV-2 can suppress IFN induction (Y. Konno, I. Kimura, K. Urie, M. Fukushi, T. Irie, Y. Koyanagi, S. Nakagawa, and K. Sato, manuscript posted on bioRxiv) and drive IL-6, IL-8, and IL-10 production (24), which to varying degrees inhibits APC maturation (25, 26) and up-regulates inhibitory receptors on NK and CD8⁺ T cells (27). Additionally, severe COVID-19 patients have a profound pan lymphopenia characterized by lymphocyte dysfunction, apoptosis, and exhaustion (22, 28, 29). This phenomenon is multifactorial and most likely reflects viral infection of lymphocytes (K. Wang, et al., manuscript posted on bioRxiv), inhibitory cytokine production (27), synapse formation with immature APCs (26, 30), and the age-related depletion of naive T cells (31). Together, these defective antiviral processes allow the formation of a persistent viral niche, which chronically activates the innate immune system and causes significant collateral damage.

COVID-19 ARDS

In severe COVID-19, this maladaptive response drives ARDS, which may be defined as an acute onset noncardiogenic pulmonary edema requiring mechanical ventilation (32). Without an effective antiviral response, SARS-CoV-2 causes sustained pathogen recognition receptors stimulation, which leads to the production of large quantities of chemokines and cytokines that recruit various leukocytes and elicit a proinflammatory reaction

(33). Indeed, single-cell transcriptomic studies on bronchoalveolar lavage fluid from moderate and severe patients showed the latter group had enrichment of three distinct macrophage subsets including group 1, which expressed monocyte-like markers (i.e., S100A8, FCN1, and CD14); group 2, which expressed high levels of chemokines (i.e., CCL2, CCL3, and CXCL10); and group 3, which expressed immunoregulatory (i.e., A2M, GPR183, and CCL13) and profibrotic genes (i.e., TREM2, TGFB1, and SPP1) (33). Collateral damage to host cells then creates a self-perpetuating cycle of necrosis and inflammation, which results in hyaline membrane formation, blood–air barrier breakdown, and fibrinous exudate production, which all undermine gas exchange (34, 35). Additionally, unlike in bacterial sepsis and influenza, monocyte and perhaps other leukocyte production of cytokines is sustained. Thus, as the disease progresses, excess cytokines (i.e., IL-1 β , IL-1 α , IL-1ra, IL-2, IL-6, IL-7, IL-10, IL-12, G-GCSF, M-CSF, TNF- α , IFN- α 2, and IFN- γ) (12, 36, 37) and chemokines (i.e., CCL2, CCL3, and CXCL10) (12) begin to accumulate in the bloodstream, which at a critical mass causes MOF in what is termed a cytokine storm (38). Currently, there are no treatments that can reverse COVID-19 ARDS, and thus, there is an urgent need for research into anti-inflammatory medications in this space.

COVID-19 coagulopathy

COVID-19 is also associated with a thrombogenic coagulopathy with a range of presentations. In this context, patients consistently present with mild thrombocytopenia (39) and increased D dimer levels (40) that correlate with disease severity, whereas other coagulation measurements are more varied (12, 41). In line with this, thromboses can occur in all severities of COVID-19 and range from small to large vessel clots, in which case reports have documented DIC (42), microvascular thromboses (43), pulmonary emboli (44, 45), aorto-iliac and mesenteric thrombi (19), and large vessel strokes (18, 46). However, the mechanisms that underlie this coagulopathy are enigmatic. Foremost, there is the well-known connection between chronic inflammation and coagulation (47), but other viral-specific processes may be at play. For example, SARS-CoV-2 can infect and damage endothelial cells (48) and cause cytokine storms and systemic inflammatory syndromes (49), which both promote coagulation. Moreover, the relationship between COVID-19 liver dysfunction and coagulopathy has yet to be clarified (50). Nevertheless, given that severe thrombotic complications occur in up to 85% of intensive care patients (51), there remains a pressing need for drug development in this space.

Comparison with severe acute respiratory syndrome coronavirus and Middle Eastern respiratory syndrome coronavirus

Prior to discussing the role of complement in COVID-19, it may be necessary to provide some background on the two other major coronaviruses that offer some related insight. Severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle Eastern respiratory syndrome coronavirus (MERS-CoV) are closely related to SARS-CoV-2, and together, they are classed in the β -coronavirus family (52). The former emerged in 2004 in Southern China, whereas the latter emerged in 2012 in Jordan, and they share 79 and 50% sequence homology with SARS-CoV-2, respectively (52, 53).

SARS-CoV, like its contemporary, binds to the angiotensin converting enzyme II host receptor, whereas MERS-CoV binds to dipeptidyl peptidase 4, which is expressed on pneumocytes, vascular endothelium, and a subset of mononuclear leukocytes. Nevertheless, both cause similar histopathology, including a thrombogenic coagulopathic state (54–57). In line with this, SARS and MERS have comparable clinical features, including a flu-like presentation, a triphasic clinical progression, similar rates of lympho- and thrombocytopenia, and rare coagulopathic events (14, 58–60). SARS-CoV and MERS-CoV also have the capacity to undermine IFN responses and the adaptive immune system (61–65) and induce similar leukocyte and cytokine profiles to that of SARS-CoV-2. Indeed, patients with severe SARS show upregulation of IL-1 β , IL-6, IL-8, IL-12, IL-18, IL-33, TNF- α , TGF- β , CCL2, CCL3, CCL5, CXCL8, CXCL10, and IFN- γ (38, 66), whereas those with severe MERS have elevated levels of IL-1 α , IL-1 β , IL-6, IL-8, IL-10, IL-15, TNF- α , TGF- β , and CXCL10 (59, 67, 68). Thus, although there are obvious differences between the three viruses, these similarities allow studies on SARS-CoV and MERS-CoV to provide some insight into the pathology of COVID-19.

The complement system

The complement system is a collection of over 40 serine proteases, receptors, and inhibitors that amplify danger signals and augment inflammatory responses. In this review, we provide a short summary of complement activation and effector mechanisms, although detailed information can be found elsewhere (69–71). These serine proteases are inactive in their native state but, once triggered, undergo proteolytic cleavage and take part in a number of chain reactions that are organized into three major pathways (Fig. 1). The classical and mannose-binding lectin (MBL) pathways are canonically activated by IgM/G and carbohydrate moieties on pathogens, respectively. They converge at C2bC4b, which acts as a C3 protease or convertase that facilitates the cleavage of C3 into C3a and C3b (72–75). Downstream, the alternative pathway is a C3 amplification loop that can contribute up to 80% of complement activation from upstream pathways and in itself can be triggered by “altered” surfaces, such as damaged or foreign tissue. The resultant C3b then joins C2bC4b from the classical/MBL pathways or C3bBb from the alternative pathway to form a C5 convertase, which cleaves C5 into C5a and C5b, the latter of which associates with C6, C7, C8, and C9 to form C5b9 or membrane attack complex (MAC) (76–78). Superimposed over these three pathways, the extrinsic pathway is a more recently recognized collection of proteases that have isolated convertase activity, some of which are part of the coagulation cascade (79–82). Once triggered, complement’s key effectors include the opsonins, which mark cargo for phagocytosis (i.e., C1q, C3b, and C4b) (83); the anaphylatoxins (i.e., C3a and C5a), which are broad spectrum immune activators (84–86); and MAC, which causes cell lysis and can be an immunomodulator (87–89). Emerging evidence suggests that complement is chronically activated in severe COVID-19 and, given its potency, ubiquity, and rapidity, is well placed as a drug target in this disease.

