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Inflammation, immunity, and antigen persistence in post-acute sequelae of SARS-CoV-2 infection

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SARS-CoV-2 infection is known to result in a range of symptoms with varying degrees of acute-phase severity. In a subset of individuals, an equally diverse collection of long-term sequelae has been reported after convalescence. As survivorship and therefore the number of individuals with 'long-COVID' continues to grow, an understanding of the prevalence, origins, and mechanisms of post-acute sequelae manifestation is critically needed. Here, we will explore proposed roles of the anti-SARS-CoV-2 immune response in the onset, severity, and persistence of SARS-CoV-2 post-acute sequelae. We discuss the potential roles of persistent virus and autoantigens in this syndrome, as well as the contributions of unresolved inflammation and tissue injury. Furthermore, we highlight recent evidence demonstrating the potential benefits of vaccination and immunity in the resolution of post-acute symptoms.

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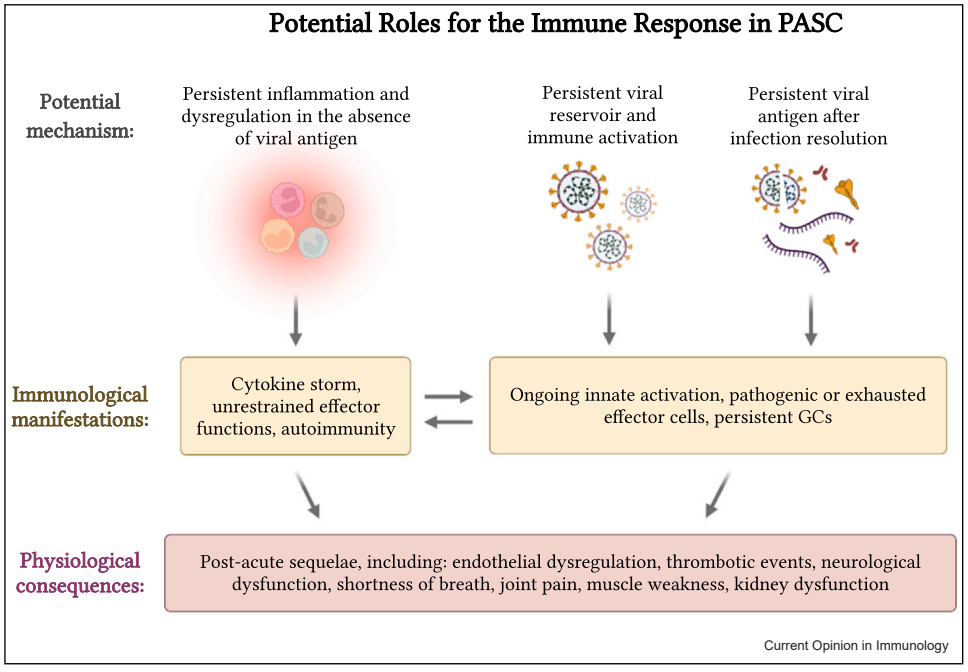
Introduction: the phenomenon of 'long COVID'

Post-acute sequelae of SARS-CoV-2 (PASC), colloquially referred to as 'long COVID', is a poorly understood condition characterized by prolonged Coronavirus Disease 2019 (COVID-19) symptoms and/or the development of new symptoms following the resolution of acute SARS-CoV-2 infection. While there is still no formal clinical definition of PASC, findings reported in individuals experiencing PASC include persistent shortness of breath, sleep disorders, hyperlipidemia, fatigue, gastroesophageal reflux disease (GERD), cough, muscle weakness, joint pain, thromboembolism, kidney disorders, neurological impairments, and cardiopulmonary abnormalities [1••–3]. PASC is differentiated from acute COVID-19 primarily by the timing of symptoms relative to the onset of illness, with acute COVID-19 defined as symptoms persisting for up to four weeks post onset, and PASC restricted to symptoms persisting or developing more than two months after symptom onset. The spectrum of symptoms associated with PASC is not fully unique to SARS-CoV-2 convalescence and includes features associated with recovery from other viral illnesses or sepsis. Nevertheless, an estimated 7–10% of convalescent COVID-19 patients — or 23–33 million people as of January 2022 — are thought to have experienced PASC [4••,5], with roughly 40% of these individuals stating that PASC significantly impacts their ability to perform basic daily tasks. Both the frequency and overall number of convalescent COVID-19 patients that experience post-acute sequelae of infection make it a significant public health concern.

