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Long COVID and post-acute sequelae of SARS-CoV-2 pathogenesis and treatment: A Keystone Symposia report

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Abstract

In 2023, the Keystone Symposia held the first international scientific conference convening research leaders investigating the pathology of post-acute sequelae of COVID-19 (PASC) or Long COVID, a growing and urgent public health priority. In this report, we present insights from the talks and workshops presented during this meeting and highlight key themes regarding what researchers have discovered regarding the underlying biology of PASC and directions toward future treatment. Several themes have emerged in the biology, with inflammation and other immune alterations being the most common focus, potentially related to viral persistence, latent virus reactivation, and/or tissue damage and dysfunction, especially of the endothelium, nervous system, and mitochondria. In order to develop safe and effective treatments for people with PASC, critical next steps should focus on the replication of major findings regarding potential mechanisms, disentangling pathogenic mechanisms from downstream effects, development of cellular and animal models, mechanism-focused randomized, placebo-controlled trials, and closer collaboration between people with lived experience, scientists, and other stakeholders. Ultimately,

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AUTHOR CONTRIBUTIONS

Durstenfeld wrote the first draft with input from Weiman. Holtzman, Blish, Pretorius, and Deeks reviewed and provided critical input.

COMPETING INTERESTS

M.S.D., none; S.W., Keystone Symposia employee; M.H., Founder, Nupeak Therapeutics Inc.; C.B., DeepCell, Inc., Catamaran Bio, Immunebridge; R.P., none; S.G.D., Pfizer, Enantha.

by learning from other post-infectious syndromes, the knowledge gained may help not only those with PASC/Long COVID, but also those with other post-infectious syndromes.

Keywords

conference report; Long COVID; pathogenesis; post-acute sequelae of COVID-19; post-viral conditions; treatment

In late summer of 2023, Keystone Symposia held the first scientific conference convening research leaders investigating the pathology behind Long COVID. The symposium “Long COVID and Post-Acute Sequelae of SARS-CoV-2 (PASC): Pathogenesis and Treatment” was held at the Eldorado Spa and Resort in Santa Fe, New Mexico August 27–30, 2023. The conference was organized by Michael Holtzman, Steven Deeks, Resia Pretorius, and Catherine Blish.The overall objective was to bring together world experts in the pathogenesis of post-acute sequelae of COVID-19 to connect, collaborate, and advance the understanding of what drives this chronic illness, which is estimated to impact millions of people worldwide. Specifically, the conference focused on five aims (Table 1).

The meeting brought together experts and stakeholders with diverse perspectives, including basic scientists, clinicians, patient advocates with lived experience, and funders including the National Institutes of Health to collectively integrate their knowledge and tackle this debilitating disease. The program covered a broad scope of scientific and medical disciplines, to get at the heart of the wide spectrum of symptoms experienced by people with Long COVID, from brain fog to orthostatic intolerance, and most notably, debilitating fatigue. Many discoveries were unveiled, revealing novel targets and new strategies for potential treatments, which are currently lacking for the many who suffer with the disease (Figure 1).

In the welcome address, **Michael Holtzman** and **Steven Deeks** highlighted that this symposium was the first international, abstract-driven meeting focused on Long COVID. Deeks commented that the meeting brought together many people from different backgrounds and fields of science who have never met in person before. He emphasized that this in-person meeting was critical to foster connections and interactions that will advance interdisciplinary insights and clinical advances.

In the opening keynote, **Gary Gibbons**, Director of the National Heart, Lung, and Blood Institute at the National Institutes of Health (NIH), spoke about the NIH RECOVER Initiative. He highlighted the incredible speed, scale, and scope of RECOVER, the world’s largest, most diverse, deeply characterized cohort of patients with PASC, leading to initial insights on PASC epidemiology, pathogenesis, and disparities.[1,2] The goal of the RECOVER Initiative is to rapidly improve our understanding of and ability to predict, treat, and prevent PASC, with four key scientific aims: (1) understand the clinical spectrum/biology underlying recovery over time; (2) define risk factors, incidence/prevalence, and distinct PASC subphenotypes; (3) study pathogenesis over time and possible relation to other organs dysfunction/disorders; and (4) identify interventions to treat and prevent PASC. Major areas under investigation include viral persistence/reactivation, immune

dysregulation, organ damage/dysfunction, vascular endothelium activation or dysfunction, and tissue pathology. He discussed the overlap between PASC, myalgic encephalitis/chronic fatigue syndrome (ME/CFS), and multisystem inflammatory syndrome in children and underscored the importance of understanding the biology of post-exertional malaise, highlighting metabolomics and mitochondrial studies. Finally, Gibbons discussed how the initial RECOVER Clinical Trials focus on the priorities of patients with lived experience.

