Is long COVID a complement dysregulation disease?

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Abbreviations: Coronavirus disease, COVID; severe-acute-respiratory-syndrome-related coronavirus, SARS-CoV-2; post-acute sequelae of SARS-CoV-2 infection, PASC; computed tomography, CT; terminal complement component, TCC; enzyme linked immunosorbent assay, ELISA; complement component 2, C2; complement factor B, FB; Complement component 5, C5; Complement component 7, C7; membrane attack complex, MAC; thrombospondin-1, TSP-1; von Willebrand factor, vWF, complement factor D, FD; complement component 3, C3.

In a recent report in *Science*, Cervia-Hasler identified the complement system as the top dysregulated biological pathway in long Coronavirus disease (COVID), thus implicating complement in the disease process and raising the prospect of blocking complement activation as a promising therapeutic intervention.[1] However, this finding has not been independently validated, which emphasizes the need for data replication in order to draw a reliable conclusion. In this brief commentary I seek to set this observation in context.

The legacy of COVID-19 extends far beyond the acute phase with ~20% of cases (in the UK ~1.9 million people or 2.9% of the population) experiencing symptoms more than 12 weeks after the acute infection, referred to as long COVID or post-acute sequelae of severe-acute-respiratory-syndrome-related coronavirus (SARS-CoV-2) infection (post-acute sequelae of SARS-CoV-2 infection; PASC). Typical symptoms include persistent fatigue, shortness of breath, muscle pains and brain fog. Long COVID imposes a substantial burden on the economy, as individuals grappling with the condition are often incapacitated, unable to work, and cannot contribute fully to society. In our recent study, almost 90% of individuals with long COVID were not in employment.[2] The long-term repercussions of long COVID may extend to future health complications such as diabetes, cardiovascular disease, and dementia. [1,2] Understanding mechanisms to guide therapy is thus an urgent priority.

Persistent inflammation is a key feature in long COVID, perhaps a consequence of virus persistence in endothelial cells. Increased levels of inflammatory cytokines along with C-reactive protein and serum amyloid A were found in long COVID patients 6-9 months post SARS-CoV-2 infection. In a systematic review including 22 studies, increased concentrations of plasma interleukin-6 in plasma were consistently associated with long COVID.[3] Conversely, the presence of autoantibodies against inflammatory chemokines during the convalescent phase

correlated with improved outcome and a reduced likelihood of developing long COVID. Activation of innate immune cells has also been identified as a contributing factor to lung fibrosis and inflammation in a humanised mouse model of long COVID (reviewed in ref 2). All these reports provide evidence of immune system dysregulation with accompanying inflammation as crucial factors in long COVID, yet none pinpoint an obvious inflammatory trigger or target for therapy.

Although complement, a key driver of inflammation, is strongly implicated in acute COVID-19, its contribution to long COVID is underexplored. One recent study reported that levels of the small complement component C4 (C4) fragment C4d were associated with chest computed tomography (CT) changes at 3 months in long COVID patients and that increased levels of complement activation products were present in plasma at 3 months and 1 year in long COVID individuals compared to healthy controls.[4] We identified a set of complement activation markers in long COVID cases implicating the activation loop (complement small factor B (FB) fragment (Ba), complement small complement component (C3) fragment (iC3b), complement small complement C5 (C5) fragments (C5a)) and downstream terminal pathway (terminal complement complex (TCC)), providing a diagnostic tool for long COVID.[2] Notably, we previously showed that the amplification loop is dysregulated in acute COVID-19 subjects with a single amplification loop-specific biomarker, Ba, being the best death predictor (reviewed in 2).

