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Review article

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The immunology of long COVID

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Abstract

Long COVID is the patient-coined term for the disease entity whereby persistent symptoms ensue in a significant proportion of those who have had COVID-19, whether asymptomatic, mild or severe. Estimated numbers vary but the assumption is that, of all those who had COVID-19 globally, at least 10% have long COVID. The disease burden spans from mild symptoms to profound disability, the scale making this a huge, new health-care challenge. Long COVID will likely be stratified into several more or less discrete entities with potentially distinct pathogenic pathways. The evolving symptom list is extensive, multi-organ, multisystem and relapsing–remitting, including fatigue, breathlessness, neurocognitive effects and dysautonomia. A range of radiological abnormalities in the olfactory bulb, brain, heart, lung and other sites have been observed in individuals with long COVID. Some body sites indicate the presence of microclots; these and other blood markers of hypercoagulation implicate a likely role of endothelial activation and clotting abnormalities. Diverse auto-antibody (AAB) specificities have been found, as yet without a clear consensus or correlation with symptom clusters. There is support for a role of persistent SARS-CoV-2 reservoirs and/or an effect of Epstein–Barr virus reactivation, and evidence from immune subset changes for broad immune perturbation. Thus, the current picture is one of convergence towards a map of an immunopathogenic aetiology of long COVID, though as yet with insufficient data for a mechanistic synthesis or to fully inform therapeutic pathways.

Sections

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Introduction

From the early days of the COVID-19 pandemic at the start of 2020, it became apparent that many aspects of recovery from SARS-CoV-2 infection were dissimilar to infections by the related, common cold, human coronaviruses. Individuals who had COVID-19 in the first wave, including patients who had only mild or asymptomatic infections, frequently reported lingering symptoms and a failure to return to baseline health[1–3]. Patient-led accounts of this experience accumulated on social media sites and many support groups were formed. These patient groups coined the term 'long COVID'[4]. Although attempts continue to re-designate it with other terms such as PASC (post-acute sequelae of SARS-CoV-2 infection), the case for retaining the patient-led term long COVID has been persuasively argued[5]. Moving forward, it may well be that, as long COVID stratifications are better defined, more formal, clinical terminology will add value, including the potential to target trials to the most appropriate individuals. In 2021, a WHO working group put in place a Delphi consensus definition of long COVID (Box 1). Without a clear and reportable diagnostic test, assessing the prevalence of long COVID is contentious but is generally agreed to be over 10% of all SARS-CoV-2 infections (Box 2).

There has been relatively little detailed analysis of the differential risk, symptomology or mechanisms of long COVID ensuing from infections by SARS-CoV-2 variants of concern. Because of the chronological separation of waves due to the different variants of concern, it is hard to differentiate between the various intrinsic properties of the viruses and changes in the immune status of the infected populations at a given time point. The key point has been that, even with vaccination and milder presentations of acute infection across recent variant waves, the cumulative burden of long COVID has continued to rise (Fig. 1). Progressing from long COVID cases of the first wave into those from the Alpha wave, a change in symptomology was observed, with increased myalgia (muscle-ache), brain fog and anxiety but decreased anosmia and dysgeusia (loss of smell and altered sense of taste)[6]. An analysis in Norway indicated that the Delta and Omicron waves carried an equal risk of subsequent long COVID, though post-Omicron long COVID tended to resolve more rapidly and was less likely to involve myalgia[7]. By contrast, a larger analysis of 41,000 individuals who had COVID-19 during the Delta wave and 56,000 from the Omicron wave in the UK, found reduced odds of long COVID in the latter case by 0.24–0.5, depending on age and time from vaccination[8]. However, the lower odds of long COVID per infected case were offset by the enhanced population caseloads of the Omicron subvariant waves. That a somewhat conserved symptomology endures across variants of differing severities and even in vaccinated populations and, thus, across differing viral loads, points to the presence of virus intrinsic mechanistic drivers of pathology. In the more recent period of relatively commonplace reinfections, a number of health-care record studies have looked at the risks of long COVID after these events, considering cohorts in the USA, UK and the Netherlands[9–11]. The studies in the USA and Netherlands each show an increased risk of persistent symptoms with reinfection, for example, in the USA, a hazard ratio (HR) of 3.54 for pulmonary sequelae after reinfection was observed. The UK study found no evidence of enhanced risk[11].

Here, we focus on current thinking about the pathophysiology of long COVID and the evidence for potential underpinning mechanisms. It will be evident from this that, although great progress has been made, we are far from a coherent schema linking SARS-CoV-2 infection to the diverse array of persistent symptoms that have been described. Hypothesized mechanisms include end-organ damage, a persistent SARS-CoV-2 reservoir, an abnormal immune response to acute COVID-19, an effect of reactivation of other viruses such as Epstein–Barr virus (EBV), altered systemic immunity, auto-immunity (including effects on the autonomic nervous system), coagulopathy and microbiome dysbiosis. Different disease descriptors may clearly impact those tests chosen for analysis and, in turn, whether particular participants are selected for a given clinical trial.

Long COVID symptoms

The lists of long COVID symptoms were initially derived through reports from patient groups, leading to a keynote online survey conducted at the end of 2020 from nearly 4,000 individuals across 56 countries that supplied symptom data spanning neuropsychiatric, systemic, reproductive, cardiovascular, musculoskeletal, immunological, head-ear-eye-nose-throat, pulmonary, gastrointestinal and dermatological symptoms[12]. A systematic review from 2021 covering 39 studies highlighted weakness (41%), general malaise (33%), fatigue (31%), impaired concentration (26%) and breathlessness (25%) as the most common symptoms[13]. That is, long COVID is a truly multi-organ, multisystem disease, with symptoms that appear to indicate a pathological process beyond and distinct from just the ACE2-positive tissues targeted for viral ingress during the acute infection[14–16]. Unsurprisingly for such wide symptomology, there is some overlap with symptoms in 'long SARS', myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and other post-acute infection syndromes[17–19] (Box 3). For a disease process with such a diverse array of symptom combinations, the challenge has been whether and how to stratify patients into

Box 1

Defining long COVID

WHO Delphi Consensus definition of long COVID:
Post-COVID-19 condition that occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19, with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include fatigue, shortness of breath, cognitive dysfunction and others, which generally have an impact on everyday functioning. Symptoms may be new onset, following initial recovery from an acute COVID-19 episode, or persist from the initial illness. Symptoms may also fluctuate or relapse over time. A separate definition may be applicable to children.

UK National Institute for Health and Care Excellence (NICE) definition:
Long COVID is a multisystem condition with a range of debilitating symptoms — signs and symptoms continue or develop after acute COVID-19, continue for more than 4 weeks, and are not explained by an alternative diagnosis. It includes both ongoing symptomatic COVID-19 (from 4 to 12 weeks) and post-COVID-19 syndrome (12 weeks or more). Long COVID may consist of a number of distinct syndromes, which can include post-intensive care unit syndrome, post-viral fatigue syndrome, long-term COVID syndrome and permanent organ damage.

Box 2

How common is long COVID after acute infection?

Many individuals affected by long COVID never seek medical advice for their condition, presumably assuming that nothing can be done for them. Indeed, among the lowest published estimates for long COVID prevalence is the inference from a Danish population survey based on prescription records initiating new drugs or new GP visits or hospital referrals during the 6 months after acute infection[140]. Another recent report that based imputation of time to recovery from long COVID on patient attrition in terms of electronic health records[141] has been debated on the grounds that concerns about methodology and interpretation mean that the study offers an overly optimistic view of long COVID recovery[142]. Counting long COVID cases remains complex due to the use of different methodologies and definitions, especially defining long COVID as symptoms persisting for at least 4 weeks versus 12 weeks from the start of acute infection. Counting method differences largely account for the array of long COVID frequencies cited, from below 10% to around 60% across studies. The 'COVID in Scotland' study including ~33,000 individuals with laboratory-confirmed COVID-19 found that, by 18 months, 6% had not recovered at all and 42% had partially recovered[143]. From a recent meta-analysis covering approximately 0.75 million cases of COVID-19 across many countries, an overall figure of 45% experienced persistent symptoms at 4 months, irrespective of hospitalization status[144]. The REACT-2 survey (Imperial College, UK) is illuminating, being a longitudinal community study of over 606,000 people in England surveyed about their symptoms[145]. Long COVID prevalence varied with time after acute infection and the number of symptoms. People gradually recovered with time from onset but, at 4 weeks after first symptoms, around 40% still reported one or more symptoms. By 12 weeks, this had dropped minimally to 38%. Prevalence was essentially sustained at this level through the next 6 months of the survey period. The results from this random survey in the community show that most of the observed recovery for the lucky 60% occurs during the first 4 weeks, but those still showing symptoms at 12 weeks find themselves on the poorly charted plateau to becoming 'long-haulers.' Another study analysed data from 10 longitudinal study samples of patients with COVID-19 and 1.1 million individuals from electronic health-care records, finding that 7.8% to 17% of participants had long COVID symptoms at 12 weeks[146]. In the UK Office for National Statistics data, 24% of the current national caseload of 2.1 million individuals who had SARS-CoV-2 infection reported persistent symptoms for over 2 years. That is, over half a million people in the UK alone have had long COVID for more than 2 years.