ACUTE TO CHRONIC DISEASE

The first session, "Acute to Chronic" (Chairs: **Michael Peluso** and **Avi Nath**), explored the link from acute illness to chronic disease under the premise that something about the body's response to the initial infection could set some people up for developing Long COVID. Defining these initial responses that promote long-term pathology may provide targets for early intervention to prevent progression to Long COVID, and/or be used to identify those most likely to develop the disease for such treatments.

Michael Holtzman, of Washington University in St. Louis and NuPeak Therapeutics, presented "Defining and Fixing Viral Reprogramming." Holtzman noted that many types of respiratory viral infections can trigger chronic disease in a susceptible subset of patients. This pattern is well described for respiratory syncytial virus (RSV) infections that result in chronic asthma in the majority of severe pediatric cases. In these cases, and similarly in COVID-19, the infection can induce an overactive repair response in epithelial stem cells. In turn, this response leads to lung remodeling disease characterized by inflammation, mucus production, and fibrosis. Holtzman showed that this disease process depends on the activation of a relatively orphan and previously undrugged kinase known as MAPK13 that is localized to this stem cell population and its lineage cells. Holtzman's group is characterizing a novel MAPK13 inhibitor that corrects post-viral lung disease in human cell and mouse models[3]; their clinical candidate drug is at the stage of investigational new drug (IND) application to the Food and Drug Administration (FDA). Holtzman remarked that this therapeutic strategy could modify disease pathology to prevent or even reverse the progression to Long COVID and PASC. Holtzman also noted that respiratory viral infections cause associated disease at sites outside of the lung, using the example of skeletal muscle atrophy. These multisystem consequences might also be preventable or treatable with the same kinase-inhibitor strategy.

Andrea Lynn Cox, of Johns Hopkins University, presented "Sex, Obesity, Immunometabolism, and Viral Persistence in Post-acute Sequelae of SARS-CoV-2 Infection." Cox showed that patients with pre-COVID obesity and hyperlipidemia were more likely to develop Long COVID, even after mild to moderate initial infection. Altogether, approximately one-third of the cohort experienced Long COVID symptoms, with those who took longer to clear the virus more likely to experience brain fog and muscle pain long-term. Cox's team has studied the immunological markers of symptom clusters, including antibody responses, cellular subsets, cytokine/chemokine levels, and immunometabolic characteristics. They found that polymorphonuclear myeloid-derived suppressor cells, especially those expressing lectin-type oxidized low-density lipoprotein receptor 1, and intermediate memory B cells are elevated and correlate with Long COVID

symptoms and severity overtime. Whether these cell types are markers or drivers of disease is yet unknown, but they could potentially serve as novel diagnostic or therapeutic targets.