The study by Cervia-Hasler et al. comprised a longitudinal study of 268 serum samples obtained from 39 healthy controls and 113 COVID-19 patients followed for up to one year after acute SARS-CoV-2 infection. They utilised a non-targeted approach to identify the best predictive biomarker(s) associated with long COVID by measuring >6500 proteins in these

serum samples using the SomaScan proteomics platform. At the 6-month follow-up, 40 patients exhibited long COVID symptoms. Machine learning and other computational tools were applied to identify candidate biomarkers predictive of long COVID; these were then validated in wet lab settings (using ELISA and mass spectrometry). Best hits in this analysis included elevated levels of complement activation products (terminal complement complex (TCC), complement small C3 fragment C3d) and complement proteins (complement component C2 (C2), FB, complement component C5 (C5)); increased complement activity was also noted. The most informative biomarkers were the complement component C7 (C7) and C7 containing complexes, both markedly reduced in long COVID, implicating the terminal complement pathway.[1] Whether the observed reduction in terminal pathway markers reflected decreased synthesis or increased consumption secondary to membrane attack complex (MAC) formation in tissues was not tested, although the latter might explain the observed tissue damage. The authors suggest that monitoring C7 levels in acute COVID-19 cases could provide a predictive biomarker for long COVID, supporting prediction of disease course and associated tissue damage. Regardless of the precise mechanism these analyses provide compelling evidence of ongoing complement dysregulation in long COVID, a likely driver of the observed persistent inflammation.

Our study implicated complement activation products Ba, iC3b, C5a and TCC as informative biomarkers for long COVID,[2] while Cervia-Hasler et al. identified C7 and C7-containing complexes as most relevant biomarkers of long COVID. Unfortunately, these markers are not routinely measured in clinical laboratories and assays are poorly validated even in specialist laboratories, a major limitation to their utilisation as potential disease biomarkers. If these assays are to be used in assessment of long COVID, efforts are needed to further develop and standardise them to allow their use in routine settings.

Cervia-Hasler and colleagues did seek further insight into the underlying pathological mechanism. They showed correlation of complement dysregulation with markers of thromboinflammation, a hallmark of long COVID**;** these included coagulation factor VIII, thrombospondin-1 (TSP-1), von Willebrand factor (vWF), fibrinogen beta, factor XI, protein C, and heparin cofactor II.[1] The observed link between complement dysregulation and thromboinflammation confirms the importance of cross-talk between complement and coagulation systems, a finding seen in other contexts; cross-talk is bidirectional, tightly controlled, and crucial for driving the immune response, inflammation, and hemostasis.[1,5] The authors propose that after acute COVID-19 infection, localised activation of complement and coagulation systems persists across various tissues in those who progress to long COVID. The endothelial cell damage is mediated by complement terminal pathway complexes perturbing cell membranes and leading to the release of thrombotic markers, including vWF and TSP1. These in turn induce platelet activation, facilitate thrombin generation and promote interactions between monocytes and platelets, resulting in microclot formation, a common feature of long COVID. Accumulating vWF aggregates in turn activate the amplification loop of complement resulting in the small complement component of C3 fragment C3b deposition and sustaining local complement activation and inflammation.

Currently, there are no specific therapies available for long COVID; available treatments primarily involve alleviating symptoms and rehabilitation. Some ongoing clinical trials are exploring medications for specific symptoms, such as ivabradine for cardiac damage, pirfenidone and inhaled interferon-1 for fibrotic lung injury, and leronlimab for inflammation triggered by acute SAR-CoV-2 infection. The demonstration that complement dysregulation is a core feature of long COVID highlights the potential for use of anti-complement drugs in therapy of the condition. Therapeutic complement inhibition might break the vicious cycle of

complement activation and tissue damage and restore normal homeostasis. Several complement inhibitors are already in the clinic,[5] and could be repurposed for long COVID therapy (Figure 1). Given the evidence implicating the amplification loop detailed above, drugs targeting amplification such as iptacopan (targeting FB), danicopan (targeting complement factor D (FD)) or pegcetacoplan (targeting C3) might be most effective. However, targeting the terminal pathway, for example with the long established C5-blocking antibody eculizumab, may also be beneficial. Anti-complement drugs used in trials for acute COVID-19 showed limited success with a single drug, the anti-C5a antibody vilobelimab, gaining limited FDA approval. [2,5] Long COVID is a very different disease, manifesting with low-grade inflammation as opposed to the acute hyperinflammatory state typifying some cases of acute COVID-19. Anti-complement therapies may prove to be more effective in this scenario, particularly if used in conjunction with reliable complement biomarkers (e.g. complement activation products; Ba, iC3b, TCC) to identify patients likely to benefit from the treatment. A reliable biomarker would inform treatment response, particularly indicating a reduction in complement activation. A proof-of-concept study or clinical trial utilising complement inhibitors is needed to validate this hypothesis.