Although there are anecdotal reports of individuals with gradual improvement and remission beyond 2 years, few studies have yet explored full recovery or the factors that favour this. The UK Office for National Statistics data is informative to the extent that, although new long COVID cases continued to stack up in large numbers during the Omicron wave of 2022, the cumulative total reporting long COVID plateaued or fell only slightly, to around 2–2.3 million, equivalent to around 3% of the population. There is remarkable convergence in long COVID prevalence and symptom profiles reported globally and cross-culturally[147].

specific clusters[20]. Proponents of the approach would argue that this is a prerequisite for the tailored management of such a heterogeneous disease entity; the opposing view would be that such clusters impose an artificial structure on a highly fluid process whereby an individual may be prioritized for referral for neurocognitive symptoms yet may, at other times, suffer considerably from, for example, breathlessness. One study used unsupervised machine learning to stratify a cohort of 6,469 patients with long COVID into clusters, yielding a six-cluster classification[20]. A Delphi process involving 1,535 participants across 71 countries arrived at a core list of 11 consensus outcomes for long COVID[21]. While acknowledging the diversity and relapsing–remitting range of the full gamut of symptoms, many studies now focus on the common list of post-exertional malaise, fatigue or tiredness, myalgia, dyspnoea, chest pain, and cognitive dysfunction or brain fog. In summary, as the field moves towards consensus stratifications that may be valuable for clinical referral pathways, the pressing challenge is to navigate, via specific biomarker testing, the relation of clusters to differential mechanisms and pathologies.

Possible mechanisms of long COVID

From the time that persistent sequelae of COVID-19 were first noted, efforts to build a mechanistic framework have been in progress[22], to some extent building on prior experience of other post-viral sequelae as well as seemingly related conditions such as ME/CFS. Viral infections that seem to offer potentially informative pathways to persistent phenotypes include EBV, with its sometimes persistent bouts of debilitating infectious mononucleosis (also known as glandular fever) and associated immune perturbations[23], measles virus with its post-viral immune amnesia[24], and Chikungunya virus with its significant aftermath of autoimmune-like and chronic arthralgia[25]. Several hypotheses are under investigation in long COVID, many of these potentially interrelated in the disease pathway and not mutually exclusive. A number of these topics of investigation are listed in Table 1. Across the very large COVID-19 data sets, ranging from population-level epidemiological end points to findings from individual lab assays, the field still lacks the evidence to build formal, mechanistic bridges.

Evidence for organ damage

Since the start of the pandemic, SARS-CoV-2 viral infection has been known to cause radiological organ changes, even in the absence of symptomatic disease[26]. Several large studies have considered long-term imaging changes after infection[27–39] (Box 4). Although changes are often seen and often show correlation with the presence of persistent symptoms, radiological damage is possible without persistent symptoms, and vice versa. At present, this makes imaging a poor option for any formal, clinical diagnosis of long COVID. Nevertheless, the literature currently contains a rich data set on multisystem organ damage caused by mild-to-severe SARS-CoV-2 infection and, in many cases, the observations, which include brain volume changes, neuroinflammation, and olfactory bulb and cardiac

changes, are potentially informative with respect to their relationship with long COVID symptoms. However, the data set on actual changes in patients with long COVID compared to those with a speedy recovery after acute infection is much smaller — that is, although COVID-19 clearly causes long-term radiological changes, evidence of a causal association with long COVID in most cases is still awaited. The Coverscan protocol examined multi-organ MRI data in a sample of 201 non-hospitalized individuals with long COVID, finding 70% to have impairment in one or more organs at 4 months after initial COVID-19 symptom onset. The most commonly affected organ was the heart, followed by the lungs[31]. In a recent follow-up of 536 individuals with long COVID at 1 year after acute infection, 59% were found to have MRI evidence of single-organ impairment[37]. However, a study using cardiovascular magnetic resonance (CMR) and 31-phosphorus CMR spectroscopy in patients with long COVID identified no differences from control individuals[38].

A persistent SARS-CoV-2 reservoir as a long COVID driver

Given the enduring presence of debilitating, persistent disease symptoms, a parsimonious explanation for long COVID would be that the virus itself persists. However, persistent reservoirs of infection had not been a common notion in the coronavirus literature until the analysis of COVID-19. Evidence of persistent virus reservoirs may come from biopsy or autopsy tissue analysis by PCR, tissue culture or staining, from stool or other samples by PCR or antigen detection, and by imputation from immune correlates such as nucleocapsid (N) antibody levels. The gastrointestinal tract has been the most studied candidate site to house a possible residual depot of virus. This is not to imply that other sites will not be identified, merely that it is a site that is both accessible through biopsy or through the proxy of faecal samples and has many cell types that are ACE2 positive and therefore permissive for infection. There are many reports of persistently positive quantitative PCR (qPCR) from stool samples several months after acute infection, but this has been difficult to correlate with other measures of persistent infection, or indeed with increased long COVID, though it certainly seems to indicate a subset of patients in convalescence harbouring a form of gastrointestinal virus reservoir for several months[40–45]. Viral antigen can be found in stool samples post SARS-CoV-2 infection regardless of long COVID status[40,43,44]. A study of a cohort with inflammatory bowel disease (IBD) stratified participants for the presence or absence of persistent symptoms after COVID-19 (ref. 45). Importantly, they used qPCR and immunofluorescence of colon biopsies to show that the presence of virus indeed correlated with persistent symptoms. However, virus-positive biopsies and long COVID were not necessarily associated with faecal shedding, suggesting that stool qPCR may not be a useful proxy for gut viral persistence. Key support for the idea of a gut reservoir comes from B cell repertoire analysis and, especially, from detection of virus in gut biopsies[43]. In a study of a cohort of 82 patients in convalescence at 1.3 and 6.2 months post-infection, neutralizing antibody levels fell fivefold over this period, whereas memory B cell frequency was stable and showed increased BCR somatic hypermutation and response potency. This suggested an ongoing antigenic stimulus. Upper and lower gastrointestinal biopsies were obtained from 14 people and virus was detected by qPCR and immunofluorescence in 7. Interestingly, the presence of persistent virus seemed not to have elicited any inflammatory infiltrate and also occurred at a time when these individuals were clear of virus by nasopharyngeal PCR swab. This implied a covert, poorly immunogenic, residual antigen depot. Another study used SIMOA (single molecular arrays; an antibody-bead enrichment approach for high sensitivity detection of low abundance proteins) to detect SARS-CoV-2 antigens in serum from 37 individuals with long COVID and compared these to 26 controls who had recovered fully after acute infection[46]. Even at 1-year post-infection, the SARS-CoV-2 protein spike (S) was detected in 60% of patients with long COVID and not in controls; the greater the number of organ systems involved in symptoms, the greater was the amount of detectable S. At face value, this is another piece of corroborative evidence that long COVID symptoms could be due to some form of uncleared viral reservoir. Moreover, there is evidence that vaccination can ameliorate symptoms in some individuals with prior long COVID, which would argue for clearance of a viral reservoir (Box 5).

A study of post-mortem tissues offers a far broader list of potential sites for persistent viral reservoirs, though with the caveat that most tissues studied tend to be from people who had serious, acute, fatal, COVID-19, and therefore should be extrapolated to general long COVID with caution[47]. However, principles of the tissues capable of harbouring persistent virus and the associated pathological changes seem to be relevant to any discussion of viral repertoires in long COVID.

In summary, there is evidence that, at least in a subset of patients with long COVID, symptoms may be associated with persistent virus. This, in turn, highlights a number of unknowns requiring further investigation. We need clarity on where virus may be sequestered in the body. Importantly, what might be the profile of viral antigen expression that could account for an ability to evade clearance by host adaptive immunity — indeed, most do not find evidence of raised immunity to N — while at the same time being sufficiently pro-inflammatory to provoke the cytokine and chemokine pathways invoked as mediators in long COVID pathogenesis? In any case, viral persistence suggests a potential therapeutic strategy with monoclonal antibodies or other antivirals.

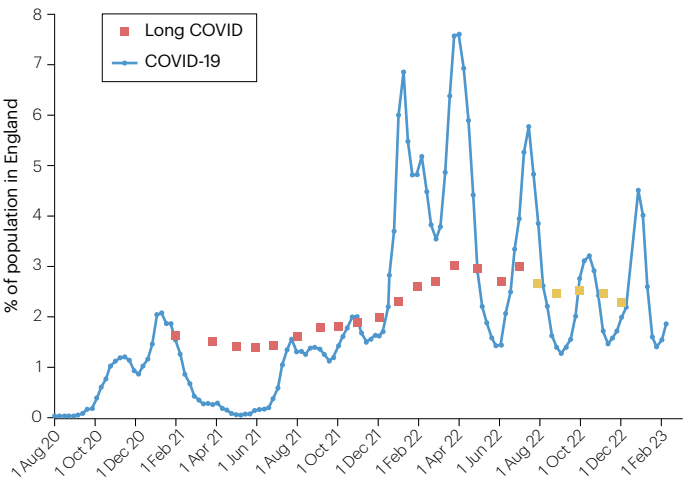

Fig. 1 | COVID-19 and long COVID prevalence in England. Weekly estimates of prevalence of COVID-19 and long COVID in England, according to the Office of National Statistics infection survey. Estimates of long COVID lasting 4 weeks or more are shifted to 4 weeks before data collection date. The Office of National Statistics (ONS) moved from interviews in person to online only data collection in the autumn of 2022. ONS estimated that online only reporting gives estimates 1.3× higher than in person, and so the yellow squares correspond to estimates adjusted downwards to be comparable to previous reporting practice.