Akiko Iwasaki, of Yale University School of Medicine, presented "Distinguishing Features of Long COVID Identified through Immune Profiling," noting that there are many unexplained post-acute infection syndromes that occur after encounters with many distinct pathogens, including Ebola, Dengue, SARS-1, Ebstein Barr Virus (EBV), West Nile Virus, Influenza, and many other viruses, which display overlapping symptoms with Long COVID and often culminate in ME/CFS. Iwasaki argued that Long COVID has simply amplified the public awareness of this phenomenon, while providing a unique opportunity to investigate the origins of such syndromes. She outlined four possible root causes that she is currently investigating: viral persistence, latent virus reactivation, autoimmunity, microbiome dysbiosis, and tissue damage. Examining the origins of long-term cognitive symptoms, Iwasaki's team, in collaboration with Michelle Monje's group at Stanford, found that mild COVID-19 infection can cause prolonged changes in the CNS, including increased reactive microglia and reduced neurogenesis, oligodendrocytes, and myelination of axons.[4] No virus was detected in the central nervous system, suggesting that these impacts are driven instead by the immune response, with increases in CCL-11 as the suspected culprit, a chemokine that is also associated with neurological deficits during aging. Looking at other immune and hormonal abnormalities in people with Long COVID, in collaboration with David Putrino's team at Mount Sinai School of Medicine, Iwasaki's team found many elevated cytokines and chemokines. Among the findings were altered hypothalamic–pituitary–adrenal axis hormone levels, in particular lower cortisol. Many differences existed between males and females; for example, women with Long COVID have elevated T cell percentages and autoantibody presence with lower testosterone levels, while males have higher natural killer (NK) cells and Transforming growth factor-beta (TGF-β) levels with lower estradiol. These distinctions could explain sex differences in symptoms, pathology, and prevalence of Long COVID, indicating that diagnosis and treatment may need to be sex-specific.

Michael Peluso, of the University of California, San Francisco, presented "Multimodal Assessment of Antigen Persistence in the Post-Acute Phase of SARS-CoV-2 Infection," sharing new data on antigen persistence generated in a collaboration with Harvard University. Among participants in the UCSF-based Long-term Impact of Infection with Novel Coronavirus (LIINC) cohort, spike, S1, and/or nucleocapsid protein could be detected in the blood for up to 14 months after infection, including in individuals without interval vaccination or suspected reinfection.[5] Interestingly, antigen tended to be more prevalent among those with the highest symptom burden, with trends toward associations with specific symptoms appeared to be emerging from a preliminary analysis. However, only a subset of people with Long COVID showed persistent antigen (primarily those who are most symptomatic), while a subset of those who report full recovery also demonstrated persistent antigen. In a small pilot study, Peluso's team looked at potential tissue antigen reservoirs and identified spike RNA for up to 2 years post-infection in gut biopsies of people with Long COVID.[6] However, it remains unknown if the antigen is also present in the gut tissue of those without Long COVID. Peluso described plans at UCSF and elsewhere to

examine whether antivirals and/or monoclonal treatments against these persistent antigens might alleviate Long COVID symptoms, which would validate the clinical relevance of these observations.

Finally, **Ella Borberg**, of Harvard University, presented "Uncovering the Link between SARS-CoV-2 Antigen Persistence and Post-Acute Sequelae of COVID-19," presenting data that suggest PASC may be correlated with the persistence of viral antigens in some cases.[7] Across multiple cohorts, one or more of full spike protein, spike subunit S1, and nucleocapsid protein were detected in 37% of all samples collected after 1-month post-infection, and all three antigens were detected up to 14 months post-infection. Persistent antigenemia was detected at higher rates in participants who reported cardiopulmonary, musculoskeletal, or neuropsychiatric symptoms.

PATHOGENESIS WORKSHOP

The workshop "Pathogenesis" (Chairs: **Harlan Krumholz** and **Julio Silva**) explored many potential drivers of PASC pathology and symptoms, including age, sex, hormones, fibrosis, and other immunological factors.

Michael J. Patton of the University of Alabama at Birmingham Hugh Kaul Precision Medicine Institute presented "Characteristics and Determinants of Pulmonary Post-acute Sequelae of SARS-COV-2 (PASC): A Single-Center Retrospective Study." Patton showed that persistent diffusion impairment and restrictive lung disease measured by longitudinal pulmonary function testing were common among patients with Long COVID with unresolved cough, dyspnea, or chest discomfort.

Lung fibrosis as a contributor to longer-term pulmonary dysfunction was further examined by **John Powers**, of the University of North Carolina at Chapel Hill, who presented "Omicron Lineage Variants Display Acute and Chronic Fibrotic Pathologic Lung Disease in BALB/c Mice." Powers showed that the severity of lung fibrosis correlated with the severity of initial infection, with certain SARS-CoV-2 variants being more likely to induce both acute and long-term damage. Thus, patients infected with these variants of concern could potentially be treated with interventions, such as antivirals or antifibrosis drugs, at the time of infection to prevent progression to PASC.