Complement-mediated hyperinflammation

As an added layer of complexity, complement has a dichotomous nature spanning a range of concentrations. In response

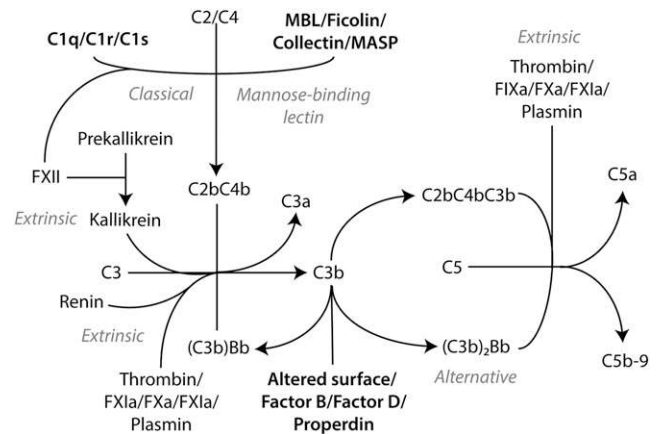


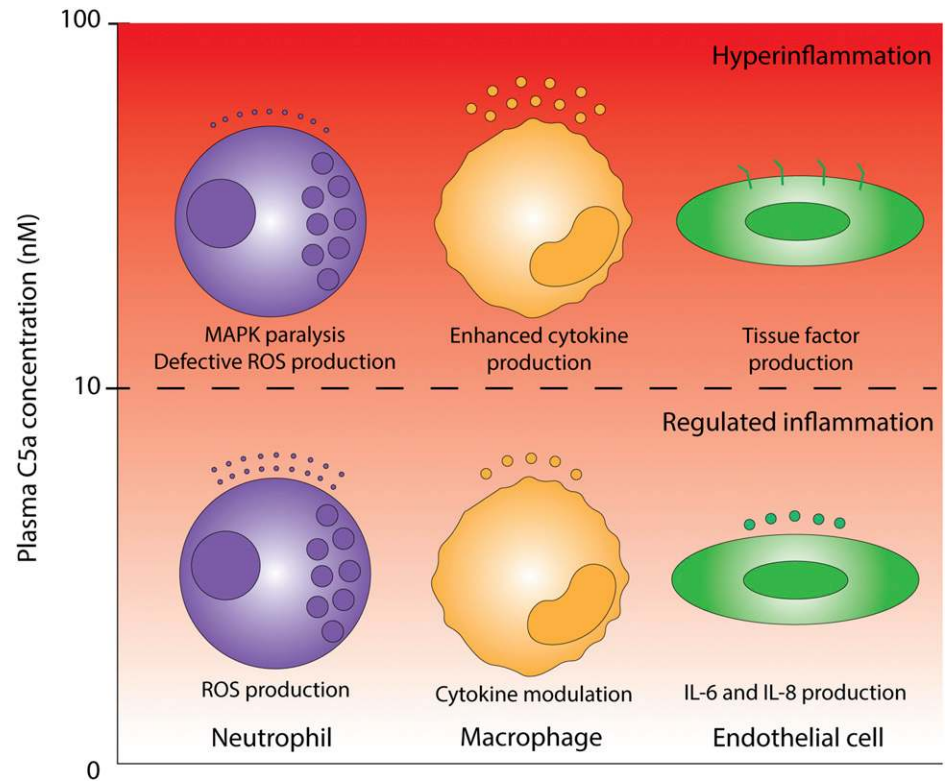
FIGURE 1. Complement activation pathways. Inactive molecules and cleavage products at tails and heads of arrows, respectively; intersecting lines denote proteolytic cleavage; pathways are given in gray italics; and pathogen- and DAMP detection mechanisms are given in bold.

to low to moderate immune stimulation, complement induces a localized inflammatory response that removes dangerous agents and promotes tissue restoration. These processes are tightly regulated and contained, and indeed, low complement concentrations can inhibit cytokine production (90). Thus, serum/plasma C5a concentrations seldom exceed 10 nM in this context (91). By contrast, in response to high levels of immune stimulation, complement can become uncontrolled and cause hyperinflammation. In this state, complement becomes maladaptive and can cause significant collateral damage. Specifically, neutrophils become unresponsive to anaphylatoxins, which undermines their antimicrobial activity; macrophages produce excess cytokines, which promotes the formation of cytokine storms; and endothelial cells produce tissue factor (TF), which drives DIC (Fig. 2). These processes are associated with serum/plasma C5a concentrations above 10 nM, as seen in patients with severe COVID-19 (Ref. 91 and T. Gao, M. Hu, X. Zhang, H. Li, L. Zhu, H. Liu, Q. Dong, Z. Zhang, Z. Wang, Y. Hu, Y. Fu, Y. Jin, K. Li, S. Zhao, Y. Xiao, S. Luo, L. Li, L. Zhao, J. Liu, H. Zhao, Y. Liu, W. Yang, J. Peng, X. Chen, P. Li, Y. Liu, Y. Xie, J. Song, L. Zhang, Q. Ma, X. Bian, W. Chen, X. Liu, Q. Mao, and C. Cao, manuscript posted on medRxiv). Indeed, SARS-CoV-2 can induce the transcription of complement (i.e., C1r, C1s, factor B, and C3) and coagulation genes (i.e., fibrinogen) in pneumocytes and hepatocytes (B. Yan, T. Freiwald, D. Chaus, L. Wang, E. West, J. Bibby, M. Olson, S. Kordasti, D. Portilla, A. Laurence, M.S. Lionakis, C. Kemper, B. Afzali, and M. Kazemian, manuscript posted on Research Square), and genetic defects in complement regulator genes (i.e., CD55 and factor H) have been linked to SARS-CoV-2 susceptibility (V. Ramlall, P. Thangaraj, N.P. Tatonetti, and S.D. Shapira, manuscript posted on medRxiv). Thus, complement is likely to drive a form of hyperactive and dysregulated inflammation in severe COVID-19.

