Towards a cure for long COVID: the strengthening case for persistently replicating SARS-CoV-2 as a driver of post-acute sequelae of COVID-19

New insights into post-acute sequelae of coronavirus disease 2019 (PASC) or long COVID are emerging at great speed. Proposed mechanisms driving long COVID include the overlapping pathologies of immune and inflammatory dysregulation, microbiota dysbiosis, autoimmunity, endothelial dysfunction, abnormal neurological signalling, reactivation of endogenous herpesviruses, and persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2] In this commentary, we describe some of these advances that indicate that long COVID may be driven by "long infection" and that persistent replicating SARS-CoV-2 may be the potentially mechanistically unifying driver for long COVID.

Long COVID is a large and growing concern

The United Kingdom (UK)[3] and United States (US)[4] report that substantial proportions of their populations are affected by long COVID, and that these proportions have remained at similar or slightly elevated levels across the past year at around 3% in the UK, and 5.5% in the US. Factors likely driving this include the chronic nature of long COVID lasting several years in some, and the high number of ongoing infections and cumulative risk of long COVID with each infection,[5] even in highly vaccinated populations.[6] Individuals in low income countries also suffer a substantial, albeit less defined, long COVID burden.[7] Moreover, children are not spared,[8] with up to 5.8 million children estimated to have the disease in the US alone.[8] Using the UK and US figures to extrapolate the global prevalence of long COVID generates an estimate of several hundred million people with long COVID.

Common symptoms of long COVID include fatigue, brain fog and post-exertional malaise (PEM).[9] Long COVID is also highly associated with cardiovascular and autonomic dysfunction, particularly postural autonomic tachycardia syndrome (POTS) and a vast range of fluctuating symptoms,[5,10] and shares overlapping symptomology with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). These symptoms can make undertaking typical activities extremely difficult with implications for workforce access and productivity, and school participation. There are several definitions of long COVID and this creates a barrier to timely diagnosis and access to care, in addition to the research and epidemiological challenges this creates. Clearer terminology for the distinction between increased risk of specific health conditions (eg, type 1 diabetes, cardiac events) and the syndrome of long COVID is also important.

Long COVID and pathophysiology: key recent advances

The long term impacts of COVID on the brain are becoming clearer. Sustained inflammation disrupting the blood–brain barrier has been shown to be a key mechanism driving the cognitive and related symptoms in long COVID.[11] A recent landmark study demonstrated a lowering of IQ by 6 points in individuals with long COVID relative to unaffected individuals. Individuals with mild acute infection showed a 3-point drop in IQ.[12] Given how widespread long COVID is, the implications for societies are substantial.

A hallmark study revealed new insights into the pathophysiological mechanisms of PEM, demonstrating damaged skeletal muscle in people with long COVID; damage that worsened with exercise.[9] The longitudinal nature of their approach was especially significant, underscoring specific physiological and metabolic pathologies that drive PEM, and related exercise intolerance. It has implications warning against graded exercise as a therapeutic approach in people with long COVID and ME/CFS.

Avenues for long COVID diagnostics via an inflammatory signature have advanced significantly.[13] Cervia-Hasler and colleagues implicated a persistent, dysregulated complement cascade as a cause of thrombo-inflammation-driven tissue damage in long COVID. Their work addresses the link between chronic complement-activation and amyloid fibrinogen particles ("microclots"), vascular inflammation, and cardiovascular complications in long COVID. Further, individuals with symptom resolution by six months also had normalisation of their complement levels, whereas individuals with a thrombo-inflammatory signature at six months were more likely to have long COVID beyond 12 months.

Inflammation and immune dysregulation have long been seen as key aspects of SARS-CoV-2 pathophysiology,[14] with a study by Yin and colleagues[15] on disrupted acquired cellular and humoral immunity in long COVID a recent standout. Here, long COVID patients had systemic inflammation and immune dysregulation, consistent with ongoing immune responses. Notable was that SARS-CoV-2-specific $CD8^+$ T cells in long COVID patients commonly expressed "exhaustion" markers, and these patients had higher SARS-CoV-2 antibody levels. Both observations are consistent with ongoing exposure to viral antigens.

Persistent immune dysfunction as a feature of long COVID is not a new concept, nor is it limited

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to adaptive immunity; innate immunity is also impacted.[16] A strength of the study by Phetsouphanh and colleagues was to show that the innate and adaptive immune effects in long COVID patients seen eight months after mild/moderate acute infections were specific to SARS-CoV-2 as they did not occur with other common-cold coronaviruses. Recently, these same authors reviewed the same cohort at 23 months.[17] Encouragingly, 62% of people with long COVID had vastly improved immunological biomarkers and this correlated with improvement in quality-of-life scores. The observation that over one-third of this group had not recovered after two years demonstrates a large ongoing challenge, even without considering re-infections. Immune dysfunction has obvious implications for susceptibility to other pathogens and chronic morbidities at an individual level and at a population scale.

Does replicating virus persist in the body?

Long term inflammation is a common feature of the long COVID pathologies described above. A key question is whether persistent SARS-CoV-2 drives this inflammation and hence is causal to long COVID. A large body of evidence shows that SARS-CoV-2 antigens (fragments of RNA and/or protein) persist in many sites in the body, at least in a subset of people.[18,19] However, what remains to be determined is how common viral persistence following acute infection is, and crucially, if the source of antigen is from replicating virus. If the latter is true, antiviral treatment and vaccination strategies could be used not just to prevent long COVID, but potentially also as "cures". Below we describe several recent studies that are starting to address these gaps and add weight to the notion that persistently replicating SARS-CoV-2 is an underlying driver of long COVID, and perhaps even the only driver.