Box 3

Lessons from and for ME/CFS and the case of 'long SARS'

It is hard to discuss or present data on long COVID without being challenged about the relationship of findings to mechanisms in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), not least by patient groups who understandably feel that their condition has endured decades of neglect in terms of biomedical research prioritization. In light of clear overlaps with long COVID, there now exists an opportunity for cross-hybridization, with much to be learnt from the long, past experience and investigations in ME/CFS, and from the new momentum of long COVID investigations over the past few years. The symptom overlap is self-evident, encompassing the key features of post-exertional fatigue, neurocognitive symptoms, dysautonomia and postural orthostatic tachycardic syndrome. A systematic review found that 25 of 29 CFS symptoms were reported by at least one long COVID study[18], whereas another study compared genes common between the two conditions in a number of ways, including pathway and network analysis[148]. This study found common hub proteins, such as IL-6 and IL-1β, between the two conditions. Another review focused on their similarities through the link of TGFβ signalling and circadian rhythms[148]. There is resonance in the post-acute viral infection symptomology across the two conditions[19]. ME/CFS has commonly been described as a post-viral condition that may ensue following a range of infections, including pandemic H1N1 influenza[149], Varicella zoster virus[150], enteroviruses and SARS-CoV-1[17]. Overlap in the immunopathological analyses is particularly interesting. It is noteworthy that raised CCL11, which has credentials as a long COVID serum biomarker functionally linked to neurocognitive symptoms, is also a biomarker of ME/CFS[151]. Revisiting the ME/CFS data also raises the possibility of investigating some of the implicated biomarkers, such as CXCL10 and leptin, in more detail in long COVID[152]. Furthermore, the ME/CFS data set may offer a reference framework to consider a role for Epstein–Barr virus reactivation in long COVID, noting that CFS can ensue from infectious mononucleosis associated with an enhanced imprint of T cell activation[153].

Long SARS, caused by SARS-CoV-1 infection, is a forerunner of long COVID and thus more helpfully considered as a variant of long COVID rather than a category of ME/CFS. A study from Canada and another from Hong Kong reported findings very similar to long COVID, with many people reporting chronic fatigue and psychiatric illnesses[17,154]. The comparative experience and lessons were recently reviewed and summarized[155]. Reviewing the Toronto University HCW cohort from 2003 to 2004, and with the caveat that it may be hard to extrapolate to long COVID generally as this was a group with severe disease and who were hospitalized, it was concluded that many of the changes were permanent and irreversible, with most patients unable to return to their former occupations.

Association with an abnormal antiviral immune response during acute infection

A number of studies considered parameters of antibody and T cell immunity to SARS-CoV-2 antigens, especially S and N, to evaluate the hypothesis that the risk of long COVID might be associated with an abnormally high or low immune response to acute infection[48–54]. Enhanced immunity would be compatible either with a high or sustained viral load, or simply with a poorly regulated response, whereas reduced immunity might be indicative of a failure to adequately clear the virus. At this stage in the evolution of this literature, studies encompass first-wave immune analysis following acute infection (and pre-vaccination) to look at predictors of long COVID, through to samples collected during ongoing long COVID in the post-vaccine period. A systems immunology approach to look for responses following acute infection that predicted subsequent persistent symptoms found raised anti-N antibodies preferentially in those progressing to predominantly neurological symptoms[48]. A study looking at the initial anti-N antibody response during acute infection found an inverse correlation between antibody level and likelihood of symptoms at 3 months or beyond, supporting the view that an inadequate initial response may predispose to long COVID[49]. Analysis of a cohort of hospitalized patients of whom a fifth would go on to experience long COVID symptoms at 1-year post-infection found that the long COVID group showed significantly lower antibody levels to S, with no difference in T cell responses[50]. A study looking at SARS-CoV-2-specific antibodies and T cell responses at 1–2 months post-infection and 4 months post-infection showed minimal differences between individuals with persistent symptoms[51]. The only significant difference was a lower percentage of N-specific IFNγ/CD107a$^+$ memory CD8$^+$ T cells in those with persistent symptoms following SARS-CoV-2 infection. A cohort with ongoing pulmonary long COVID showed quite substantially enhanced CD4$^+$ and CD8$^+$ T cell responses to peptide pools from S, N and membrane (M) proteins[52], whereas a similar study showed little difference in peptide response between people with long COVID and those who had recovered[53]. A recent study considered immunity to SARS-CoV-2 among parameters within a multi-faceted machine learning approach to long COVID diagnosis[54] and found increased IgG responses to S. To summarize studies in rather different settings and assessed at different times after acute infection: findings are divergent and include evidence for immunity to S and N which may be equal, reduced or enhanced relative to those without persistent symptoms. However, there is perhaps an emerging theme of somewhat enhanced adaptive immunity to SARS-COV-2 in some with long COVID. This may argue either for ongoing viral stimulation and/or for a less regulated initial response to acute infection. The somewhat diverse findings are likely another indicator of the poorly charted heterogeneity within long COVID cohorts.

A potential role of latent virus reactivation

Since the beginning of efforts to narrate and decode the symptomology of long COVID, it has been apparent that there are similarities to some features of severe infectious mononucleosis. Although most primary infections by EBV are largely asymptomatic, infectious mononucleosis represents primary infections that become symptomatic in terms of mild-to-severe sore throat, cervical lymphadenopathy, fever and fatigue lasting for weeks to months[23]. The response to acute infection during infectious mononucleosis involves profound innate and adaptive

immune activation, most notably, a dramatic $CD8^+$ T cell response with large peripheral expansions of the global $CD8^+$ compartment, reflected in high cell frequencies picked up by tetramers, sometimes with half the entire repertoire targeted to EBV lytic epitopes. There is an assumption that the exaggerated $CD8^+$ T cell response is implicated in pathogenesis more than any direct effect of viral load itself. An important early clue as to a potential role in long COVID came from a study using a battery of omics approaches to track 309 patients with acute COVID-19 looking for predictive correlates of persistent symptoms at 8–12 weeks. One such predictive biomarker came from qPCR to measure EBV viral load, suggestive of EBV reactivation[48]. Potentially corroborative data has since come from other studies. Evidence for EBV reactivation as determined by early antigen diffuse and viral capsid antigen seroreactivity, comparing patients after COVID-19, with or without long COVID, found a highly significant increase in the long COVID group[55]. Another study showed very similar findings, with evidence of EBV reactivation in terms of early antigen diffuse seroreactivity strongly associated with fatigue[56]. Interestingly, reactivation of cytomegalovirus, a latent herpesvirus with similar effects on the immune system, was, if anything, negatively associated with long COVID. An analysis of patients with long COVID who had EBV and human herpesvirus 6 reactivation found that this group was significantly more likely to present with fever, headache, myalgia, neurological disorders and pulmonary disorders[57]. Multi-dimensional immune profiling of patients with long COVID found a positive correlation between EBV p23 seroreactivity and T effector memory and $IL\text{-}4^+/IL\text{-}6^+$ $CD4^+$ T cell populations as well as correlations between reactivity against EBV gp42 and $IL\text{-}4^+/IL\text{-}6^+$ double-positive $CD4^+$ T cell populations among those with long COVID[54].

While noting that any relationship between long COVID and EBV reactivation remains correlative, without evidence of causality, the profound effect of active EBV infection on perturbation of immune subsets offers one mechanism that could potentially contribute to long COVID immunopathogenesis. This could encompass effects at the level of expanded $CD8^+$ T cells targeting EBV antigens and/or effects through activation of B cell plasmablasts[58]. Epidemiological and immunological studies have long linked EBV status to an enhanced risk of various autoimmune diseases such as the relationship between EBV serology and lupus AABs[59] and, most notably, recent evidence for an association with the risk of multiple sclerosis[60]. There are hopes that the convergence of evidence across diseases for a causal, immunopathogenic role of EBV infection will supply the required momentum for investment in prophylactic and therapeutic measures against EBV[61].

Perturbations in systemic immunity

Arguably one of the most contentious areas in the discussion of long COVID aetiology has been the extent to which pathogenesis may be associated with long-term disruption of adaptive immunity or, at least, perturbed immune subsets (or consequences of this in skewed serum cytokine or chemokine profiles). As with other aspects of long COVID research, extrapolation from the large, initial data set of findings in severe, acute infection to the context of persistent symptoms can offer both clues and confounders. In the longer term, profound immune perturbations might be unexpected outside the realms of lymphotropic infections such as HIV, EBV or measles. On the other hand, the COVID-19 pandemic has been notable for revealing a whole series of phenotypes that had not been anticipated for coronavirus infection. It had been assumed that a coronavirus would not trigger any of the persistent phenotypes more commonly associated with a lifelong, latent resident such as EBV. Such certainties are now much weakened given our lack of clarity on the possibility of persistent SARS-CoV-2 reservoirs and, subsequently, the possibility that long-term perturbations may be the secondhand, downstream consequence of acute SARS-CoV-2 infection through reactivation of latent EBV[55]. Lastly, any textbook expectations of what is 'normal' following viral infections must be set against the point that we have never before had a global pandemic subjected to this level of investigation, especially when the approach of choice is catch-all systems immunology. Relatively few studies have been able to compare samples from individuals with SARS-CoV-2 with matched samples for other viral infections; therefore, we are often left uncertain of whether the changes seen are unique to this infection. As with other aspects of long COVID immunopathogenesis, much has been learnt, but we are still some way off any mechanistic consensus that might feed into therapeutic strategies. In considering analyses of global immune repertoire changes, such as global increases in PD1 expression by T cells or depletion of naive subsets, the intuitive tendency has been to reach to conclusions about the viral infection having depleted immune populations. However, when one considers the relatively low frequency of cells within the $CD4^+$ or $CD8^+$ T cell compartment that are specifically involved in responses to the SARS-CoV-2 proteome[62,63], it is hard to envisage a mechanism for gross subset perturbations except through the intermediary of some form of larger bystander activation or cytokine activation.