S. J. Sahagun, of MaineHealth Institute for Research, presented "Endotrophin and PASC Symptoms at 12 Months After Acute COVID-19 Infection." However, the extracellular matrix protein endotrophin, which is associated with fibrosis in the setting of chronic inflammation, was not associated with PASC although it positively correlated with respiratory support in the acute phase of infection.

Other workshop presenters investigated demographic, genetic, and immunological factors that might predispose certain individuals for Long COVID and PASC.

Ling Li, of the University of Minnesota, presented "Capturing the Impact of Age, Sex, and APOE Genotype on the Progression of SARS-CoV-2 Infection and Subsequent Neuroinflammation in Mouse Models," during which she reported that older age, male

sex, and APOE4 are risk factors for severe neuroinflammatory response to SARS-CoV-2 in mouse models, resulting in brain transcriptomic perturbations. This was true even without a virus detected in the brain. Sex hormones may play a role in the different manifestation and prevalence of Long COVID between males and females, via the influence on immune activation.

Julio Silva, of Yale School of Medicine, presented "Testosterone is a Key Differentiator of Sex-specific Immune Profiles and Symptomology in Long COVID." A machine learning approach revealed that females with Long COVID have more autoreactivity, T cell exhaustion, and other cytokine expression profiles that differed from the male predominant myeloid signature.

Other immunological anomalies were presented by **Sasha Tabachnikova** from Yale University, who presented "Identification of Elevated and Altered Humoral Responses to Epstein-Barr Virus in Long COVID," which indicated how latent herpesviruses reactivation–especially EBV reactivation–in Long COVID patients may drive their symptoms via immunopathology, causing an increase in inflammatory IL-4^+/IL-6^+ $CD4^+$ T cells that correlates with symptom burden.

Amber Wolabaugh, of Columbia University, presented "A Novel Human Immune System Mouse Model for COVID-19" in which she argued that the adaptive T cell response may dampen the innate immune response to SARS-CoV-2 infection, providing additional insight and tools to further study immune implications of Long COVID.

IMMUNOPATHOLOGY OF LONG COVID/PASC

The session "Immunopathology of Long COVID/PASC" (Chairs: **Janko Nikolich-Zugich** and **Sara Moron-Lopez**) further expanded on this theme, looking specifically at immune dysregulation in people with Long COVID, the origins of this dysregulation, and how these might contribute to various symptoms.

Catherine Blish, of Stanford University School of Medicine, presented "Adipose Tissue Inflammation as a Potential Driver of Long COVID." Given that adipose tissue expresses the ACE2 receptor that SARS-CoV-2 uses to enter cells, Blish investigated adipocytes as a potential reservoir for long-term virus persistence that could drive Long COVID symptoms.[8] Her team has concluded that the virus does indeed infect adipose tissue during acute infection, as well as the macrophages that reside there. Macrophages appear to be a reservoir of viral replication in the lung as well and initiate tissue-wide inflammation through the release of cytokines and interferons in both cell types. How long adipocytes and macrophages continue to produce viral antigens after acute infection is unknown, but these data could explain why obese individuals are at higher risk for both severe acute infection and Long COVID. Macrophages may carry the virus throughout the body, propagating infection and driving inflammatory responses systemically.

Anna Aschenbrenner, of Systems Medicine, DZNE, Germany, presented "Immunologic Correlates of Long COVID Following Mild Disease," reporting systemic proinflammatory changes and transcriptional activation of proinflammatory programs in monocytes, NK

cells, and cytotoxic $CD16^+$ T cells in the patients of their cohort. Further, with a study on the clinical effects of dexamethasone in acute COVID-19 using high-resolution single-cell multi-omics and the cell type-derived gene signatures being validated in larger clinical cohorts, Aschenbrenner exemplified how studying the effect of pharmacological interventions at single-cell resolution can help to stratify patients by outcome and thus help with therapeutic strategies. Finally, Aschenbrenner projected ahead on single-cell multi-omics approaches in clinical trials currently running in Germany under the umbrella of the National Clinical Study Group Post-Covid-Syndrome and ME/CFS.[9]