Complement in COVID-19 ARDS

The critical role of complement in COVID-19 ARDS continues to emerge as more studies are published. Initially, studies showed that complement activation can occur as early as day 1 of symptom onset (92). However, complement’s pathogenicity is probably confined to the middle to late stages of

FIGURE 2. Complement-mediated hyperinflammation. In uncontrolled circumstances, complement induces a hyperactive and dysregulated inflammatory response that is associated with plasma C5a concentrations above 10 nM (top). In controlled circumstances, complement induces a limited inflammatory response that is associated with plasma C5a concentrations below 10 nM (bottom).



severe disease, in which patients have markedly elevated levels of serum C5a and MAC (Ref. 93 and T. Gao, et al., manuscript posted on medRxiv). In line with this, post mortem lung samples from severe COVID-19 patients showed strong immunohistochemical staining for MBL, MBL-associated serine protease 2 (MASP2), C4a, C3, and MAC that colocalized with SARS-CoV-2 nucleocapsid (N) protein (Ref. 43 and T. Gao, et al., manuscript posted on medRxiv), which is also seen in the kidneys of a subset of patients with disseminated infection (B. Diao, C. Wang, R. Wang, Z. Feng, Y. Tan, H. Wang, C. Wang, L. Liu, Y. Liu, Y. Liu, G. Wang, Z. Yuan, L. Ren, Y. Wu, and Y. Chen, manuscript posted on medRxiv). These findings suggest that SARS-CoV-2 can directly activate complement in ARDS, and indeed, a preprint study showed that its N protein is able to potentiate MASP-2-dependent complement activation (T. Gao, et al., manuscript posted on medRxiv). Moreover, transcriptomic studies on bronchoalveolar lavage fluid from severe COVID-19 patients found elevated ficolin 1 expression in monocyte-derived macrophages, which may further support MBL pathway activation (33). Downstream of these pathways, the anaphylatoxins are thought to promote a host of immune processes, including chemotaxis; NETosis; degranulation; and the production of cytokines, inflammasomes, reactive oxygen species, and eicosanoids (85, 94–97), many of which are upregulated in severe COVID-19 (e.g., chemotaxis, NETosis, IL-1 β , IL-6, IL-8, IL-12, TNF- α , and CCL3) (Refs. 98–101 and P. Skendros, A. Mitsios, A. Chrysanthopoulou, D.C. Mastellos, S. Metallidis, P. Rafailidis, M. Ntinopoulou, E. Sertaridou, V. Tsironidou, C. Tsigalou, M. Tektonidou, T. Konstantinidis, C. Papagoras, I. Mitroulis, G. Germanidis, J.D. Lambris, and K. Ritis, manuscript posted on medRxiv). Moreover, MAC-mediated cell death and other forms of collateral damage may cause the release of damage-associated molecular patterns (DAMPs) (e.g., hyaluronan

and ATP) that can further activate complement in a self-perpetuating cycle (Fig. 3) (102, 103). However, these findings should be interpreted with caution as the relationship between complement and ARDS is still a matter of debate (104). Nevertheless, COVID-19 ARDS patients who were treated with anti-C5 Abs ($n = 2$ and $n = 4$) and C3 inhibitors ($n = 1$) all recovered with no adverse effects (Refs. 105, 106, and T. Gao, et al., manuscript posted on medRxiv). Thus,

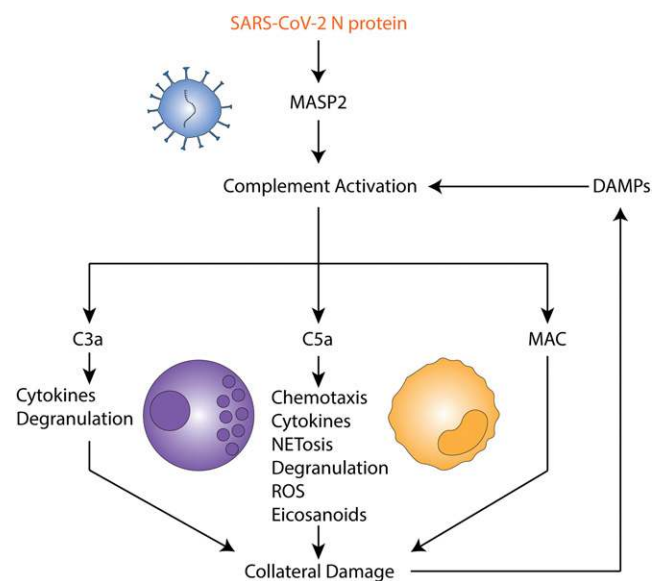


FIGURE 3. Complement-mediated inflammation in COVID-19 ARDS. SARS-CoV-2 N protein binds to MASP2 and activates the complement cascade; complement effectors then drive a proinflammatory response through granulocytes and monocytes; and collateral damage establishes a positive feedback loop of cell death and inflammation.

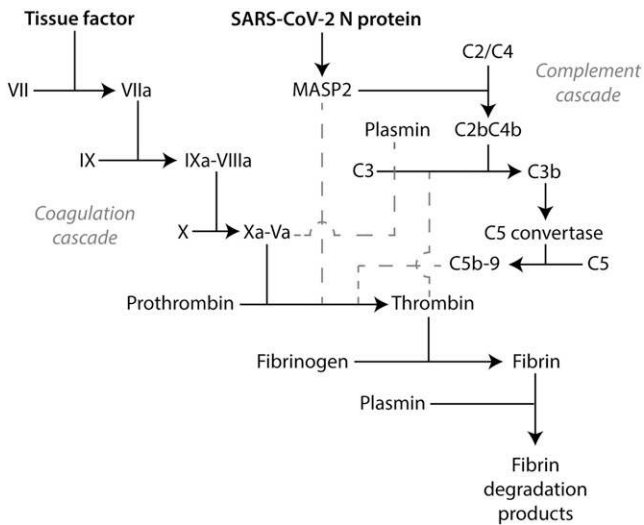


FIGURE 4. Complement–coagulation cross-talk in COVID-19 coagulopathy. Inactive molecules and cleavage products are at tails and heads of arrows, respectively; intersecting lines denote proteolytic cleavage; pathways are given in gray italics; and complement activation of coagulation factors and vice versa are given in gray dashed lines.

these promising results suggest that further research into complement in COVID-19 is warranted.

Currently, the mechanisms that underlie these processes are unknown; however, inferences may be gleaned from studies in SARS and MERS. Like in COVID-19, SARS patients have elevated serum complement levels (107–109), and in New Zealand white rabbits, MERS-CoV can cause a defective adaptive immune response and complement-mediated hyperinflammation (110). These similar findings probably reflect the shared ability of these coronaviruses to activate the MBL pathway. Indeed, all their N proteins can activate MASP2 and exacerbate LPS-induced pneumonia in mice (T. Gao, et al., manuscript posted on medRxiv). Given these similarities, mechanistic studies on complement in SARS and MERS may provide additional insight into COVID-19 and suggest that complement has broad immune functions that affect multiple organs during a coronavirus infection. C3 knockout mice infected with SARS-CoV had decreased weight loss, respiratory dysfunction, lung pathology, and levels of lung and serum cytokines (e.g., IL-1 α , IL-5, IL-6, TNF- α , and G-CSF) compared with their wild type littermates (111). Similarly, anti-C5 Ab treatment in MERS-CoV-infected human dipeptidyl peptidase 4 transgenic mice reduced their lung and spleen pathology, serum cytokine levels (e.g., IL-1 β , TNF- α , IFN- γ , IL-12, and CCL2), and viral replication in their lungs and brain (112).

Taken together, these findings highlight complement's potency and the wide range of effects in coronavirus-mediated inflammation.