Despite the prevalence and growing awareness of PASC, the physiological mechanisms underpinning the phenomena remain poorly defined. This is in part due to the diverse array of symptoms associated with PASC, the fundamental complexities of COVID-19 pathogenesis, and the lack of a concrete and consistently applied clinical definition of PASC. Only recently have large-scale studies started to shed light on the full clinical burden of PASC and on the risk factors associated with PASC severity and persistence. Both premorbid risk factors — such as hypertension, obesity, and immunosuppression — and the duration and severity of acute COVID-19 illness appear to correlate with the severity and persistence of PASC [3,4••,6–9]. However, a common theme is the presence of persistent inflammation and immune activation beyond the acute infectious insult. Here, we will explore the association between persistent inflammation and PASC, as well as several potential mechanisms for the induction and maintenance of dysregulated antigen-specific immune responses following the resolution of acute SARS-CoV-2 infection (Figure 1).

Figure 1



Graphical abstract of potential roles of the immune system in PASC.

Immunological misfiring, inflammatory storms, and persistent inflammation in post-acute sequelae of SARS-CoV-2

There is mounting evidence that PASC is accompanied by dysregulated and persistent multiorgan inflammation [10•–13••]. Elevated serum levels of C-reactive protein, TNFα, IFNγ, and IL-6 have been observed in convalescent COVID-19 patients who progressed to develop PASC, with the quantity of these inflammatory markers correlating with the number of patient-supplied PASC symptoms [9,10•]. Notably, Phetsouphanh et al. have described persistent immunological dysfunction in individuals at 8 months after nonsevere SARS-CoV-2 infection characterized by highly activated innate immune signatures and higher expression of both type-I and -III IFN, as well as CXCL9, CXCL10, IL-8, and sTIM-3 [14•]. In individuals with PASC, this unresolved infection-attendant inflammation is postulated to drive tissue damage, endothelial dysregulation, hypoxia-induced injury, and activation of pathogenic effector lymphocyte subsets [15–17].

Unchecked inflammation and dysregulated adaptive immune responses have been associated with long-term sequelae following the resolution of many other viral pathogens, including Ebola, Lassa, Chikungunya, and influenza viruses [18–20]. For SARS-CoV-2, persistent immune activation has been shown to result in endothelial dysfunction in convalescent COVID-19 patients [16•]. This study additionally identified circulating activated endothelial cells following the resolution of acute infection, postulating that this indicator of vascular injury was a consequence of proinflammatory cytokine production by activated cytotoxic $CD8^+$ T cells. Notably, they also observed higher frequencies of effector T cells in subjects with pre-existing cardiovascular conditions and other comorbidities associated with increased COVID-19 severity and, consequently, increased probability of developing PASC. In support of this lymphocyte-activation model of PASC, Patterson and colleagues [21] utilized a machine-learning approach to predict the time to resolution of SARS-CoV-2-infection symptoms. The resultant predictive signature of PASC chronicity included elevated levels of plasma IFN-γ and IL-2, suggesting that untempered immune activation may contribute to PASC symptoms after viral clearance.