Anthony Kelleher, of the University of New South Wales, Australia, presented "Resolution of Immune Dysregulation and Improvement in Health-related Quality of Life in Individuals with Long COVID at 2-years Following SARS-CoV-2 Infection." Kelleher presented data from a cohort in Sydney, Australia. Following up on an impactful study published by their group,[10] they found that quality of life scores improved over time and a significant majority reported full recovery. At 8 months, people with Long COVID had persistent inflammation and immune dysfunction, with high levels of type I IFN (IFN-β), type III IFN (IFN-λ1), activated plasmacytoid dendritic cells (pDCs), activated monocytes, and dysregulated proportions of CD4 and CD8 T cells. Long COVID symptoms and most of these abnormalities typically resolved by 24 months. Recovery rates correlated with serum levels of Pentraxin-related protein 3 (PTX3), C reactive protein (CRP), various interferons, and platelet counts, based on machine learning; however, the levels of each of these were within the normal range. Long-term follow-up was challenging due to reinfections and varying vaccination status and timing.

Tim Henrich, of the University of California San Francisco, presented "Imaging the Immune Response to SARS-CoV-2 Infection in Long COVID." Henrich's team has reported on a novel radiopharmaceutical agent [18]F-AraG, a highly selective tracer that allows for anatomical quantitation of activated T lymphocytes throughout the body.[6] Henrich showed that tracer uptake in the post-acute COVID group, which included those with and without Long COVID symptoms, was significantly higher compared to pre-pandemic controls in many anatomical regions, including the brain stem, spinal cord, bone marrow, nasopharyngeal and hilar lymphoid tissue, cardiopulmonary tissues, and gut wall. Activated lymphocytes were higher among those imaged closer to the time of acute infection but still noticeably present among those imaged 2.5 years after acute infection. The connection to Long COVID symptoms remains to be defined, as activated lymphocytes were common even among those who fully recovered from infection.

Jennifer Liu presented "Sex Differences in SARS-CoV-2 during Acute and Post-acute Infection in a Mouse Model of COVID-19," in which she showed that male mice experienced greater acute morbidity and had lower pulmonary immune activation than females, despite similar viral loads. During the post-acute phase, at 6 and 12 weeks post-infection, while males and females both showed evidence of anosmia, females experienced greater memory deficits and neuropsychiatric impairment consistent with the neurologic phenotype of Long COVID.

Brent Appelman presented “Muscle Abnormalities Contribute to Post-Exertional Malaise in Long COVID” reported that post-exertional malaise was associated with mitochondrial dysfunction and amyloid deposits in the skeletal muscle. The muscle also showed evidence of severe myopathy with atrophy and increased necrosis after exercise.

VASCULAR DISEASE

In the session “Vascular Disease” (Chairs: **Karl Morton** and **Jane Mitchell**), scientists focused on endothelial dysfunction, possibly related to chronic thrombotic endothelialitis, as a potential contributor to PASC. Given the role of the vascular system in delivering oxygen and nutrients to tissues, dysfunction of this system could explain the vast array of symptoms, affecting nearly every organ system in the body.

Resia Pretorius, of Stellenbosch University, South Africa, kicked off the session with the talk “Microclots and Platelet Hyperactivation as Key Pathologies in Long COVID.” She argued that Long COVID is a chronic thrombotic endotheliitis, at least in some people. Microclots form when platelets interact with circulating inflammatory molecules, forming a microscopic clot. Pretorius has reported on this phenomenon in other chronic autoimmune and autoinflammatory diseases, including lupus, rheumatoid arthritis, Parkinson’s disease, Alzheimer’s disease, and diabetes. In the case of COVID, the S1 spike protein interacts directly with fibrinogen to induce microclots and platelet hyperactivation, which then in turn causes widespread endotheliitis that might persist even after the virus is cleared. These microclots contain fibrin, amyloid, hyperactivated platelets, and possibly inflammatory cytokines, and are resistant to degradation.[11] Pretorius hypothesized that anticoagulant therapy may improve symptoms, but this will need to be studied rigorously in randomized clinical trials given concerns about safety. The advance leads the way as a novel approach that might be considered in treating other chronic inflammatory diseases where microclotting is identified.