Complement in COVID-19 coagulopathy

Moreover, a small case series has implicated complement in COVID-19 coagulopathy, which has a spectrum of presentations that are ostensibly linked to a patient's individual inflammatory state. Specifically, mild patients appear to develop thromboses secondary to an insidious form of chronic inflammation, whereas severe patients form clots in response to complement activation. In line with this, placentas from pregnant mothers with mild COVID-19 showed large vessel thromboses with unremarkable staining for complement and viral Ags (i.e., C3d, C4d, C5b, viral spike protein, and viral RNA) (113). By contrast, severe patients may present with a variety of thromboses, including retiform purpura with extensive colocalizing deposition of C3d, C4d, MAC, and viral spike protein (43). It is not clear as to why this spectrum of pathology occurs; however a likely hypothesis is that N protein-mediated complement activation is dependent on the absence of an enveloping membrane. In this sense, in mild disease, N proteins are hidden within viral envelopes and infected cells, whereas in severe disease, collateral damage to host cells causes the release of large quantities of immature viral components that are free to activate the complement cascade. Nevertheless, these initial studies suggest that complement plays a key role in the coagulopathy of severe COVID-19.

Although mechanistic data are lacking, a potential model for complement–coagulation cross-talk in COVID-19 can be devised from the literature. In reality, these thromboses are probably driven by a combination of systemic and locally activated complement (43, 114), but for simplicity, in this review, we will focus on the latter. In such a model, viral infection of the endothelium would lead to the production and release of N proteins that would activate complement and drive neutrophil, monocyte, and potentially endothelial production of TF (Refs. 115, 116, and P. Skendros, et al., manuscript posted on medRxiv) as well as cause intimal damage that would expose subendothelial TF (48). The complement and coagulation cascades would then cross over at two key points: 1) MASP2 and MAC can cleave prothrombin (117–119), and 2) factor Xa, plasmin, and thrombin can cleave C3 (Fig. 3) (79). This complement–coagulation cross-talk in theory facilitates a powerful positive feedback loop and may explain the striking inflammation that surrounds the retiform purpura in severe COVID-19 (Fig. 4) (43). This analysis highlights the therapeutic potential of complement

Table I. Clinical trials of complement therapeutics in COVID-19

Drug	MOA	Drug Type	ROA	Company	Clinical Phase
Conestat alfa	Recombinant C1 esterase inhibitor	Protein	i.v.	Pharming Group	II
APL-9	C3 inhibition	Peptide	i.v.	Apellis	III
AMY101	C3 inhibition	Peptide	s.c., i.v.	Amyndas	II
Zilucoplan	C5 inhibition	Peptide	s.c.	Ra Pharma	II
Eculizumab	C5 inhibition	Ab	i.v.	Alexion	Expanded access
Ravulizumab	C5 inhibition	Ab	i.v.	Alexion	III

MOA, mechanism of action; ROA, route of administration.

in COVID-19 coagulopathy, and thus, further research is needed.

Therapeutic developments

Currently, 13 clinical trials are underway in the field of complement therapeutics in COVID-19 (120). The field consists mainly of trials for C3 and C5 inhibitors in COVID-19 ARDS and include zilucoplan, AMY101, APL-9, eculizumab, and ravulizumab (Table I). These drugs should cause widespread complement inactivation despite high levels of viral proteins and DAMPs, and some have been used safely in the clinic for almost 15 y (121). Thus, we expect patients enrolled in these trials to show improvement in clinical end points including PaO₂/FiO₂, sequential organ failure assessment scores, requirements for supplemental oxygen and mechanical ventilation, and survival. Indeed, severe COVID-19 patients treated with eculizumab (anti-C5 Ab) and ruxolitinib (JAK1/2 inhibitor) showed clinical improvement within 3 d of treatment, which may in part be due to their ability to inhibit the pathways (i.e., NF-κB and STAT1/2) that drive local and hepatic complement synthesis (Ref. 122 and B. Yan, et al., manuscript posted on Research Square). To supplement these trials, a small number of studies are also characterizing the time profile of serum complement in COVID-19. This would assist in the optimization of therapeutic doses and the time window for treatment, which probably should occur sometime after symptom onset, as complement may have beneficial effects during the first phase of disease. These clinical trials provide much needed hope, and we greatly anticipate their outcomes.

Future research directions

Early SARS-CoV-2 studies and prior research in other respiratory viral infections suggest that complement therapeutics would be highly effective in severe COVID-19. However, two questions remain unanswered. First, the time window for treatment has yet to be established. Complement is rarely used as a biomarker in this fashion as most fragments have a very short $t_{1/2}$, and studies suggest that complement distributions overlap in moderate and severe patients (93). However, a preprint study suggests that erythrocyte-bound C3 may be a useful marker in this respect (L.M. Lam, S.J. Murphy, L. Kuri-Cervantes, A.R. Weisman, C.A.G. Ittner, J.P. Reilly, M.B. Pampena, M.R. Betts, E.J. Wherry, W.C. Song, J.D. Lambris, D.B. Cines, N.J. Meyer, and N.S. Mangalmurti, manuscript posted on medRxiv). Thus, further research is needed to identify clinical and inflammatory markers that could be used to guide therapeutic intervention. Second, the precise mechanisms by which complement drives severe COVID-19 have yet to be validated and include complement-driven cytokines and viral protein activation of complement-coagulation cross-talk. Given that future outbreaks of similar viruses are inevitable (123), defining these underlying mechanisms will enhance our understanding of complement in coronavirus and other viral infections, which will support drug development and their clinical application for years to come.

Conclusions

COVID-19 serves as a reminder of the modern world's vulnerability to infectious diseases. Nations are now heavily

interconnected and interdependent, which allows pandemics to rapidly form and become difficult to manage without severe economic consequences. However, by developing treatments that can reduce the lethality of these infections, we may simultaneously minimize their death rates and economic fallouts. The development of complement therapeutics in COVID-19 may therefore be a critical step toward managing coronavirus outbreaks now and into the future.

Disclosures

The authors have no financial conflicts of interest.