Among the most important of these recent studies is a community surveillance study recently published in *Nature*.[20] Ghafari and colleagues followed more than 90 000 people, taking regular nasopharyngeal swabs regardless of symptoms or test history and sequenced viral genomic fragments from these samples to distinguish persistent from new infections. Remarkably, viral genomic RNA could be detected in the respiratory tract for one to six months after initial infection and this was surprisingly frequent, between 1/200 to 1/1000 of all infections. Ghafari and colleagues clarified that their approach only detected "high titre" infections (with a polymerase chain reaction cycle threshold value of < 30) and referenced other work that used more sensitive detection methods (cycle threshold > 30) and found 6% of infections persisted more than a month following symptom onset. This indicates that Ghafari and colleagues likely underestimated the frequency of viral persistence.

Although this does not prove that the detected virus was viable and replicating, the relatively frequent observation of "rebounding" viral genome loads, and the clear signatures of positive selection pressure in the persistent genomes (each sample had at least 50% genome sequence coverage), strongly point to the presence of virus that is replicating over several months at least. Importantly, this persistent infection was associated with a 55% higher chance of long COVID symptoms at 12 weeks or more after infection. This work convincingly demonstrates that virus clearance from the respiratory tract is delayed in many people for much longer than previously thought and that this persistence is associated with long COVID.

Adding to this with a very different approach, Menezes and colleagues performed a differential transcriptome analysis in the whole blood of 60 well characterised and matched donors, comprising 48 long COVID cases and 12 controls,[21] with samples taken almost two years after acute infection. Viral genes were distinctly upregulated in individuals with long COVID relative to controls, and this included the presence of antisense viral RNA, which can only be present if RNA replication has occurred. The authors also found a positive association between the amount of viral RNA detected and symptom severity.

In another related advance, Peluso and colleagues[22] detected persistent SARS-CoV-2 antigens in blood plasma up to 14 months after acute infection. Their approach used a highly sensitive detection method and powerfully controlled for important potential confounders by looking at samples obtained before vaccination or re-infection and compared these to samples obtained before 2020. This study shows that viral antigens (spike, NC and S1 proteins) persist in plasma with antigens detected in 11% of samples at six to ten months and 7.4% at ten to 14 months after acute infection. While there was no attempt to link persisting antigen to long COVID, the frequency of antigen detection, and its link to severity of disease (patients hospitalised with acute COVID were more likely to have persistent viral antigen in their plasma) is consistent with such an association.

Recent work by Zuo and colleagues[23] adds to the many tissues from which viral antigens have been identified,[24] with viral RNA found in ten different tissue types from 225 participants at one, two and four months following acute infection. Although sampling was only performed four months after infection, the authors demonstrated an association between high viral RNA and increased likelihood of developing long COVID symptoms.

Together with extensive earlier work, these recent advances constitute a powerful body of evidence demonstrating that SARS-CoV-2 infection can persist for extended periods, and that this persistence is linked to long COVID.[25] The gastrointestinal tract is one leading example of a potential viral reservoir,[26] while megakaryocytes in the bone marrow and platelets are others.[27] Culturing virus from reservoirs would help provide the gold standard proof that persistent virus causes long COVID, but this is technically challenging to achieve.

Implications for treatment and prevention

If persistent infection is a driver of long COVID, then specific anti-SARS-CoV-2 approaches should improve outcomes. Some recent evidence suggests that this is

the case. In perhaps the most important study[28] of its type, involving over 20 million people across three countries, vaccination was shown to reduce the risk of long COVID by 29–52%. Very recently published work spanning March 2020 to January 2022 also shows that vaccination provided substantial protection against long COVID; with unvaccinated individuals more than twice as likely to develop long COVID.[29]

On the therapeutic front, a randomised control trial confirmed prior findings that the common drug metformin has anti-viral properties.[30] Bramante and colleagues demonstrated that, compared with placebo, treatment with metformin for acute COVID-19 substantially reduced the overall viral load at Day 5 and Day 10 (overall effect -0.56 $\log_{10}$ copies/mL, 95% confidence interval, -1.05 to -0.063, $P = 0.027$). Remarkably, this reduction in viral load was associated with a 41% reduction of long COVID outcomes at ten months post-infection. Although there remains no cure for long COVID, an increase in randomised controlled trials for its prevention and treatment has begun.

Importantly, there are evidence-based treatments and symptom control approaches for some of the many comorbidities that occur in long COVID (eg, POTS), and it is critical that these are pursued to maximise people's quality of life and reduce their symptom burden.

Conclusions

Evidence is mounting that at least some of the potential mechanisms driving long COVID mentioned herein (immune and inflammatory dysregulation, microbiota dysbiosis, autoimmunity and endothelial dysfunction) may themselves have the common denominator of persistent SARS-CoV-2 infection. This has several important implications:

- Prioritise long COVID: the notion of "long infection" should help further demystify long COVID, validating individuals who live with this illness (and post-acute infection syndromes in general) and have it move into the mainstream of surveillance and strategies for diagnosis, treatment and prevention.
- Ramp-up existing antiviral approaches: existing approaches, including vaccines and therapies, appear to decrease the risk of long COVID and should be more actively included in trials for long COVID. This recognition and the potential cost-effectiveness implications should prompt reassessment of eligibility requirements for access to therapies and vaccines to promote their use in younger people and to individuals even with milder forms of acute infection.
- Urgently develop new antiviral approaches and long COVID diagnostics: there is now increased impetus for the development of improved therapeutics and vaccines for SARS-CoV-2 in addition to definitive biological long COVID diagnostic tests to complement clinical diagnoses.
- Strengthen prevention approaches: long COVID incidence remains high, and this carries a large health, labour and economic impact. It is therefore critical that sustainable prevention strategies are strengthened, especially in higher transmission-risk settings. Major recent advances towards safer indoor air environments offer one clear avenue to achieve this.

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