Jane Mitchell, of Imperial College London, expanded on this theme of endotheliitis in her talk “COVID19 Brings Endothelitits and Systemic Vascular Manifestations of Infection into the Spotlight.” Mitchell noted that endothelliitis induced by acute COVID-19 impairs endothelial function, which regulates vasodilation and vasoconstriction across all organ systems. This impairment lingers at least 3–4 weeks after initial infection, but whether it persists longer or to a greater degree in those who develop Long COVID is unknown. Mitchell suspects that endothelitis may contribute to increased incidence of cardiovascular events post-COVID.

Avi Nath, of the National Institute of Neurologic Diseases and Stroke, National Institutes of Health, presented “Neuroinflammatory Syndromes and Microvascular Disease with COVID-19,” during which Nath discussed how autopsies of acutely infected patients who experienced sudden death with minimal respiratory symptoms revealed the presence of microclots, activated platelets, and microinfarcts in the brain.[12] Nath suggested that an antibody-mediated breakdown in the blood–brain barrier due to COVID-related inflammation with perivascular macrophage infiltration and associated neuronal injury may be central to the development of these brain-specific abnormalities. These are extreme cases

but if similar underlying pathology could be to blame for “COVID brain” in acute infection and brain fog in people with Long COVID experiencing both short- and long-term cognitive symptoms.

In the short talks session, **Massimo Nunes**, of Stellenbosch University, South Africa, presented “Long COVID and ME/CFS: Shared Dysregulation of Coagulation, Complement Machinery, and the Endothelium Revealed by Data Independent Acquisition of LC-MS/MS Analysis of Plasma.” Nunes indicated that Long COVID vascular pathology is also observed in ME/CFS. Roughly, half of their 30 participants with ME/CFS exhibited hypercoagulability and hyperactive platelets, similar to those with Long COVID. Proteomic analysis revealed the upregulation of prothrombotic pathways involving three key coagulation system proteins: thrombospondin-1, platelet factor 4, and protein S, as well as differences in complement C1s, complement C9, ficolin-3, lactotransferrin, and protein S100-A9.

Kailin Yin, of the University of California, San Francisco, presented “T cell dysregulation, inflammation and an uncoordinated adaptive immune response to SARS-CoV-2,” which highlighted work using multi-omics approaches. Individuals with Long COVID harbored increased levels of SARS-CoV-2 antibodies, exhausted SARS-CoV-2-specific $CD8^+$ T cells, and $CD4^+$ T cells poised to migrate to inflamed tissues.[13] T and B cell coordination is impaired, which could lead to immune dysregulation, systemic inflammation, and clinical symptoms.

Lavanya Visvabharathy, of Northwestern University, presented “Mild SARS-CoV-2 Infection Promotes Autoantibody Production in Long COVID Despite Vaccination.” Visvabharathy showed that patients with primarily neurologic symptoms of Long COVID vigorously produce autoantibodies commonly found in systemic lupus erythematosus and inflammatory myopathies, such as anticytokine, antineuronal, and antinuclear autoantibodies. The presence and level of these autoantibodies was correlated with the severity of cognitive dysfunction and neurologic symptoms and is not impacted by COVID vaccination.

Finally, **Jon Izquierdo-Pujol**, of IrsiCaixa AIDS Research Institute, presented “PBMC Immunophenotyping, Plasma Inflammatory Profile and Antibody Levels of Children with Long COVID.” Izquierdo-Pujol ▫ s team is following a cohort of more than 200 children and adolescents with Long COVID,[14] finding higher numbers of memory B cells ($IgA^+CD21^+CD27^+$ and $IgA^+CD21^-CD27^-$) and lower levels of IgG and IgA in children with Long COVID. A less mature immune system in children may lead to unique immune signatures, highlighting the importance of continuing to include children in research studies to identify mechanisms of PASC.

ORGAN-SPECIFIC SYNDROMES

The session “Organ-Specific Syndromes” (Chairs: **Braeden Charlon** and **Mitch Miglis**) explored how post-acute sequala of COVID (PASC) has lasting impacts on different systems and processes throughout the body, from the autonomic nervous system to metabolism.

Many of these syndromes remain poorly documented clinically, and researchers are working to unravel their pathology, prevalence, and susceptibility to these various manifestations of PASC.