Several teams have reported substantive efforts to apply omics screening approaches to the prediction of those who will progress to persistent symptoms following acute infection[42,48]. For example, mass

Table 1 | Hypothetical mechanisms underlying long COVID pathogenesis

Proposed consequence of COVID-19	Potential contribution to long COVID	Refs.
Organ damage in targeted tissue	Loss of function, such as in olfactory lobes, or effect of lung tissue changes on gas exchange	16–22, 26–39
Persistent virus or antigen reservoirs	Ongoing immune or inflammatory activation	39–47
Reactivation of Epstein–Barr virus or other latent viruses	Downstream effects of Epstein–Barr virus reactivation such as perturbed immune subsets or impact on auto-antibody release	48,54–57
Changes in inflammatory activation, systemic immunity, immune subsets and their transcriptional profiles	Downstream impacts ranging from endothelial activation to altered allergic profiles	48,54, 65–68,70
Vascular endothelium activation or dysfunction	Impacts on platelet activation, clotting, microclots and gas exchange	88–91, 103–115
Mast cell activation	Downstream immune changes and allergic programme	73
Autoimmunity	Causal role of autoimmune auto-antibodies or T cells to specific phenotypes, including endothelial activation, postural orthostatic tachycardic syndrome, myocarditis and neuroinflammation	48,54,76, 79–84, 89–96
Microbiota dysbiosis	Altered metabolomic interactions with body systems including the immune system	122–125

Box 4

Evidence for end-organ damage from imaging studies

Lung

A central conundrum in the decoding of long COVID has been the disconnect between breathlessness as one of the defining symptoms and a tendency for those with long COVID to show profound desaturation with even mild exertion in the face of often unremarkable lung CT and MRI findings. Insights have been gained through the use of ^{129}Xe MRI and CT pulmonary vascular measurements to investigate the relationship of persistent symptoms and exercise limitation in long COVID[39]. ^{129}Xe MRI measurements were lower in the long COVID group than in controls, associated with CT pulmonary vascular density, lung carbon monoxide diffusing capacity, exercise capacity and dyspnoea. These studies are evidence of a failure of gas transfer in the vascular compartment as a pathogenic mechanism in long COVID. There is also increasing acceptance of more routine imaging modalities for the investigation of respiratory symptoms of long COVID. Dual-energy CT demonstrates perfusion defects in the lungs of approximately 60% of those with respiratory symptoms of long COVID at 3 months after infection and demonstrates persistent macrothrombosis (visible clots evidenced by filling defects in the pulmonary arteries) in 5% of patients[30].

Heart

If lung imaging studies remain some way from offering a pathological correlate for exercise intolerance and breathlessness, one might make a similar case for the less-than-clear relationship between symptoms and cardiac imaging. Chest pain is a hallmark long COVID symptom in most of the symptom cluster studies. From large health-care data sets, there is an enhanced risk of cardiovascular disease in the 12-month period after COVID-19 infection[130]. Even individuals with SARS-CoV-2 infection who were not hospitalized show an increased 1-year risk of stroke, transient ischaemic attack, myocarditis, angina, pulmonary embolism and heart failure. However, identifying the radiological correlates of this enhanced risk has been controversial, with divergent findings[29,32,156]. Cardiac imaging studies across individuals with asymptomatic, mild and severe COVID-19 report abnormal MRI findings spanning a wide range, from 0% to 78%. The nature of this divergence remains unclear, but may relate to differences in the use of baseline, pre-COVID-19 data[29,32]. Late gadolinium enhancement indicative of myocarditis was similarly observed in 0–45% of those imaged[156]. Further studies are warranted to relate specific long COVID cardiac symptoms, such as chest pain, to radiological findings.

Brain

In terms of MRI brain imaging, the UK Biobank cohort study has the methodological advantage of matched images for each participant, spanning a 3-year interval before and after infection[35]. The findings were not specifically linked to the reporting of long COVID symptoms in this population. Significant changes following infection were reported for brain and cerebrospinal fluid volume, lateral ventricle volume, and the temporal cortex functional network. Notably, there was also cognitive decline, which correlated with changes in the volume of cognitive lobule crus II of the cerebellum[35]. Such longitudinal studies highlight a confounder intrinsic to our long COVID data sets, in that we generally lack individual comparator data sets prior to infection. Long-term changes to smell and taste in long COVID have been considered by a large number of imaging studies[36]. Again, findings are rather variable, but a number show significant reduction in olfactory bulb volume, which is in line with changes shown following acute disease by histopathology and transcriptomics, potentially indicating ongoing T cell immunopathology in the absence of detectable virus[157].

spectrophotometric analysis of a plasma proteome panel was used to compare the response to acute infection in health-care workers with mild infection and stratified those with or without persistent symptoms at 1 year[64]. This identified a long COVID predictive biosignature comprising 20 analytes, including APP, often considered an anticoagulant, the Hsp90 chaperonin and the iron–sulfur cluster biogenesis protein HSCB, which may be released into plasma as a consequence of mitochondrial disruption.

A multi-omics approach was used to look for predictors as to who of 309 patients with acute COVID-19 would progress to persistent symptoms[48]. The comprehensive battery of assays encompassed anti-SARS-CoV-2 immune analysis, viral load, cytomegalovirus and EBV load, serum proteomics and metabolomics, single-cell RNA sequencing, AAB screening, and TCR and BCR analysis. This approach related specific response patterns during acute infection to patterns of subsequent persistent symptoms and found that differential immune phenotypes correlated with diverse downstream long COVID phenotypes. Individuals who went on to report persistent respiratory symptoms showed low serum cortisol, whereas those going on to develop neurological symptoms had an elevation of proteins involved in circadian regulation of the sleep cycle. Evidence of EBV reactivation detectable at the time of acute infection (though not at later time points) predicted subsequent fatigue. Individuals who would progress to persistent gastrointestinal symptoms showed development of expanded T cell populations, including specific SARS-CoV-2 clonotypes. The ADAPT study recruited 147 participants with SARS-CoV-2 infection who were followed for 8 months, focusing in particular on a comparison between 31 who developed persistent fatigue, dyspnoea or chest pain, compared to 31 matched controls in the cohort who made a complete recovery[65]. Again, through use of a wide range of approaches, several differences in immune parameters were found. This study also benefited from having a comparison with groups of individuals who had had other human coronaviruses. Looking at serum biomarkers, IFNβ and IFNγ remained elevated at 8 months post-infection in the long COVID group. The authors proposed a long COVID predictive model based on the analytes IFNβ, IFNγ, PTX3 and IL-6. By contrast, a study looking for a serum cytokine diagnostic biosignature at 2 years post-infection proposed a triad of raised IL-1β, IL-6 and TNF[66]. The ADAPT study additionally used cluster analysis to look at results from peripheral blood mononuclear cell (PBMC) analysis at 3 and 8 months using a 19-parameter

flow panel[65]. They defined 24 subsets within PBMCs, with 3 clusters absent at 8 months in individuals with long COVID, highlighting effects on $CD127^{low}CD8^+$ cells, $CD4^+$ cells and B cells. However, $CD38^+HLA\text{-}DR^+$ activated myeloid cells were elevated in patients with long COVID. A subset of $CD8^+$ T cells had elevated expression of the exhaustion markers PD1 and TIM3. A profile compatible with T cell exhaustion was confirmed by another study using multi-parameter flow to look at a long COVID cohort[67]. This observation, among others, has led to the hypothesis that T cell exhaustion due to SARS-CoV-2 infection may be causal in long COVID. This is clearly of interest and will require further investigation. In terms of whether subset alterations in PD1 expression equate to differences in functional responses, it is worth noting that the PD1 ligand PDL1 is downregulated on antigen-presenting cells at 6 months after infection[68]. Furthermore, PD1 upregulation cannot be considered synonymous with loss of T cell function in the absence of demonstrable changes in specific response phenotypes[69]. A litmus test of whether even mild COVID-19 can mediate long-term reprogramming of adaptive immunity, as suggested by these long COVID studies, would be whether subsequent responses to other vaccine immunogens are normal. This has been investigated using a systems biology approach, with surprising results[70]. The study identified long-term, altered transcriptomic changes in PBMCs, both from SARS-CoV-2 and from influenza H1N1 infection. The authors then found that men but not women who had recovered from COVID-19 6 months previously showed an enhanced response to flu vaccination, especially in terms of $CD8^+$ T cell-derived IFNγ and a heightened B cell plasmablast and antibody response.

In another study, multi-dimensional immune phenotyping focused on 99 non-hospitalized individuals with long COVID and a comparator cohort of 40 healthy convalescent individuals[54]. Several potentially informative differences were found. Machine learning analysis of the data highlighted reduced serum cortisol — around half of levels in healthy controls — as the significant defining serum biomarker. There was also an increase in IL-8 and CCL4. Flow cytometry of PBMCs, like the other studies, also showed a number of differences. As in other studies, there was evidence of an activated B cell subset ($CD86^{hi}HLA\text{-}DR^{hi}$) but increased PD1 and TIM3 expression on T cells as well as a decreased subset of central memory $CD4^+$ T cells. They found no specific correlation between long COVID symptoms and AAB profiles.