References:

1. Cervia-Hasler C, Brüningk SC, Hoch T, Fan B, Muzio G, Thompson RC, et al. Persistent complement dysregulation with signs of thromboinflammation in active Long COVID. Science. 2024;383(6680):eadg7942.
2. Baillie K, Davies HE, Keat SBK, Ladell K, Miners KL, Jones SA, et al. Complement dysregulation is a prevalent and therapeutically amenable feature of long COVID. Med. 2024;5(3):239-253.e5.

3. Yin JX, Agbana YL, Sun ZS, Fei SW, Zhao HQ, Zhou XN, et al. Increased interleukin-6 is associated with long COVID-19: a systematic review and meta-analysis. Infect Dis Poverty. 2023;12(1):43.
4. Barratt-Due A, Pettersen K, Børresdatter-Dahl T, Holter JC, Grønli RH, Dyrhol-Riise AM, et al. Escalated complement activation during hospitalization is associated with higher risk of 60-day mortality in SARS-CoV-2-infected patients. J Intern Med. 2024.
5. Mastellos DC, Hajishengallis G, Lambris JD. A guide to complement biology, pathology and therapeutic opportunity. Nat Rev Immunol. 2024;24(2):118-141.

Figure 1. The complement cascade and complement drugs in clinics. The complement system is activated via the classical and lectin pathways and amplified by the amplification loop of the alternative pathway. The classical pathway is initiated by complement component C1 (C1) binding to antigen-antibody complexes. A small fragment of C1 C1s in the C1 complex cleaves C4 and C2 to form the C3 convertase C4b2a. The lectin pathway is activated by mannose binding lectin (MBL) or other lectins binding surface carbohydrates; attached (mannan binding lectin serine proteases) MASPs are activated to cleave C4 and C2 to generate C4b2a. The C3 convertase cleaves C3 to C3b (a small fragment of C3) and C3a. C3b, C4b (a small fragment of C4) and their degradation products are important opsonins. C3b also binds FB enabling its cleavage by FD to form the C3bBb convertase that cleaves more C3 in a feedback cycle, the alternative pathway amplification loop. Binding of a further C3b to either C3 convertase creates a C5 convertase which cleaves C5 to initiate the terminal pathway culminating in formation of the soluble (terminal complement complex; TCC) and membrane-inserted (membrane attack complex; MAC) complexes. Complement small fragment of C3, C3a and C5a, are anaphylatoxins that signal via their receptors to recruit immune cells. FDA (Food and

Drug Administration) approved complement drugs in clinics are shown in red. Numerous other complement inhibitors are currently in development, undergoing preclinical and clinical trials, with a focus on targeting diverse pathways. Figure created with BioRender (BioRender.com).