Mitch Miglis, of Stanford University, presented "Dysautonomia and POTS." He compared common symptoms in Long COVID with previously documented conditions of autonomic dysfunction and postural orthostatic tachycardia syndrome (POTS), which are most common in females and in the past were often dismissed as psychosomatic. Cardiovascular abnormalities like blood volume dysregulation, blood pressure dysregulation, baroreflex impairment, high CO_2 and alkalinity in the blood, and many others are common to these syndromes and Long COVID.

John Wood, of the Children's Hospital of Los Angeles, presented work in collaboration with The RECOVER Post Acute Sequelae of SARS-CoV-2 (PASC) Pediatric Cohort Study in the talk "Brain Fog in Children." Wood described plans to utilize functional magnetic resonance imaging (MRI) to examine blood flow and metabolism in the brain, which might account for many of the neurologic symptoms of Long COVID in children, such as trouble concentrating. Wood's team will also characterize soluble factors in the blood and within exosomes that may traverse the blood-brain barrier to drive neuroinflammation and impair cognitive performance.

Clifford Rosen presented "Metabolic Aspects of PASC: The Impact of high-densitity lipoprotein (HDL) and Lipid Peroxides," which showed how acute SARS-COV-2 infection drives mitochondrial dysfunction, lipid and adipocyte dysregulation, lipid toxicity (which drives inflammation), and insulin resistance. These acute impacts persist and even worsen over time, and those with PASC exhibit hyperlipidemia, insulin resistance, and even type 2 diabetes. Acute and lasting metabolic impacts can be traced back to HDL levels; reduced HDL during acute infection is a predictor of Long COVID, and HDL levels are inversely correlated with symptoms burden in Long COVID patients.

In the short talk session, **Matthew Durstenfeld** presented "Cardiopulmonary Exercise Testing and Heads up Tilt Table Testing in Cardiopulmonary PASC," which described reduced exercise capacity (peak VO_2) and orthostatic symptoms in cardiopulmonary exercise testing in those with PASC. IL-6 inflammatory cytokine levels were linearly correlated with reduced exercise capacity and inability to increase heart rate adequately during exercise (chronotropic incompetence), which may be a particular interesting clue as to underlying mechanisms related to inflammation or autonomic dysfunction.[15] Durstenfeld also presented tilt table results indicating that many people with Long COVID and orthostatic symptoms do not meet diagnostic criteria for POTS.[16]

Finally, **Kristin Sauter** presented "Effect of Obesity on Long-term Response to SARS-CoV-2 Infection in Rhesus Macaques," which showed data from her team on developing a primate model to study the impact of obesity on the outcomes up to 6 months after SARS-CoV-2 infection. While these studies are still in progress, Sauter said that they have demonstrated abnormalities in glucose metabolism during and after acute infection among obese compared to lean nonhuman primates, consistent with human studies.

LESSONS LEARNED FROM OTHER POST-INFECTIOUS COMPLICATIONS

The session “Lessons Learned from other Post Infections Complications” (Chair: **Leonard Calabrese**) revealed insights from ME-CFS, post-Lyme, EBV, and other longstanding diseases that had been neglected and dismissed as psychosomatic until Long COVID appeared on the scene to validate these patients’ experiences. Much can be learned from those who have been studying these diseases for decades, despite limited funding and public interest. There is hope that the surge in Long COVID funding will finally provide some insights and relief for patients suffering with these related conditions.

Lisa McCorkell, co-founder of Patient-Led Research Collaborative, provided a unique perspective in her talk “Patient Insights into Long COVID and Associated Conditions” by discussing the patient experience of Long COVID, its impact on daily life, and the minimal rates of recovery. Mobilizing the patient community, her group has published six open-access papers thus far, revealing important insights into symptoms, comorbidities, and quality of life measures over time.[17–19] For example, she notes overlooked impacts on female reproductive conditions like menstrual cycles, menopause, and other gynecologic pathologies.[20] McCorkell argued that research in partnership with patients prioritizes agendas that directly serve patients, respond to patient community insights, and ensure research is reflective of lived experiences with impact-driven incentive structures. She also believes that cross-illness research that builds off and learns from existing research on infection-associated chronic condition and similar conditions like ME/CFS and POTS is needed. McCorkell argued that it is critical to have a representative cohort of patients in studies that is reflective of populations most impacted by Long COVID and does not rely on a positive COVID test for inclusion given the lack of current testing. She stressed the importance of accounting for illness in study design to ensure relevance, such as sampling during post-exertional malaise, separating phenotypes/symptom clusters in analysis, considering illness duration, what treatment and supplements participants are taking, and other factors that can influence patient symptoms and likely biological states.