In a condition as complex and heterogeneous as long COVID, it is not surprising that somewhat differently targeted studies produce rather divergent answers. Moreover, these studies span different time

Box 5

A persistent virus reservoir — evidence from responses to COVID-19 vaccination

By nature of the COVID-19 timeline, the global caseload of individuals with very-long-term long COVID is skewed to those who had Wuhan Hu-1 and Alpha wave SARS-CoV-2 infection during the pre-vaccine era. Given the considerable efficacy associated with the vaccine programmes, it might have been anticipated that there would be few additional long COVID cases in the post-vaccine era. This has not been the case. The UK Office of National Statistics data shows that, of the approximately 2 million individuals with long COVID, over half had COVID-19 during the post-vaccination, Delta and Omicron waves. Although some of these individuals would belong to the ~20% of the population that was unvaccinated, the evidence is clear that long COVID can result from breakthrough infections in vaccinated individuals. The breadth and severity of persistent symptoms of long COVID in those who were vaccinated and had a breakthrough infection look no more mild or limited than in the unvaccinated[158]. Questions around long COVID and vaccination are thus: (1) to what extent does two-dose vaccination reduce long COVID risk in the event of breakthrough infection, (2) in those already with long COVID, to what extent does vaccination (and boosting) ameliorate extant symptoms, and (3) can vaccination ever be associated with symptom exacerbation or, even, with causing de novo long COVID? While vaccination with two or more vaccine doses clearly reduces the risk of infection per se, data across several, large, health record studies and app surveys indicate only a moderate reduction in risk of long COVID symptoms in the event that breakthrough infection does occur[159–161]. Office of National Statistics data found that long COVID symptoms of any severity were reported by 9.5% of double-vaccinated study participants compared with 14.6% of socio-demographically matched unvaccinated participants, that is, a 41% decrease in the chance of long COVID at 12 weeks. Studies have also considered the possibility that vaccination ameliorates symptoms in those with prior long COVID[161–163]. One study found an overall 13% reduction in long COVID symptoms over the 9 weeks following vaccination, with improvements most common in fatigue, headache and poor sleep[162]. Improvement was greatest in those with long COVID of shorter duration, that is, more recent acute infection. A similar overall reduction was reported in a smaller French study, which also found that, among those with residual long COVID symptoms, the impact of symptoms on the lives of patients was less after vaccination[164]. A feature common to the studies is the relatively short follow-up to track remission and, thus, the possibility that the effect is transient[161]. The Occam razor hypothesis for the finding that a small but significant subset of people with long COVID show improvement after vaccination is that this marks out a disease endotype suffering symptoms due to specific inability to clear a chronic viral reservoir. With the boosted adaptive immunity of additional vaccine doses, virus clearance may be enhanced. The proportion of individuals in these studies who, on the other hand, report symptom exacerbation is small though notable in that such responses can deter other people with long COVID from coming forward for vaccination. In an observational questionnaire of 900 respondents, 58% reported symptom improvement after vaccination, 18% reported exacerbation and 24% reported no change[165]. A sizeable study found no significant effect, positive or negative, of vaccination on long COVID symptoms[166].

points since acute infection, different symptomatology, and varying long COVID definitions and measure different immune parameters, from specific responses to SARS-CoV-2 or other viruses to systemic subset analysis. The field needs bigger, deeper and more comprehensive analyses.

It is noteworthy that each of two major multi-omic cohort studies[48,54] identified low serum cortisol as a long COVID biomarker. In drug-induced pituitary suppression or in Addison disease, low cortisol would commonly be associated with fatigue, dizziness, muscle weakness and mood changes, thus offering a contextual framework for considering the potential contribution of cortisol levels to pathogenesis, though the association remains one of correlation rather than causation.

Another serum biomarker that may be of particular interest because of its known links to pathology is the chemokine CCL11 (also known as eotaxin). An elegant study looked at CNS neuropathological consequences of mild respiratory SARS-CoV-2 infection of ACE2-transgenic mice[71]. Cerebrospinal fluid (CSF) analysis showed persistently elevated CCL11 at 7 weeks. Similar observations were made following influenza infection. When CCL11 was administered to mice, it induced microglial/macrophage reactivity in hippocampal white matter. The findings were, in some respects, reminiscent of the hippocampal changes seen in the longitudinal study of MRI CNS changes in the UK Biobank study of mild COVID-19 infection[35]. In a human long COVID cohort, the study then found elevated CCL11 in the plasma of individuals with brain fog, particularly in male patients. Interestingly, CCL11 has long been recognized as a mediator involved in neurocognitive decline in ageing[72].

There are anecdotal reports in long COVID support groups stating that many people with long COVID observed changes to their allergic profiles and benefit from taking over-the-counter antihistamines — what clues can be drawn from these observations? Systems immunology studies so far offer no decisive clues of switching to any immune programme that is more skewed to a T helper 2 transcriptome beyond the important observations of a novel IL-4$^+$/IL-6$^+$ expanded population as described above[54], and the possibility that CCL11 may be induced and regulated via a pathway of T helper 2 activation. Some investigators have highlighted features of the long COVID immune response that are reminiscent of mast cell activation, including elevated serum levels of active tryptase and carboxypeptidase 3 (CPA3)[73].

Induction of AABs

Early studies of immune mechanisms in acute COVID-19 showed that infection is more severe in those with a prior repertoire of anti-type I interferon AABs[74] and that SARS-CoV-2 also seems capable of de novo activation of a relatively large and diverse repertoire of additional AABs[75–84] (Table 2). A central lesson from many studies of autoimmunity following SARS-CoV-2 infection has been that a large repertoire of AABs is induced. This is, to an extent, correlated to disease severity, which has also been observed (albeit to a lesser extent) in influenza infection. It has been suggested that COVID-19 may be similar to lupus insofar as it favours an increase in DN2 B cells — negative for IgD, CD27, CXCR5 and CD21 — and that this facilitates an extrafollicular B cell activation pathway with less stringent tolerance checkpoints[76].

Patients with severe, acute COVID-19 show extrafollicular B cell activation concomitant with the collapse of Bcl-6$^+$ germinal centres[85] and potential failure of negative selection[86], which may predispose to autoimmunity. Other aetiologies of enhanced autoimmunity that will require investigation include the possibility of an EBV–autoimmunity link as postulated in multiple sclerosis and lupus, potentially through altered activation of EBV-infected B cells. The backdrop to any consideration of whether and by what mechanism specific AABs may have a causal role in long COVID is the relatively high prevalence of diverse AABs during acute infection, without specific reference to persistent sequelae. Human proteome arrays expressing 21,000 targets were used to screen for IgM AABs, comparing patients with acute COVID-19 and hospitalized (treated either in an intensive care unit (ICU) or normal ward), compared to samples from patients treated in ICUs who did not have COVID-19 (ref. 77). More than 260 candidate auto-antigens were identified, though this tended to be a feature of critical illness in general, not limited to COVID-19. There was a tendency for these to be expressed in pulmonary, vascular, renal and gastrointestinal tissue. No longitudinal follow-up was possible in this study and therefore no conclusions could be drawn about the risk of long COVID. Another study looked for IgG AABs in hospitalized patients with acute COVID-19, who were screened using bespoke auto-antigen arrays designed to detect responses relevant to connective tissue disease or anti-cytokine responses[75]. The study found AABs in half of patients with COVID-19 but rarely in healthy controls. This screen had been prompted by a preliminary analysis for antinuclear antibodies and detected these in a quarter of the patients with COVID-19. Auto-antigens commonly recognized in the arrays included ribosomal P proteins, P0, P1 and P2, RPP14, Ro/La, U1-snRNP and thyroid antigens. A number of studies on autoimmunity during acute infection have focused on patients with neurological symptoms. One analysis focused on seven patients with acute COVID-19 who had neurological symptoms spanning encephalopathy, seizures and intractable headaches, and from whom paired samples of peripheral blood and CSF had been obtained[79]. When monoclonal antibodies were derived from CSF B cells, the majority showed a degree of neuronal (neuropil) staining within CNS sections. Conducting human proteome array screening with the T7 bacteriophage-expressed PhIP-seq platform, the authors were able to identify THAP domain-containing 3 (THAP3) and intraflagellar transport protein 88 homologue (IFT88) as common auto-antigens shared between two of the patients. AABs were analysed in CSF of 11 patients in the ICU critically ill with COVID-19 showing neurological symptoms, including myoclonus, delirium and seizures. By immunofluorescence on murine CNS sections, all the samples showed the presence of anti-neuronal AABs targeted to neuronal surface proteins, endothelium, astrocytic proteins and neuropil of the olfactory bulb, basal ganglia or hippocampus[80]. Another study used a human proteome array to map AABs in 122 patients hospitalized with COVID-19 with brain injury evidenced by serum markers, neurofilament light (NFL) and glial fibrillary acidic protein (GFAP). The proteome arrays were selected for expression of CNS and blood–brain barrier antigens as well as lung, heart and coagulation antigens[81]. Patients with severe disease were positive for AABs to several targets, including relatively common responses to surfactant protein A (SFTPA1) and myelin-associated glycoprotein (MAG) — one of the myelin target antigens that has been implicated in multiple sclerosis immunopathogenesis. However, the study did not show substantial differences between patients with COVID-19 and a matched influenza cohort. Another study compared tissue-staining patterns by AABs in serum from individuals sampled during ICU admission for severe COVID-19, an ICU cohort sampled 3–6 months later, and a group of health-care workers who had recovered sampled 1–3 months after mild-to-moderate COVID-19 (ref. 82). More than half of the individuals in each of these groups showed the presence of tissue AABs, with the widest spectrum seen in the most severe cohort. Tissue-staining patterns highlighted persistent autoimmune responses to epidermal intercellular cement as well as skeletal, cardiac and smooth muscle. Among the

findings regarding AAB induction during acute disease are functional effects that may be of interest for investigation in the specific context of long COVID. For example, anti-ACE2 IgM is found in 27% of individuals with severe COVID-19, which can initiate complement binding and alter the permeability of vascular microvessel endothelium[83].