References

1. Worldometer. 2020. COVID-19 coronavirus pandemic. Available at: <https://www.worldometers.info/coronavirus/>. Accessed: June 30, 2020.
2. International Monetary Fund. 2020. World economic outlook, April 2020: the great lockdown. In *World Economic Outlook Reports*. G. Gopinath, ed. International Monetary Fund, Washington, DC, p. 1–25.
3. Wu, Z., and J. M. McGoogan. 2020. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA*.
4. Andersen, K. G., A. Rambaut, W. I. Lipkin, E. C. Holmes, and R. F. Garry. 2020. The proximal origin of SARS-CoV-2. *Nat. Med.* 26: 450–452.
5. Zhang, H., J. M. Penninger, Y. Li, N. Zhong, and A. S. Slutsky. 2020. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* 46: 586–590.
6. Hamming, I., W. Timens, M. L. C. Bulthuis, A. T. Lely, G. J. Navis, and H. van Goor. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J. Pathol.* 203: 631–637.
7. Zang, R., M. F. Gomez Castro, B. T. McCune, Q. Zeng, P. W. Rothlauf, N. M. Sonnek, Z. Liu, K. F. Brulois, X. Wang, H. B. Greenberg, et al. 2020. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci. Immunol.* 5: eabc3582.
8. Mousavizadeh, L., and S. Ghasemi. 2020. Genotype and phenotype of COVID-19: their roles in pathogenesis. *J. Microbiol. Immunol. Infect.*
9. Day, M. 2020. Covid-19: four fifths of cases are asymptomatic, China figures indicate. *BMJ* 369: m1375.
10. Day, M. 2020. Covid-19: identifying and isolating asymptomatic people helped eliminate virus in Italian village. *BMJ* 368: m1165.
11. Lauer, S. A., K. H. Grantz, Q. Bi, F. K. Jones, Q. Zheng, H. R. Meredith, A. S. Azman, N. G. Reich, and J. Lessler. 2020. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann. Intern. Med.* 172: 577–582.
12. Huang, C., Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, et al. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. [Published erratum appears in 2020 *Lancet* 395: 496.] *Lancet* 395: 497–506.
13. Chen, T., D. Wu, H. Chen, W. Yan, D. Yang, G. Chen, K. Ma, D. Xu, H. Yu, H. Wang, et al. 2020. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. [Published erratum appears in 2020 *BMJ* 368: m1295.] *BMJ* 368: m1091.
14. Berlin, D. A., R. M. Gulick, and F. J. Martinez. 2020. Severe covid-19. *N. Engl. J. Med.*
15. Tan, L., Q. Wang, D. Zhang, J. Ding, Q. Huang, Y. Q. Tang, Q. Wang, and H. Miao. 2020. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct. Target. Ther.* 5: 33.
16. CDC COVID-19 Response Team. 2020. Preliminary estimates of the prevalence of selected underlying health conditions among patients with coronavirus disease 2019 — United States, February 12–March 28, 2020. *MMWR Morb. Mort. Wkly. Rep.* 69: 382–386.
17. Bellani, G., J. G. Laffey, T. Pham, E. Fan, L. Brochard, A. Esteban, L. Gattinoni, F. van Haren, A. Larsson, D. F. McAuley, et al; LUNG SAFE Investigators; ESICM Trials Group. 2016. Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 countries. [Published erratum appears in 2016 *JAMA* 316: 350.] *JAMA* 315: 788–800.
18. Oxley, T. J., J. Mocco, S. Majidi, C. P. Kellner, H. Shoirah, I. P. Singh, R. A. De Leacy, T. Shigematsu, T. R. Ladner, K. A. Yeager, et al. 2020. Large-vessel stroke as a presenting feature of covid-19 in the young. *N. Engl. J. Med.* 382: e60.
19. Vulliamy, P., S. Jacob, and R. A. Davenport. 2020. Acute aorto-iliac and mesenteric arterial thromboses as presenting features of COVID-19. *Br. J. Haematol.* 189: 1053–1054.
20. Viner, R. M., and E. Whittaker. 2020. Kawasaki-like disease: emerging complication during the COVID-19 pandemic. *Lancet* 395: 1741–1743.
21. Guo, Y. R., Q. D. Cao, Z. S. Hong, Y. Y. Tan, S. D. Chen, H. J. Jin, K. S. Tan, D. Y. Wang, and Y. Yan. 2020. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil. Med. Res.* 7: 11.