In addition to triggering unchecked and dysregulated inflammation, it has also been suggested that both severe COVID-19 and PASC are accompanied by the development of self-reactive immune responses. Multiple studies have now demonstrated that the production of autoantibodies can be triggered by SARS-CoV-2 infection and track with the development of anti-SARS-CoV-2 humoral immunity [22,23]. This appears to be especially evident following severe COVID-19 and extends beyond the anti-IFN antibodies that have been identified as playing a role in driving acute COVID-19 severity [24]. Additionally, it has been suggested that SARS-

CoV-2 infection can trigger an inflammatory response against other, non-SARS-CoV-2 organisms, thereby resulting in the reactivation of latent viral infections and increasing the risk of opportunistic infections [13••,25,26]. These observations suggest that SARS-CoV-2-driven inflammation can cause a persistent disruption of immunological homeostasis that results in the propagation of both tissue-level and systemic sequelae.

Persistent antigen production by noninfectious viral RNA

Dysregulated and persistent inflammation does not require the continuous presence of viral antigen after an acute viral illness. However, a growing number of studies posit the existence of a persistent noninfectious SARS-CoV-2 antigen reservoir. While infectious SARS-CoV-2 can generally only be observed in the airway for the first week after symptom onset, viral RNA and protein antigens have been detected in sites such as the central nervous system, intestine, and secondary lymphoid organs for weeks to months after the resolution of acute symptoms [27•–29••]. Autopsies of individuals historically positive for SARS-CoV-2 have detected SARS-CoV-2 RNA up to 230 days post infection in the lung and a wide range of extrapulmonary tissues [27•,28]. However, SARS-CoV-2 RNA found outside of the lung late after acute infection was not accompanied by cytopathic tissue damage or marked inflammation in these individuals [28]. Therefore, while SARS-CoV-2 genetic material may persist long after the initial acute infection, it is unlikely that this represents a truly persistent or latent viral infection. A provocative hypothesis proposed by recent reports is the possibility that SARS-CoV-2 is capable of partial genomic integration, resulting in a somatic source of persistent antigen and subgenomic RNA [30]. This speculation has been intensely disputed [31–33], and will demand further examination as a potential source of persistent viral antigen that may be capable of driving protracted inflammation.

Some of the strongest evidence supporting a role for SARS-CoV-2-specific immunity in PASC lies in the observed impact of vaccination on PASC prevalence and durability. Recent work by Antonelli and colleagues [34•] demonstrated a 49% reduction in the risk of long-term SARS-CoV-2-infection symptoms in individuals who were vaccinated before infection compared with unvaccinated controls. Moreover, preliminary studies suggest that in some individuals, SARS-CoV-2 vaccination may help to resolve, or abate the worsening of, PASC symptoms [35]. Indeed, some evidence suggests that the duration of acute SARS-CoV-2 infection symptoms is associated with the persistence, but not magnitude, of antigen-specific adaptive immune responses [36,37]. It is therefore plausible that clearance of persistent antigen or a return to immunological baseline as a consequence of vaccination contributes to the resolution of PASC.

Antigen persistence in the absence of viral persistence

While there is evidence suggesting the persistence of SARS-CoV-2 genomic material beyond the acute infection phase, persistent immune activation directed toward SARS-CoV-2 is not dependent on prolonged viral replication or *de novo* antigen production. Multiple immunological mechanisms exist to retain antigen for the purpose of maturing immunological memory after the resolution of an acute infectious insult [38,39]. Most notably, follicular dendritic cells can retain protein antigens in germinal centers (GCs) within lymph nodes or spleen for months after initial antigen production. Indeed, the long-term retention of SARS-CoV-2 antigen within GCs has been suggested to drive clonal and mutational maturation of memory B cells isolated from convalescent COVID-19 patients [29••,40•]. A separate study identified reduced total IgM or IgG3 levels as a predictor of PASC risk [41], suggesting that not just the magnitude and affinity, but also the isotype of SARS-CoV-2 Ig may play a role in PASC.