What about evidence from studies looking specifically for SARS-CoV-2-associated AAB profiles that might be functionally related to subsequent persistent symptoms? Although much of the literature in this area, as described above, is concerned with predisposition to and consequences of severe COVID-19 infection, some reports are

Table 2 | AABs characterized following acute COVID-19 or in long COVID cohorts

AAB target	Method	Participants, *n*	Infection status at assessment	AAB-related disease or functional association or imputation	COVID status	Ref.
Type I interferon pathway IFNω, IFNα (13 types)	ELISA, Luminex, multiplex particle-based assay for AAB	Several cohorts	Acute infection	Impaired viral clearance and increased disease severity	Critical COVID-19	74
ACE2	IgM ELISA against recombinant ACE2	66	Acute infection	Complement activation and vascular endothelial activation	Acute COVID-19	83
CNS neuronal antigens	Cell-based assays and tissue section staining	11	Day 12 of acute infection	Neuronal intracellular and cell surface antigens, including Yo, NMDA receptor	Acute COVID-19 with neurological symptoms	80
CNS neuronal antigens, including THAP3 and IFT88	CSF-derived monoclonal antibodies tested by mouse CNS tissue staining and on PhIP-seq arrays	5	Day 12 after hospital admission	Potential relationship to neurological symptoms	Acute COVID-19 with neurological symptoms	79
Double-stranded DNA	ELISA (of CSF)	40	0–13 months after infection	Association with neurological symptoms	Acute COVID-19	34
MAG, SFTPA1	Custom proteome array	250	Up to 4 months after infection	Potential relationship to COVD-19-induced brain injury	Acute COVID-19	81
Skin, skeletal muscle and cardiac antigens	Tissue section staining	56	Up to 4 months after infection	Novel pattern of tissue-specific AABs	ICU or convalescent	82
GPCR signalling pathway: β_2 adrenoreceptor, α_1 adrenoreceptor, angiotensin II receptor, noiceptin-like opioid receptor	Rapid extracellular antigen profiling	215	12–15 months after infection	Implicated in immunopathogenesis of cardiological and neurological autoimmunity, including dysautonomia and POTS	Long COVID	54
	Bioassay	31	Not known			91
	ELISA	246	Not known		Critical COVID-19	135
Nuclear antigens	Microarray AAB profiling	106	3, 6 and 12 months after infection	Biomarker for rheumatological symptoms, lupus and vasculitis	Long COVID	84
	Multiplexed, bead-based platform	147	Not known		Critical COVID-19	75
	Indirect immunofluorescence	175	12 months after infection		Critical COVID-19	75
	Indirect immunofluorescence	175	0–12 months after infection		Acute COVID-19	75
CCP	ELISA	119	4–8 months after infection	Rheumatoid arthritis	Long COVID	136
TG				Coeliac disease		
DSG2	Electrochemiluminescent-based immunoassay	300	6–9 months after infection	Cardiac pathology	Long COVID	137
Sex-specific antigens	Multiplexed bead-based assay using Luminex FlexMAP 3D technology	177	6 months after infection	Range of long COVID symptoms	Long COVID	138
Prothrombotic anti-phospholipids and phospholipid- binding proteins such as c ardiolipin, β2-glycoprotein I, p rothrombin, phosphatidylserine	ELISA	172	NA	Antiphospholipid syndrome	Critical COVID-19	89
	ELISA	221	120 days after infection	Dysregulation of vascular tension and worsening acute respiratory distress syndrome	Critical COVID-19	139
	ELISA	376	NA	Dysregulation of vascular tension and worsening acute respiratory distress syndrome	Critical COVID-19	139

Although these studies show diverse and common examples of auto-antibody (AAB) induction following COVID-19, few examples have yet been causally related to specific long COVID phenotypes or indeed been proven as pathogenic. CCP, cyclic citrullinated peptide; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; GPCR, G protein-coupled receptor; ICU, intensive care unit; IFT88, intraflagellar transport protein 88; MAG, myelin-associated glycoprotein; NA, not available; POTS, postural orthostatic tachycardic syndrome; SFTPA1, surfactant protein A; TG, thyroglobulin; THAP3, THAP domain-containing protein 3.

specifically pertinent to the risk of long COVID. An analysis of antinuclear AABs using a panel of 102 clinically mapped auto-antigens showed an association of antinuclear antibodies with persistent symptoms at 12 months after acute infection[84]. Lupus-like antinuclear AABs were found in half of patients severely ill with COVID-19 included in the study[87].

Coagulopathies have been a central concern both in acute COVID-19 and then in consideration of long COVID aetiology[88]. Some have looked for presence of prothrombotic AABs in serum of patients hospitalized with COVID-19 (ref. 89). This study focused on a specific panel of phospholipid-binding antibodies used in the diagnosis of antiphospholipid syndrome. Looking at a cohort of 172 individuals hospitalized with COVID-19, the study identified antiphospholipid AABs in more than half of the patients, the most common specificities being anti-phosphotidylserine prothrombin and anticardiolipin. The presence of high titres correlated with neutrophil activation, neutrophil extracellular trap release and high platelet counts. In a passive transfer model, administration of purified IgG from these patients to mice led to accelerated venous thrombosis. In a related set of studies, it was shown that antibodies in patient serum mediate endothelial cell activation, assessed by increased expression of E-selectin, VCAM1 and ICAM1 (ref. 90).

Since some specific clinical features of long COVID can be shared with classic autoimmune presentations, including vasculitis, systemic lupus erythematosus, rheumatoid arthritis, scleroderma, antiphospholipid syndrome and myositis, it is plausible that there may be aspects of common aetiology in terms of autoimmune repertoires. If the literature from studies of severe, acute SARS-CoV-2 infection makes the clear point that the virus can induce this wide spectrum of AABs, how robust is the case that, even in mild or asymptomatic infections, such autoimmunity may be a driver of long COVID symptoms? Two major studies[48,54] looking at comprehensive biomarker discovery in long COVID cohorts again found complex patterns of AABs. One found an AAB response signature inversely correlated with anti-S antibodies but some specific AABs correlate with long COVID endotypes such as between IFNα2-targeted AABs and pulmonary disease[48]. A study of AABs targeting G protein-coupled receptors (GPCRs)[91], as implicated in the aetiology of postural orthostatic tachycardic syndrome (POTS) and dysautonomia[92,93], looked at 31 individuals with long COVID. All carried AABs against between two and seven different GPCRs that could function as receptor agonists[91], although healthy control comparator data was drawn from the literature. Some correlates of long COVID symptoms were posited such as patterns of GPCR-targeted AABs found in those patients with new alopecia.

The data presented above shows that a broad array of AABs can be induced following COVID-19, though a clear correlation with long COVID was only found in few studies. This is one of the many areas of COVID-19 human immunology where observations of the response to SARS-CoV-2 far outstrip studies on virtually any other viral infection, such that we lack comparators to determine whether these findings are unique and functionally meaningful in relation to the specific pathogenesis of this disease process. Certainly, electronic health-care records are showing a significant increase in reporting of a spectrum of new-onset autoimmune diagnoses in individuals who had had SARS-CoV-2 infection compared to individuals without infection[94,95]. Across records for nearly 6 million individuals, adjusted HRs after COVID-19 were 2.98 for rheumatoid arthritis, 2.99 for lupus, 1.96 for vasculitis and 1.78 for inflammatory bowel disease[94,95]. A preprint on health-care data in Germany from 642,000 patients with COVID-19 found a 43% increased likelihood of new-onset autoimmunity[96].

Immune effects of AABs on the autonomic nervous system. In considering the gamut of AABs that have been identified following COVID-19 and in long COVID, it is especially pertinent to consider the mechanistic underpinnings of the interplay between the immune system and the autonomic nervous system in dysautonomia and, especially, the proposed pathophysiology of POTS[92,93]. Moderate-to-severe autonomic dysfunction is reported in two-thirds of patients with long COVID[97], with POTS being a frequent phenotype[92,93], and autonomic system dysfunction may be regarded as a common factor potentially underlying several long COVID symptoms. POTS is defined as experiencing orthostatic symptoms (that is, caused by upright posture), a sustained increase in heart rate of >30 bpm on standing and a reduction in blood pressure[98] due to venous pooling and the associated haemodynamic response[99]. Like long COVID in general, POTS is more common in women, likely related to hormonal effects on autoimmunity and/or on the vasculature[92]. A number of AABs have been identified as associated with and potentially causal in POTS. These include GPCR-specific AABs and AABs to the angiotensin II receptor and structural cardiac proteins[92]. There is a need for further studies to relate specific AAB profiles in long COVID to POTS symptoms.

CNS immune and pathological changes in relation to neurocognitive symptoms

One of the central dilemmas in decoding long COVID pathogenesis is the common occurrence of a wide range of highly disabling neurocognitive symptoms, notably, those contributing to brain fog, yet there is little evidence for viral infection within the CNS itself, except in the most severe acute infections. Large-scale cohort and health record-based studies indicate the large extent of the neurological sequelae following COVID-19. Analysis of US Department of Veteran Affairs follow-up data for 154,000 patients with COVID-19 (ref. 100) estimated a 42% increased risk of neurological sequelae at 1 year after infection. This spanned both mild and hospitalized acute cases, though higher in the latter group, and encompassed sensory, cognitive and memory disorders. The UK Biobank study contained a population cohort of over 400 individuals for whom two MRI scans, separated by about 3 years and spanning the episode of infection, were available[35]. Of these, 15 had been hospitalized and the others were mild cases. The data were also compared to paired imaging across the same period from 384 participants who had not had COVID-19. The data showed greater cognitive decline and several longitudinal imaging differences in the post-infection cohort, including reduced grey matter thickness and contrast in the para-hippocampal gyrus and orbitofrontal cortex as well as a greater reduction in brain size. In the absence of evidence for direct CNS viral infection, downstream effects of neuroinflammation must be a key working hypothesis. There is some evidence for this from animal models. Modelling of even mild respiratory infection in ACE2-transgenic mice resulted in profound neuroinflammatory changes that appear pertinent to neurological symptoms in human long COVID. From 1 week, several cytokines were elevated in CSF, notably CCL11, which is implicated in cognitive impairment in ageing[72,101,102]. The mouse infection model also revealed a decrease in oligodendrocyte precursors; this, in turn, was associated with decreased myelin density in subcortical white matter. Staining of subcortical white matter sections showed increased microglial/macrophage reactivity; single-cell transcriptomics characterized these cells as positive for the pro-inflammatory cytokines TNF, IL-1α and IL-1β. There were similar findings in the subcortical white matter of human CNS autopsies of individuals who had died following COVID-19, though not for reasons that appeared explicitly related to

severe COVID-19 disease. In their analysis of the human long COVID cohort, plasma CCL11 was raised in those with brain fog compared to those without[71], making CCL11 an important therapeutic target for the treatment of neurocognitive long COVID symptoms.