22. Zhou, Z., L. Ren, L. Zhang, J. Zhong, Y. Xiao, Z. Jia, L. Guo, J. Yang, C. Wang, S. Jiang, et al. 2020. Heightened innate immune responses in the respiratory tract of COVID-19 patients. *Cell Host Microbe* 27: 883–890.e2.
23. Azkur, A. K., M. Akdis, D. Azkur, M. Sokolowska, W. van de Veen, M. C. Bruggen, L. O'Mahony, Y. Gao, K. Nadeau, and C. A. Akdis. 2020. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy* 75: 1564–1581.
24. Blanco-Melo, D., B. E. Nilsson-Payant, W. C. Liu, S. Uhl, D. Hoagland, R. Möller, T. X. Jordan, K. Oishi, M. Panis, D. Sachs, et al. 2020. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 181: 1036–1045.e9.
25. Park, S. J., T. Nakagawa, H. Kitamura, T. Atsumi, H. Kamon, S.-I. Sawa, D. Kamimura, N. Ueda, Y. Iwakura, K. Ishihara, et al. 2004. IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation. *J. Immunol.* 173: 3844–3854.
26. Giamarellos-Bourboulis, E. J., M. G. Netea, N. Rovina, K. Akinosoglou, A. Antoniadou, N. Antonakos, G. Damoraki, T. Gkavogianni, M. E. Adami, P. Katsounou, et al. 2020. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe* 27: 992–1000.e3.
27. Antoniolli, L., M. Fornai, C. Pellegrini, and C. Blandizzi. 2020. NKG2A and COVID-19: another brick in the wall. *Cell. Mol. Immunol.* 17: 672–674.
28. Xiong, Y., Y. Liu, L. Cao, D. Wang, M. Guo, A. Jiang, D. Guo, W. Hu, J. Yang, Z. Tang, et al. 2020. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerg. Microbes Infect.* 9: 761–770.
29. Zheng, H.-Y., M. Zhang, C.-X. Yang, N. Zhang, X.-C. Wang, X.-P. Yang, X.-Q. Dong, and Y.-T. Zheng. 2020. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. *Cell. Mol. Immunol.* 17: 541–543.
30. Dhodapkar, M. V., R. M. Steinman, J. Krasovsky, C. Munz, and N. Bhardwaj. 2001. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J. Exp. Med.* 193: 233–238.
31. Weiskopf, D., B. Weinberger, and B. Grubeck-Loebenstein. 2009. The aging of the immune system. *Transpl. Int.* 22: 1041–1050.
32. Matthay, M. A., R. L. Zemans, G. A. Zimmerman, Y. M. Arabi, J. R. Beitler, A. Mercat, M. Herridge, A. G. Randolph, and C. S. Calfee. 2019. Acute respiratory distress syndrome. *Nat. Rev. Dis. Primers* 5: 18.
33. Liao, M., Y. Liu, J. Yuan, Y. Wen, G. Xu, J. Zhao, L. Cheng, J. Li, X. Wang, F. Wang, et al. 2020. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat. Med.* 26: 842–844.
34. Tian, S., Y. Xiong, H. Liu, L. Niu, J. Guo, M. Liao, and S.-Y. Xiao. 2020. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. *Mod. Pathol.* 33: 1007–1014.
35. Matthay, M. A., and R. L. Zemans. 2011. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu. Rev. Pathol.* 6: 147–163.
36. Aziz, M., R. Fatima, and R. Assaly. 2020. Elevated interleukin-6 and severe COVID-19: a meta-analysis. *J. Med. Virol.*
37. Liu, Y., C. Zhang, F. Huang, Y. Yang, F. Wang, J. Yuan, Z. Zhang, Y. Qin, X. Li, D. Zhao, et al. 2020. Elevated plasma levels of selective cytokines in COVID-19 patients reflect viral load and lung injury. *Natl. Sci. Rev.* 7: 1003–1011.
38. Coperchini, F., L. Chiovato, L. Croce, F. Magri, and M. Rotondi. 2020. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev.* 53: 25–32.
39. Lippi, G., M. Plebani, and B. M. Henry. 2020. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clin. Chim. Acta* 506: 145–148.
40. Lippi, G., and E. J. Favaloro. 2020. D-dimer is associated with severity of coronavirus disease 2019: a pooled analysis. *Thromb. Haemost.* 120: 876–878.
41. Wang, D., B. Hu, C. Hu, F. Zhu, X. Liu, J. Zhang, B. Wang, H. Xiang, Z. Cheng, Y. Xiong, et al. 2020. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 323: 1061–1069.
42. McGonagle, D., J. S. O'Donnell, K. Sharif, P. Emery, and C. Bridgewood. 2020. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. *Lancet Rheumatol.* 2: E437–E445.
43. Magro, C., J. J. Mulvey, D. Berlin, G. Nuovo, S. Salvatore, J. Harp, A. Baxter-Stoltzfus, and J. Laurence. 2020. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl. Res.* 220: 1–13.
44. Middeldorp, S., M. Coppens, T. F. van Haaps, M. Foppen, A. P. Vlaar, M. C. A. Müller, C. C. S. Bouman, L. F. M. Beenen, R. S. Kootte, J. Heijmans, et al. 2020. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J. Thromb. Haemost.*
45. Bompard, F., H. Monnier, I. Saab, M. Tordjman, H. Abdoul, L. Fournier, O. Sanchez, C. Lorut, G. Chassagnon, and M. P. Revel. 2020. Pulmonary embolism in patients with Covid-19 pneumonia. *Eur. Respir. J.* DOI: 10.1183/13993003.01365-2020.
46. Avula, A., K. Nalleballe, N. Narula, S. Sapozhnikov, V. Dandu, S. Toom, A. Glaser, and D. Elsayegh. 2020. COVID-19 presenting as stroke. *Brain Behav. Immun.* 87: 115–119.
47. Foley, J. H., and E. M. Conway. 2016. Cross talk pathways between coagulation and inflammation. *Circ. Res.* 118: 1392–1408.
48. Varga, Z., A. J. Flammer, P. Steiger, M. Haberecker, R. Andermatt, A. S. Zinkernagel, M. R. Mehra, R. A. Schuepbach, F. Ruschitzka, and H. Moch. 2020. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395: 1417–1418.
49. Ogura, H., S. Gando, T. Iba, Y. Eguchi, Y. Ohtomo, K. Okamoto, K. Koseki, T. Mayumi, A. Murata, T. Ikeda, et al; Japanese Association for Acute Medicine Disseminated Intravascular Coagulation Study Group. 2007. SIRS-associated coagulopathy and organ dysfunction in critically ill patients with thrombocytopenia. *Shock* 28: 411–417.