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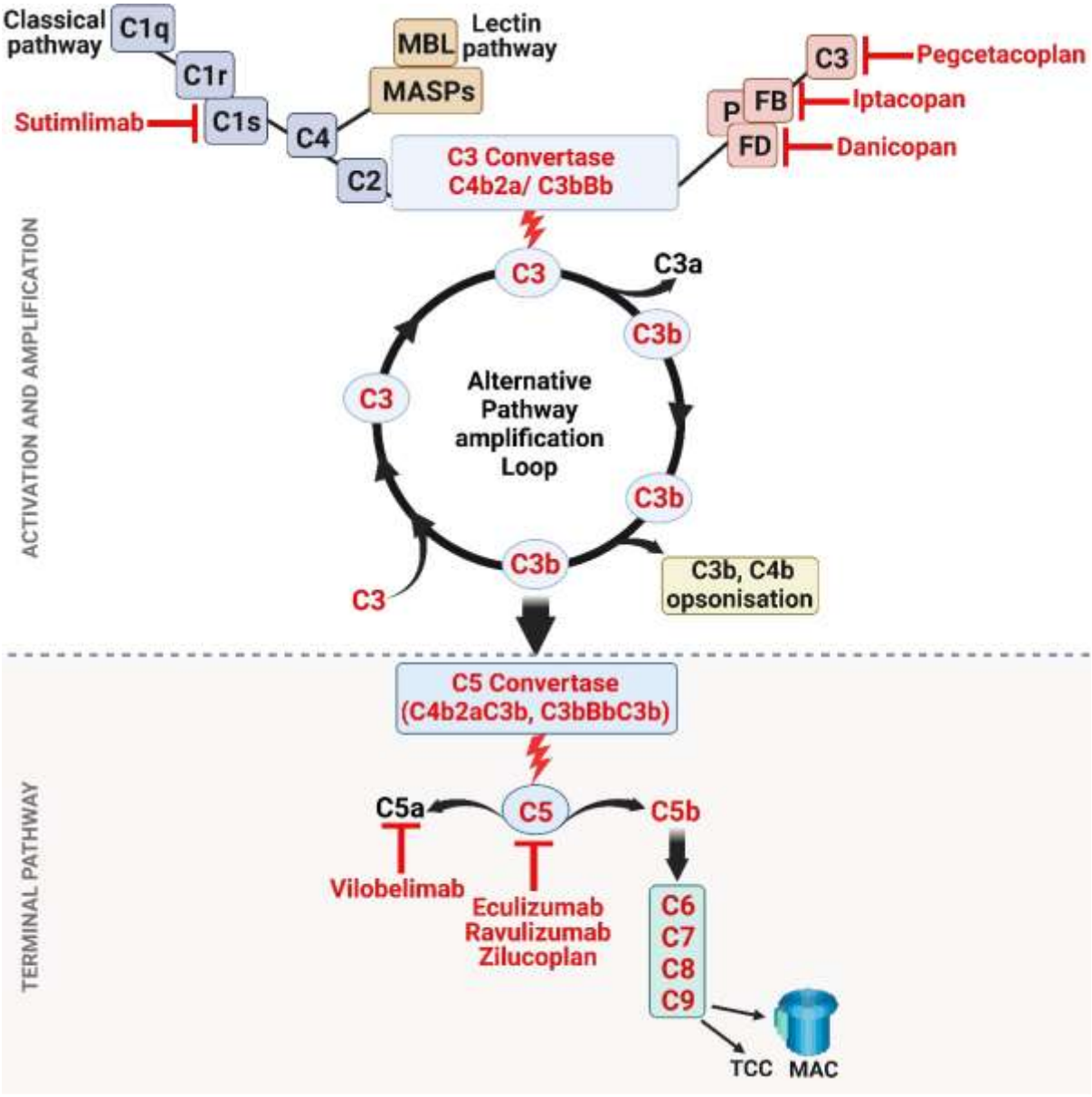
Figure 1


Classical pathway
C1q
C1r
C1s
Sutimlimab
C4
C2
MBL
Lectin pathway
MASPs
C3 Convertase
C4b2a/ C3bBb
P
FB
FD
C3
Pegcetacoplan
Iptacopan
Danicopan
ACTIVATION AND AMPLIFICATION
C3
C3a
C3b
Alternative Pathway amplification Loop
C3b
C3b
C3
C3b, C4b opsonisation
TERMINAL PATHWAY
C5 Convertase
(C4b2aC3b, C3bBbC3b)
C5a
C5
C5b
Vilobelimab
Eculizumab
Ravulizumab
Zilucoplan
C6
C7
C8
C9
TCC
MAC