Endothelial activation and microclots

From the earliest efforts to characterize the specific pathogenic features of SARS-CoV-2 infection, it was evident that tropism for infection of ACE2-positive endothelial cells made this, to a large extent, an acute infection involving hypercoagulability, endotheliopathy and potentially widespread microthrombi[103–105]. Endothelial cell damage followed by activation are considered key steps in the generation of thrombi. An early post-mortem acute infection series showed microthrombi of the small myocardial vasculature in the majority of cases[103]. Similarly, a majority of brains analysed from COVID-19 autopsies showed widespread microinfarcts and microthrombi in the neocortex[106]. From studies of acute cases during severe SARS-CoV-2 infection, it is thus apparent that COVID-19-associated coagulopathy[106] is a key aspect of pathogenesis; this is explained by the direct effects of viral infection, especially of endothelial cells. In the context of long COVID, the question has been the extent to which sustained endotheliopathy and hypercoagulability remain abnormal during the months or years following acute infection and might thus correlate with and cause persistent symptoms (Fig. 2). Clearly, perturbations of the vascular endothelium and occlusion of vessels by microthrombi have the potential to impair oxygen delivery to tissues, thus accounting for many aspects of tissue-specific dysfunction in long COVID. A plethora of studies, often derived from longitudinal follow-up of individuals discharged from hospital following severe infections during the first wave, show long-term dysregulation across a wide range of parameters of coagulation and haemostasis[103–105,107–111]. A report of 163 patients discharged from hospital showed a cumulative 2.5% incidence of thrombosis within 1 month[105]. A systematic review and meta-analysis of thrombo-embolic events over a mean follow-up period of 8.5 months from acute infection showed HRs of 3.16 for pulmonary embolisms and 2.55 for deep venous thrombosis[110]. Follow-up of 39 patients recovering from COVID-19 over a 16-month period showed a significant minority of individuals with raised D-dimer, factor VIII, IL-6 and von Willebrand factor (vWF)[104]. In terms of specific correlation with long COVID symptoms, a similar analysis of sustained endotheliopathy markers showed that vWF levels inversely correlated with performance in the 6-minute walk-test[108] or desaturation and/or an increase in lactate in a 1-minute sit-to-stand test[109]. A specific case has been argued for pathogenic microclots in long COVID based on the use of an assay to detect and quantify fibrin-based amyloid clots, termed 'fibrinaloids', by analogy to amyloid[112,113]. The case made is that the resulting occlusions may account for many long COVID symptoms, including the 'ground-glass' lung CT opacities often seen in COVID-19 imaging studies[26]. It is proposed that this process occurs to a lesser extent with Omicron[112]. The approach has triggered investigations into anticoagulation therapeutics for long COVID, though no controlled trials have yet been published[113].

The detailed molecular basis for SARS-CoV-2-induced coagulopathies remains to be resolved beyond the starting point of damage to infected endothelial cells and the potential for an intrinsic role of S itself in subverting haemostasis[114]. It is likely that the process is driven

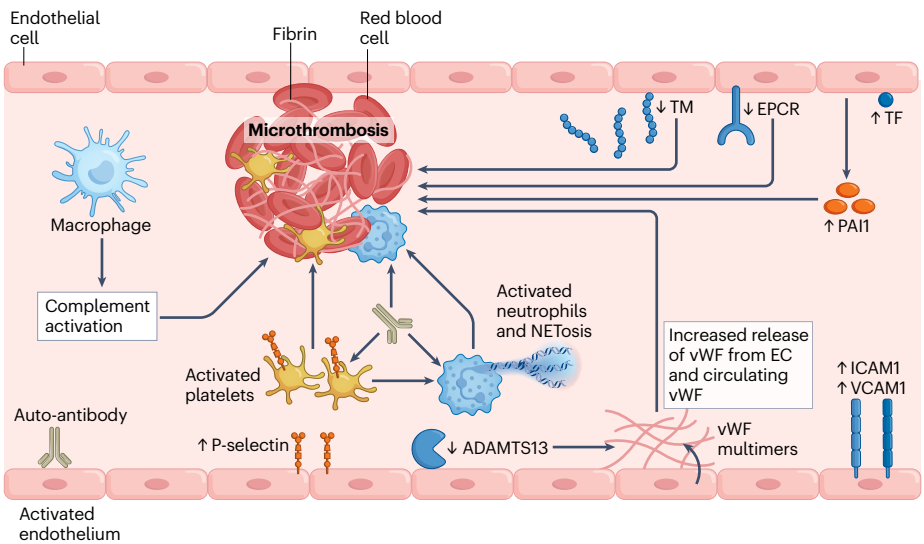

Fig. 2 | Proposed mechanisms of endothelial dysfunction and immunothrombosis in long COVID. During acute COVID-19, SARS-CoV-2 enters the respiratory epithelium via ACE2. This is followed by viral replication, the activation of macrophages and complement activation, leading to endothelial cell (EC) activation. This promotes endothelial expression of proteins, such as P-selectin and tissue factor (TF), and cell adhesion molecules, such as ICAM1, VCAM1 and plasminogen activator inhibitor 1 (PAI1), and the release of ultra-large von Willebrand factor (vWF) multimers. These are accompanied by other procoagulant changes, including downregulation of thrombomodulin (TM) and endothelial protein C receptors (EPCR), contributing to a hypercoagulable state. Reduced TM expression leads to a downregulation of the activated protein C pathway, allowing persistence of activated cofactors factor Va and factor VIIIa, thereby further promoting thrombin generation and microthrombosis. An increased level of PAI1 is a prominent feature in long COVID, leading to impaired fibrinolysis, which further promotes persistent clot formation. Auto-antibody formation, especially antiphospholipid antibodies, also contributes to activation of endothelial cells, complement and coagulation pathways, again favouring thrombus formation. Reduced levels of ADAMTS13 result in the circulation of ultra-large and prothrombotic vWF multimers. Activated platelets, complement and auto-antibodies activate neutrophils, which undergo NETosis. Neutrophil extracellular traps (NETs) are highly prothrombotic, activating and capturing platelets and vWF as well as activating the contact pathway of coagulation. The combined effects of these multiple pathways is the formation of microthrombosis with consequent organ dysfunction.

through the pro-inflammatory responses, notably increased IL-6, established as a part of the endothelial response. This makes tocilizumab a potential candidate in future long COVID randomized controlled trials. The extent to which coagulopathies in long COVID pathogenesis fall within an immunological and immunotherapeutic framework depends on any proven role of innate immune activation in the vasculature and then of AABs, either in promoting clotting abnormalities or in response to them[115]. It has been noted that the COVID-19-associated coagulopathy pattern of thrombosis in the arteries, veins and microcirculation is in some ways similar to that seen in antiphospholipid syndrome[106]. Antiphospholipid syndrome is considered to be driven by a spectrum of phospholipid-specific AABs[76,89]. A study of sera from patients hospitalized due to acute COVID-19 showed that a majority carried antiphospholipid antibodies against a range of different targets[89]. Furthermore, antibodies purified from patient sera caused accelerated venous thrombosis in murine models. Another study of sera from acute infection found an enrichment for IgM AABs against prothrombin, which correlated with the anti-S antibody response[115]. It has been posited that AAB induction may be a downstream consequence of abnormal fibrinaloid microclot formation[112].

To summarize, there is strong consensus on a role of coagulopathy as a driver of long COVID pathogenesis. However, until further data can be accrued on immunothrombosis correlates, including AABs, in long COVID, it remains uncertain to what extent this may encompass the action of antiphospholipid antibodies or other AABs.

Dysbiosis

Given the interest and important findings in recent years through the use of metagenomics to decode gut microbiota species correlated with differences in pro-inflammatory and anti-inflammatory or autoimmune disease states, this has been an obvious sphere of exploration[116–121]. Studies in long COVID have largely focused on the gut microbiome from faecal samples analysed at the level of bacterial, fungal and viral microbiome differences[122–125]. The approach has been applied prospectively to look for predictive biomarkers (and mechanisms) of long COVID development and as a diagnostic tool in ongoing long COVID[122,123]. One team has developed a diagnostic test based on their prospective study of 106 patients with acute COVID-19 who were followed up for 6 months[122]. Development of long COVID was associated with a specific microbiome enriched for *Ruminococcus gnavus* and *Bacteroides vulgatus* and with a reduced abundance of *Faecalibacterium prausnitzii*[122]. Different symptom endotypes were associated with differential microbiome repertoires. An aspect of particular therapeutic interest is that bacterial species that were most negatively associated with long COVID tended to be ones favouring the production of butyrate metabolites. This is noteworthy because the immunoregulatory roles of butyrate, for example in autoimmune disease, are well documented[126]. In arthritis, microbiota-derived butyrate was found to ameliorate disease through aryl hydrocarbon receptor (AhR) binding-dependent activation of regulatory B cells[126]. Furthermore, butyrate is one of the metabolites involved in promoting regulatory T cells[127]. That is, immune modulatory short-chain fatty acids are of interest due to the ease with which they lend themselves to the development of novel therapeutic approaches[128]. Importantly, the theme of reduced abundance of *Faecalibacterium prausnitzii* associated with butyrate deficiency and persistent disease symptoms is fully reiterated in ME/CFS, emphasizing the importance of this avenue of investigation[129]. Another longitudinal metagenomics study that followed individuals for 6 months after acute infection identified a bacterial, fungal and viral microbiome that was associated with a doubling of risk for the development of long COVID[123]. It included, once again, enrichment for *Ruminococcus gnavus* as well as several of clostridial strains, fungal species, including *Candida glabrata*, and a virome, including *Vibrio phage* and *Klebsiella phage*. A 'long COVID' microbiota has also been associated with increased serum pro-inflammatory biomarkers, IL-6 and CRP[124].