50. Zhang, C., L. Shi, and F. S. Wang. 2020. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol. Hepatol.* 5: 428–430.
51. Klok, F. A., M. J. H. A. Kruip, N. J. M. van der Meer, M. S. Arbous, D. A. M. P. J. Gommers, K. M. Kant, F. H. J. Kaptein, J. van Paassen, M. A. M. Stals, M. V. Huisman, and H. Endeman. 2020. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb. Res.* 191: 145–147.
52. Corman, V. M., D. Muth, D. Niemeyer, and C. Drosten. 2018. Hosts and sources of endemic human coronaviruses. *Adv. Virus Res.* 100: 163–188.
53. Lu, R., X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, et al. 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395: 565–574.
54. Li, W., M. J. Moore, N. Vasilieva, J. Sui, S. K. Wong, M. A. Berne, M. Somasundaran, J. L. Sullivan, K. Luzuriaga, T. C. Greenough, et al. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426: 450–454.
55. Raj, V. S., H. Mou, S. L. Smits, D. H. W. Dekkers, M. A. Müller, R. Dijkman, D. Muth, J. A. A. Demmers, A. Zaki, R. A. M. Fouchier, et al. 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495: 251–254.
56. Alsaad, K. O., Y. M. Arabi, and A. H. Hajeer. 2020. Spectrum of histopathological findings in coronavirus disease-19, middle east respiratory syndrome and severe acute respiratory syndrome. *Ann. Thorac. Med.* 15: 52–53.
57. Giannis, D., I. A. Ziogas, and P. Gianni. 2020. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J. Clin. Virol.* 127: 104362.
58. Petrosillo, N., G. Viceconte, O. Ergonul, G. Ippolito, and E. Petersen. 2020. COVID-19, SARS and MERS: are they closely related? *Clin. Microbiol. Infect.* 26: 729–734.
59. Kim, E. S., P. G. Choe, W. B. Park, H. S. Oh, E. J. Kim, E. Y. Nam, S. H. Na, M. Kim, K. H. Song, J. H. Bang, et al. 2016. Clinical progression and cytokine profiles of middle east respiratory syndrome coronavirus infection. *J. Korean Med. Sci.* 31: 1717–1725.
60. Cherry, J. D., and P. Krogstad. 2004. SARS: the first pandemic of the 21st century. *Pediatr. Res.* 56: 1–5.
61. Spiegel, M., A. Pichlmair, L. Martínez-Sobrido, J. Cros, A. García-Sastre, O. Haller, and F. Weber. 2005. Inhibition of beta interferon induction by severe acute respiratory syndrome coronavirus suggests a two-step model for activation of interferon regulatory factor 3. *J. Virol.* 79: 2079–2086.
62. Law, H. K., C. Y. Cheung, H. Y. Ng, S. F. Sia, Y. O. Chan, W. Luk, J. M. Nicholls, J. S. Peiris, and Y. L. Lau. 2005. Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. *Blood* 106: 2366–2374.
63. Niemeyer, D., T. Zillinger, D. Muth, F. Zielecki, G. Horvath, T. Suliman, W. Barchet, F. Weber, C. Drosten, and M. A. Müller. 2013. Middle east respiratory syndrome coronavirus accessory protein 4a is a type I interferon antagonist. *J. Virol.* 87: 12489–12495.
64. Hu, W., Y. T. Yen, S. Singh, C. L. Kao, and B. A. Wu-Hsieh. 2012. SARS-CoV regulates immune function-related gene expression in human monocytic cells. *Viral Immunol.* 25: 277–288.
65. Kopecky-Bromberg, S. A., L. Martínez-Sobrido, M. Frieman, R. A. Baric, and P. Palese. 2007. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. *J. Virol.* 81: 548–557.
66. Cameron, M. J., J. F. Bermejo-Martin, A. Danesh, M. P. Muller, and D. J. Kelvin. 2008. Human immunopathogenesis of severe acute respiratory syndrome (SARS). *Virus Res.* 133: 13–19.
67. Alosaimi, B., M. E. Hamed, A. Naem, A. A. Alsharif, S. Y. AlQahtani, K. M. AlDosari, A. A. Alamri, K. Al-Eisa, T. Khojah, A. M. Assiri, and M. A. Enani. 2020. MERS-CoV infection is associated with downregulation of genes encoding Th1 and Th2 cytokines/chemokines and elevated inflammatory innate immune response in the lower respiratory tract. *Cytokine* 126: 154895.
68. Min, C.-K., S. Cheon, N.-Y. Ha, K. M. Sohn, Y. Kim, A. Aigerim, H. M. Shin, J.-Y. Choi, K.-S. Inn, J.-H. Kim, et al. 2016. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. *Sci. Rep.* 6: 25359.
69. Ricklin, D., E. S. Reis, and J. D. Lambris. 2016. Complement in disease: a defence system turning offensive. *Nat. Rev. Nephrol.* 12: 383–401.
70. Noris, M., and G. Remuzzi. 2013. Overview of complement activation and regulation. *Semin. Nephrol.* 33: 479–492.
71. Bajic, G., S. E. Degn, S. Thiel, and G. R. Andersen. 2015. Complement activation, regulation, and molecular basis for complement-related diseases. *EMBO J.* 34: 2735–2757.
72. Mortensen, S. A., B. Sander, R. K. Jensen, J. S. Pedersen, M. M. Golas, J. C. Jensenius, A. G. Hansen, S. Thiel, and G. R. Andersen. 2017. Structure and activation of C1, the complex initiating the classical pathway of the complement cascade. *Proc. Natl. Acad. Sci. USA* 114: 986–991.
73. Bohlson, S. S., P. Garred, C. Kemper, and A. J. Tenner. 2019. Complement nomenclature-deconvoluted. *Front. Immunol.* 10: 1308.
74. Merle, N. S., S. E. Church, V. Fremeaux-Bacchi, and L. T. Roumenina. 2015. Complement system part I - molecular mechanisms of activation and regulation. *Front. Immunol.* 6: 262.
75. Nauta, A. J., N. Raaschou-Jensen, A. Roos, M. R. Daha, H. O. Madsen, M. C. Borrias-Essers, L. P. Ryder, C. Koch, and P. Garred. 2003. Mannose-binding lectin engagement with late apoptotic and necrotic cells. *Eur. J. Immunol.* 33: 2853–2863.
76. Harboe, M., and T. E. Molnes. 2008. The alternative complement pathway revisited. *J. Cell. Mol. Med.* 12: 1074–1084.