How the persistence of SARS-CoV-2 antigen may mechanistically lead to individual post-acute symptoms remains unclear, but a number of investigations into the immunological basis for PASC and isolated case studies have identified dysregulation of $CD8^+$ T-cell responses as a correlate of risk [42–44•]. Immune signatures of bronchoalveolar lavage (BAL) fluid in older individuals with PASC at 60–90 days post infection revealed a persistent increase in the frequency of $CD69^+$ $CD103^-$ $CD8^+$ T_{RM} cells, as well as inflammatory signatures among myeloid cells [44•]. Following peptide stimulation, the $CD69^+$ $CD103^-$ $CD8^+$ T cells in convalescent subjects were polyfunctional for cytotoxic cytokine production, and expressed granzyme K, which can promote fibroblast activation. In contrast, Peluso et al. [43•] observed PASC patients 4 months out from infection to have a reduced frequency of degranulation-prone $CD107a^+$ $CD8^+$ T cells in the peripheral blood. Moreover, the frequency of nucleocapsid-specific IFN-γ-secreting $CD8^+$ T cells declined more quickly in individuals with PASC in this study. It is possible that the preferential antigen-specific activation of pathogenic $CD103^-$ $CD8^+$ T cells leads to cytotoxic lung-tissue damage and pathology, or that cytotoxic $CD8^+$ T-cell exhaustion limits the capacity for complete viral or viral antigen clearance. Importantly, these possibilities are not mutually exclusive.

Stimulation of B cells via antigen–receptor binding is known to lead to the expansion of atypical memory B cells (atMBCs) during chronic or repeated intracellular

infection or vaccination [45]. atMBCs are also associated with the production of autoantibodies in several autoimmune diseases, and were observed to be expanded in the peripheral blood of individuals with PASC before the onset of symptoms [13]. Cheon and colleagues [44•] also observed elevated frequencies of activated resident memory-like $CD27^+$ $CD69^+$ B cells in the BAL fluid of aged convalescent subjects, and several studies have shown or suggested a potential relationship between peripheral autoantibody levels and PASC [46,47•]. Further investigation will be required to determine whether atMBCs and circulating autoantibodies detected in PASC patients contribute to symptoms, or are a consequence of PASC itself.

Conclusions and significant open questions for the field

Despite the abundance of epidemiological information about PASC, numerous critical questions remain about the role of the immune system in PASC risk, resolution, and severity. It has become clear that considerable heterogeneity exists in the presentation — and likely also the origins — of specific PASC. As has been suggested for acute SARS-CoV-2 disease, the kinetics of the immune response and the interaction of polarized subsets of immune cells are likely to be the link between identified PASC risk factors and clinical outcomes. As observed by Su et al. [13], it is possible that early or inappropriately skewed acute T- and B-cell response could cause local tissue-damage-related PASC. Alternatively, delayed activation or exhaustion of the adaptive immune response to SARS-CoV-2 infection could result in untempered inflammation and secondary viral/antigen dissemination and inflammation at distal sites. Indeed, respiratory and GI PASC were proposed to be driven by divergent early transcriptional programs [13], and although these programs may have pre-existing pathological origins, differences in the kinetics of recruitment could also account for these disparities.

Another outstanding question is the nature and mechanistic underpinnings of severe post-acute symptoms observed in some younger individuals. One subgroup of PASC symptoms, Multisystem Inflammatory Syndrome in Children (MIS-C), is a rare acute shock-like syndrome affecting pediatric populations during SARS-CoV-2 convalescence. Recent analyses have identified marked activation of vascular patrolling $CX3CR1^+$ $CD8^+$ T cells [48] and dysregulated IFN-γ responses [49] during MIS-C. It will be important to determine whether some cases of PASC in adults follow a parallel mechanism of development, considering the similarities between the infectious context and potential immunological basis for these conditions. Additionally, it will be intriguing to explore whether pre-existing cross-reactive adaptive immune cells from endemic hCoVs or heterologous SARS-CoV-2 exposures influence the development or resolution of symptoms in the post-acute phase.

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Graphics

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