Long-term disease risk following COVID-19

The clinical definition of long COVID is still a work in progress. Already there is a tendency to draw a distinction between the familiar spectrum of persistent symptoms such as fatigue, breathlessness and neurocognitive impairment, on the one hand, and increased risk of overt 'lifetime' impacts such as increased risk of stroke, myocardial infarction, and types 1 and 2 diabetes, on the other. In the absence of clear mechanistic pathways, we argue that both sets of outcomes need to be considered within the framework of long COVID. If long COVID encompasses consequences of the infection beyond 4 weeks, then increased lifetime risk of neurological, cardiovascular, renal or metabolic disease events certainly qualify within the term 'long'. The risk of new-onset autoimmunity as reported in health-care records following COVID-19 has been discussed above[94–96]. The US Department of Veteran Affairs health record data sets were used in a series of papers on altered risk conferred by COVID-19 on a wide range of disease outcomes, often viewed as HR deduced from 1-year disease burden data[100,130–133]. This finds an increased risk of dyslipidaemia across abnormal lipid outcomes, with a HR of 1.24 (ref. 131). Analysis of cardiovascular outcomes found a HR of 1.52 for risk of stroke, 2.93 for pulmonary embolism, 5.38 for myocarditis and 1.49 for transient ischaemic attacks[130]. For all diabetes across this data set, the HR was 1.4 (ref. 133).

Therapeutics and outlook

It should be clear from the above that, in the few years that it has taken to recognize and define long COVID, a huge amount of high-quality research has been conducted and many aspects of the puzzle of pathogenesis have been put in place. However, it is invidious at this stage to attempt any mechanistic synthesis that could draw the connecting lines between those parts of the picture that seem to be largely agreed: infection by SARS-CoV-2 leaves a lasting imprinting of variably apparent, radiologically detectable end-organ damage, including in the CNS, as well as immune subset perturbations, enhanced cytokine levels, including CCL11, and a range of pathologies relating to coagulopathy. There are possible roles also of EBV reactivation, persistent SARS-CoV-2, microbiota dysbiosis and low serum cortisol. It is apparent that it is premature and spurious to contort these into a simple pathway while such huge knowledge gaps remain. Meanwhile, people with long COVID feel neglected and are impatient to see these medical research findings translated into therapeutic trials. In a period where all but the most severe acute infections go largely unreported, the potential for trials into the mitigating effects of antiviral or other treatments in long COVID in this phase appears limited, such that most attention is focused on treatment of the persistent phase. In terms of treatment for existing long COVID, the UK-based STIMULATE-ICP trial is enrolling participants into study arms for colchicine, rivaroxaban (an anticoagulant), famotidine and loratadine (antihistamines), compared to no drug, with the potential to add in further drug arms[134]. According to ClinicalTrials.gov as of May 2023, there are 384 clinical trials, mostly USA based, that enrol or have recently enrolled patients with long COVID; 197 of these are interventional studies, of which 44 offer a therapeutic and the remainder tend to be behavioural or rehabilitation based (Table 3). Analysis of the list shows that interventional studies are under way to

Table 3 | Trials on long COVID

Trial	Rationale	Centre
Modulation of immunity or inflammation		
Anakinra	Anti-IL-1 mAb	AZ Sint-Jan Brugge Brugge, Belgium
Ibudilast	Anti-inflammatory phosphodiesterase inhibitor	University Health Network, Toronto, Canada
Immunoadsorption	Removal of auto-antibodies from blood	Charite, Berlin, Germany
Leronlimab	Target CCR5 receptor on T cells	Rheumatic Disease Specialties, Aventura, Florida, USA
Methylprednisolone	Synthetic glucocorticoid, anti-inflammatory, immunosuppressive	NIH Clinical Center, Bethesda MD, USA
Nivolumab or ipilumab	Immune-checkpoint blockade to enhance T cell immunity	Centro Richerche Cliniche, Verona, Italy
NT-17	Human IL-7 to promote T cell development	NIH Clinical Center, Bethesda MD, USA
Pentoxifylline	Anti-inflammatory phosphodiesterase inhibitor	University Health Network, Toronto, Canada
Plasmapheresis	Remove auto-antibodies	Hospital Europeen, Marseille, France
Rintatolimod	Poly(I:C)-like TLR stimulation	Aim Immunotech, Ocala, Florida, USA
RSLV-132	RNAse 1 IgG–Fc fusion protein to block TLR activation	Resolve Therapeutics, Florida, USA
Sargramostim	Promote immune stimulation by GM–CSF	AZ Sint-Jan Brugge Brugge, Belgium
Siltuximab	Anti-IL-6 mAb	AZ Sint-Jan Brugge Brugge, Belgium
Sirolimus	T cell inhibition	University of Chicago, Chicago, IL, USA
Temelimab	Anti-HERV-W Env	UOC Malattie Infettive, Rome, Italy
Zilucoplan	Inhibit C5 cleavage	OLVZ Aalst Aalst, Belgium
Modulate protection against SARS-CoV-2		
ARCT-021 COVID-19 vaccine	Boost immunity to augment virus clearance	Singapore General Hospital, Singapore
COVID-19 mRNA booster vaccine	Boost immunity to augment virus clearance	NCID Singapore; McMaster University, Ontario, Canada
Infliximab	mAb to clear viral reservoirs	University of Helsinki, Finland
IVIG	Antibody to clear viral reservoirs	NIH Clinical Center, Bethesda MD, USA
Paxlovid	Antiviral to clear viral reservoirs	Yale University, Duke University, Stanford University, USA
SCB-2019	COVID-19 protein vaccine to augment viral clearance	Linear Clinical Research, Australia
Vitamin D	Augmented immune function	China Medical University, Taiwan
Modulate gut microbiota		
Faecal microbiota transplantation	Reverse dysbiosis	Prince of Wales Hospital, Hong Kong
Probiotics	Reverse dysbiosis	CIUSSS de L'Estrie-CHUS Hospital Sherbrooke, Quebec, Canada
Therapeutics affecting other mechanisms		
AXA 1125	Improve mitochondrial function	University of Oxford, UK
Cannabidiol	Relief from symptoms, including pain and movement problems	University of York, UK
CoEnzyme Q10	Improve mitochondrial function	Department of Infectious Diseases, Aarhus, Denmark
Ivabradine	Treatment of heart failure	Uniformed Services University, USA
Lithium	Relief from symptoms	University at Buffalo Williamsville, New York, USA
LYT-100	Anti-fibrotic	University of Alabama, USA
Naltrexone	Used for symptom relief in opioid addiction	BC Women's Hospital, Vancouver, Canada
Niagen, vitamin B3	Effect of VitB3 on mood and cognitive function	Clinical Trials Research Unit, Boston, USA
PB1046	VIP analogue	Baptist Health Research Institute, Florida, USA
S1 226	Bronchodilator	Sol Aeromed Inc., USA
Somatropin	Human growth hormone	UTMB, Galveston, TX, USA
Vagus nerve stimulation	Anti-inflammatory and anti-pain	Mt Sinai School of Medicine, New York, USA

Current, recently completed or forthcoming long COVID therapeutics trials listed on ClinicalTrials.gov. Of 384 listed long COVID studies, 197 are interventional, of which 44 involve therapeutics and the remainder are behavioural interventions. IVIG, intravenous immunoglobulin; mAb, monoclonal antibody; TLR, Toll-like receptor; VIP, vasoactive intestinal peptide.

address the candidate disease mechanisms discussed above. The list also embodies the current lack of consensus: the immunological treatments encompass some fairly potent approaches to either boost T cell immunity, suppress T cell immunity or deplete AABs. Interestingly, none of the trials listed addresses two disease axes for which some of the most exciting data are accruing — the possibility of ameliorating any effects on neurocognitive function by targeting CCL11 or that of inhibiting EBV reactivation through therapeutic vaccination or antivirals. There is a powerful case for large, comparative, randomized control trials with the transformative potential that the Recovery trial offered for acute COVID-19. The greater the clarification of the diagnosis, clinical phenotypes and stratification of long COVID, the more powerful clinical trials will be — some individuals will indeed benefit from therapeutics against a persistent virus reservoir, but how do we differentiate between these and the subgroups that would specifically benefit from anticoagulation treatment? By merging the groups, we risk losing the power of the result.

The oncoming burden of long COVID faced by patients, health-care providers, governments and economies is so large as to be unfathomable, which is possibly why minimal high-level planning is currently allocated to it. If 10% of acute infections lead to persistent symptoms, it could be predicted that ~400 million individuals globally are in need of support for long COVID. The biggest unknowns remain the joined-up scheme of its pathogenesis and thus the best candidate therapeutics to be trialled in randomized controlled trials, along with a better understanding of the kinetics of recovery and the factors influencing this. Some countries have invested in first-round funding for the pilot investigations. From the above, far more will be needed.

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D.M.A. and R.J.B. designed and wrote the paper and head the Long COVID Research Programme. S.L. and E.W. wrote sections and helped with literature searches and tables. D.J.A. advised on coagulopathy content and the associated figure.

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