77. Thurman, J. M., and V. M. Holers. 2006. The central role of the alternative complement pathway in human disease. *J. Immunol.* 176: 1305–1310.
78. Łukawska, E., M. Polcyn-Adamczak, and Z. I. Niemi. 2018. The role of the alternative pathway of complement activation in glomerular diseases. *Clin. Exp. Med.* 18: 297–318.
79. Amara, U., M. A. Flierl, D. Rittirsch, A. Klos, H. Chen, B. Acker, U. B. Brückner, B. Nilsson, F. Gebhard, J. D. Lambris, and M. Huber-Lang. 2010. Molecular intercommunication between the complement and coagulation systems. *J. Immunol.* 185: 5628–5636.
80. Békássy, Z. D., A.-C. Kristofferson, J. Rebetz, R. Tati, A. I. Olin, and D. Karpman. 2018. Aliskiren inhibits renin-mediated complement activation. *Kidney Int.* 94: 689–700.
81. Irmscher, S., N. Döring, L. D. Halder, E. A. H. Jo, I. Kopka, C. Dunker, I. D. Jacobsen, S. Luo, H. Slevogt, S. Lorkowski, et al. 2018. Kallikrein cleaves C3 and activates complement. *J. Innate Immun.* 10: 94–105.
82. Ghebrehiwet, B., B. P. Randazzo, J. T. Dunn, M. Silverberg, and A. P. Kaplan. 1983. Mechanisms of activation of the classical pathway of complement by Hageman factor fragment. *J. Clin. Invest.* 71: 1450–1456.
83. Reis, E. S., D. C. Mastellos, G. Hajishengallis, and J. D. Lambris. 2019. New insights into the immune functions of complement. *Nat. Rev. Immunol.* 19: 503–516.
84. Li, X. X., J. D. Lee, C. Kemper, and T. M. Woodruff. 2019. The complement receptor C5aR2: a powerful modulator of innate and adaptive immunity. *J. Immunol.* 202: 3339–3348.
85. Coulthard, L. G., and T. M. Woodruff. 2015. Is the complement activation product C3a a proinflammatory molecule? Re-evaluating the evidence and the myth. *J. Immunol.* 194: 3542–3548.
86. Ricklin, D., E. S. Reis, D. C. Mastellos, P. Gros, and J. D. Lambris. 2016. Complement component C3 - the "swiss army knife" of innate immunity and host defense. *Immunol. Rev.* 274: 33–58.
87. Triantafyllou, M., T. R. Hughes, B. P. Morgan, and K. Triantafyllou. 2016. Complementing the inflammasome. *Immunology* 147: 152–164.
88. Triantafyllou, K., T. R. Hughes, M. Triantafyllou, and B. P. Morgan. 2013. The complement membrane attack complex triggers intracellular Ca²⁺ fluxes leading to NLRP3 inflammasome activation. *J. Cell Sci.* 126: 2903–2913.
89. Vlaicu, S. I., A. Tatomir, V. Rus, A. P. Mekala, P. A. Mircea, F. Niculescu, and H. Rus. 2016. The role of complement activation in atherogenesis: the first 40 years. *Immunol. Res.* 64: 1–13.
90. Li, X. X., R. J. Clark, and T. M. Woodruff. 2020. C5aR2 activation broadly modulates the signaling and function of primary human macrophages. *J. Immunol.*
91. Ward, P. A. 2004. The dark side of C5a in sepsis. *Nat. Rev. Immunol.* 4: 133–142.
92. Wei, X. S., X. R. Wang, J. C. Zhang, W. B. Yang, W. L. Ma, B. H. Yang, N. C. Jiang, Z. C. Gao, H. Z. Shi, and Q. Zhou. 2020. A cluster of health care workers with COVID-19 pneumonia caused by SARS-CoV-2. *J. Microbiol. Immunol. Infect.*
93. Cugno, M., P. L. Meroni, R. Gualtierotti, S. Griffini, E. Grovetti, A. Torri, M. Panigada, S. Aliberti, F. Blasi, F. Tedesco, and F. Peyvandi. 2020. Complement activation in patients with COVID-19: a novel therapeutic target. *J. Allergy Clin. Immunol.* 146: 215–217.
94. Guo, R. F., and P. A. Ward. 2005. Role of C5a in inflammatory responses. *Annu. Rev. Immunol.* 23: 821–852.
95. Yousefi, S., C. Mihalache, E. Kozłowski, I. Schmid, and H. U. Simon. 2009. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ.* 16: 1438–1444.
96. Ibrahim, F. B., S. J. Pang, and A. J. Melendez. 2004. Anaphylatoxin signaling in human neutrophils. A key role for sphingosine kinase. *J. Biol. Chem.* 279: 44802–44811.
97. Arbore, G., and C. Kemper. 2016. A novel "complement-metabolism-inflammasome axis" as a key regulator of immune cell effector function. *Eur. J. Immunol.* 46: 1563–1573.
98. Zuo, Y., S. Yalavarthi, H. Shi, K. Gockman, M. Zuo, J. A. Madison, C. N. Blair, A. Weber, B. J. Barnes, M. Egeblad, et al. 2020. Neutrophil extracellular traps in COVID-19. *JCI Insight* 5: e138999.
99. Schindler, R., J. A. Gelfand, and C. A. Dinarello. 1990. Recombinant C5a stimulates transcription rather than translation of interleukin-1 (IL-1) and tumor necrosis factor: translational signal provided by lipopolysaccharide or IL-1 itself. *Blood* 76: 1631–1638.
100. Pan, Z. K. 1998. Anaphylatoxins C5a and C3a induce nuclear factor kappaB activation in human peripheral blood monocytes. *Biochim. Biophys. Acta* 1443: 90–98.
101. Wang, R., H. Xiao, R. Guo, Y. Li, and B. Shen. 2015. The role of C5a in acute lung injury induced by highly pathogenic viral infections. *Emerg. Microbes Infect.* 4: e28.
102. Lo, M. W., and T. M. Woodruff. 2020. Complement: bridging the innate and adaptive immune systems in sterile inflammation. *J. Leukoc. Biol.* 108: 339–351.
103. Shi, Y., Y. Wang, C. Shao, J. Huang, J. Gan, X. Huang, E. Bucci, M. Piacentini, G. Ippolito, and G. Melino. 2020. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ.* 27: 1451–1454.
104. de Beer, F. M., M. P. V. Begieneman, J. J. T. H. Roelofs, J. Horn, H. W. M. Niessen, M. J. Schultz, and W. K. Lagrand. 2019. Pulmonary complement depositions in autopsy of critically ill patients have no relation with ARDS. *Intensive Care Med.* 7(Suppl. 1): 35.
105. Mastaglio, S., A. Ruggeri, A. M. Risitano, P. Angelillo, D. Yancopoulou, D. C. Mastellos, M. Huber-Lang, S. Piemontese, A. Assanelli, C. Garlanda, et al. 2020. The first case of COVID-19 treated with the complement C3 inhibitor AMY-101. *Clin. Immunol.* 215: 108450.
106. Diurno, F., F. G. Numis, G. Porta, F. Cirillo, S. Maddaluno, A. Ragozzino, P. De Negri, C. Di Gennaro, A. Pagano, E. Allegorico, et al. 2020. Eculizumab treatment in patients with COVID-19: preliminary results from real life ASL Napoli 2 Nord experience. *Eur. Rev. Med. Pharmacol. Sci.* 24: 4040–4047.
107. Chen, J.-H., Y.-W. Chang, C.-W. Yao, T.-S. Chiueh, S.-C. Huang, K.-Y. Chien, A. Chen, F.-Y. Chang, C.-H. Wong, and Y.-J. Chen. 2004. Plasma proteome of severe acute respiratory syndrome analyzed by two-dimensional gel electrophoresis and mass spectrometry. *Proc. Natl. Acad. Sci. USA* 101: 17039–17044.
108. Pang, R. T., T. C. Poon, K. C. Chan, N. L. Lee, R. W. Chiu, Y. K. Tong, R. M. Wong, S. S. Chim, S. M. Ngai, J. J. Sung, and Y. M. Lo. 2006. Serum proteomic fingerprints of adult patients with severe acute respiratory syndrome. *Clin. Chem.* 52: 421–429.
109. Huang, J. L., J. Huang, Z. H. Duan, J. Wei, J. Min, X. H. Luo, J. G. Li, W. P. Tan, L. Z. Wu, R. Y. Liu, et al. 2005. Th2 predominance and CD8+ memory T cell depletion in patients with severe acute respiratory syndrome. *Microbes Infect.* 7: 427–436.
110. Houser, K. V., A. J. Broadbent, L. Gretebeck, L. Vogel, E. W. Lamirande, T. Sutton, K. W. Bock, M. Minai, M. Orandle, I. N. Moore, and K. Subbarao. 2017. Enhanced inflammation in New Zealand white rabbits when MERS-CoV reinfection occurs in the absence of neutralizing antibody. *PLoS Pathog.* 13: e1006565.
111. Gralinski, L. E., T. P. Sheahan, T. E. Morrison, V. D. Menachery, K. Jensen, S. R. Leist, A. Whitmore, M. T. Heise, and R. S. Baric. 2018. Complement activation contributes to severe acute respiratory syndrome coronavirus pathogenesis. *MBio* 9: e01753-18.
112. Jiang, Y., G. Zhao, N. Song, P. Li, Y. Chen, Y. Guo, J. Li, L. Du, S. Jiang, R. Guo, et al. 2018. Blockade of the C5a-C5aR axis alleviates lung damage in hDPP4-transgenic mice infected with MERS-CoV. *Emerg. Microbes Infect.* 7: 77.
113. Mulvey, J. J., C. M. Magro, L. X. Ma, G. J. Nuovo, and R. N. Baergen. 2020. Analysis of complement deposition and viral RNA in placentas of COVID-19 patients. *Ann. Diagn. Pathol.* 46: 151530.
114. Batlle, D., M. J. Soler, M. A. Sparks, S. Hiremath, A. M. South, P. A. Welling, and S. Swaminathan. COVID-19 and ACE2 in Cardiovascular, Lung, and Kidney Working Group. 2020. Acute kidney injury in COVID-19: emerging evidence of a distinct pathophysiology. *J. Am. Soc. Nephrol.* 31: 1380–1383.
115. Gravastrand, C. S., B. Steinkjer, B. Halvorsen, A. Landsem, M. Skjelland, E. A. Jacobsen, T. M. Woodruff, J. D. Lambris, T. E. Mollnes, O.-L. Brekke, et al. 2019. Cholesterol crystals induce coagulation activation through complement-dependent expression of monocytic tissue factor. *J. Immunol.* 203: 853–863.
116. Ikeda, K., K. Nagasawa, T. Horiuchi, T. Tsuru, H. Nishizaka, and Y. Niho. 1997. C5a induces tissue factor activity on endothelial cells. *Thromb. Haemost.* 77: 394–398.
117. Gulla, K. C., K. Gupta, A. Krarup, P. Gal, W. J. Schwaebel, R. B. Sim, C. D. O'Connor, and K. Hajela. 2010. Activation of mannan-binding lectin-associated serine proteases leads to generation of a fibrin clot. *Immunology* 129: 482–495.
118. Wiedmer, T., C. T. Esmon, and P. J. Sims. 1986. Complement proteins C5b-9 stimulate procoagulant activity through platelet prothrombinase. *Blood* 68: 875–880.
119. Krarup, A., R. Wallis, J. S. Presanis, P. Gal, and R. B. Sim. 2007. Simultaneous activation of complement and coagulation by MBL-associated serine protease 2. *PLoS One* 2: e623.
120. U.S. National Library of Medicine. 2020. Clinical trials.gov. U.S. National Library of Medicine, Bethesda, MD.
121. Risitano, A. M., D. C. Mastellos, M. Huber-Lang, D. Yancopoulou, C. Garlanda, F. Ciceri, and J. D. Lambris. 2020. Complement as a target in COVID-19? [Published erratum appears in 2020 *Nat. Rev. Immunol.* 20: 448.] *Nat. Rev. Immunol.* 20: 343–344.
122. Giudice, V., P. Pagliano, A. Varella, A. Masullo, S. Poto, B. M. Polverino, R. Gammaldi, A. Maglio, C. Sellitto, C. Vitale, et al. 2020. Combination of Ruxolitinib and Eculizumab for treatment of severe SARS-CoV-2-related acute respiratory distress syndrome: a controlled study. *Front. Pharmacol.* 11: 857.
123. Cheng, V. C. C., S. K. P. Lau, P. C. Y. Woo, and K. Y. Yuen. 2007. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. *Clin. Microbiol. Rev.* 20: 660–694.