Karl Morten, of Oxford University, presented “Developing a Blood Cell-Based Diagnostic Test for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome Using Peripheral Blood Mononuclear Cells.” The lack of a specific diagnostic test can be a barrier for patients with this debilitating disease. Looking for blood biomarkers that might be used as a diagnostic, Morten’s team found some plasma metabolites that are 400–40,000 times higher in patients with ME/CFS than those without this syndrome. In addition, he has developed a novel Raman spectroscopy approach which examines peripheral blood mononuclear cells (PBMCs), and diagnoses ME/CFS with 90% accuracy based on impaired cellular bioenergetics and dysfunctional mitochondrial profiles.[21] These tests provide diagnostic possibilities and may point to metabolites and metabolic pathways that play a role in the disease pathology.

William Robinson, of Stanford University School of Medicine, presented “EBV as a Driver of Chronic Disease, including Long COVID.” He noted that EBV reactivation is common during acute infection and is associated with the development of Long COVID, particularly neurologic symptoms. EBV reactivation may be the underlying trigger for autoimmunity,

due to molecular mimicry between the virus and human proteins. Specifically, EBV encodes a viral protein that is similar to the human myelin protein GlialCam, such that antibodies produced against the virus cross-react with human oligodendrocytes and astrocytes, causing demyelination and, in some cases, multiple sclerosis.[22] The same region also encodes multiple molecular mimics, including those that may be responsible for inducing lupus and rheumatoid arthritis. Moderna is currently developing vaccines against EBV infection and reactivation, which may be an important tool to control the development of these long-term complications after EBV.

Adriana Marques, of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, presented work in the talk "Symptoms after treatment of Lyme disease," showing that many Lyme disease symptoms overlap with Long COVID symptoms. While most patients will recover with recommended antibiotic therapy, some develop persistent or intermittent nonspecific symptoms after therapy. Fatigue, joint and muscle pain, difficulties with memory and concentration, and sleep and mood disturbances are the most common post-treatment Lyme disease symptoms. The mechanisms underlying these symptoms are unknown, and it is likely that different factors play a role in an individual case.[23] There is hope that Long COVID research could help to identify the mechanisms underlying persistent symptoms after Lyme disease and facilitate the development of diagnostics and treatments for this underserved community as well.

Sarah Leist, University of North Carolina at Chapel Hill, presented "A Model of Persistent Post SARS-CoV-2 Induced Lung Disease in Mice for Target Identification and Evaluation of Therapeutic Strategies." Under the mentorship of Ralph Baric, Leist, Kinny Dinnon, and their team have adapted the SARS spike protein to bind to mouse rather than human receptors to create an infection model that recapitulates acute and chronic lung pathology, inflammation, and fibrosis seen in humans. Using this model to test treatments, they find that pathology can be prevented with early intervention antiviral molnupiravir and partially with the antifibrotic nintedanib. It can also be used to examine host genetic contributors to different disease outcomes in short- and long-term studies.

Braeden Charlton, of Vrije Universiteit, Netherlands, presented "Skeletal Muscle Structure and Function Contribute to Exercise Intolerance in Long COVID Patients." Examining muscle biopsies in people with Long COVID, Charlton's team found decreased type I fibers, which are required for endurance, and increased glycolytic type IIx fibers, which can lead to lactic acid build up, muscle fatigue, and exercise intolerance. Capillary density was not reduced, indicating that reduced capillarization (which would contribute to tissue hypoxia) does not explain the reduction in exercise capacity. Charlton's team also found amyloid deposits in the skeletal muscle but not in capillaries, suggesting that amyloid deposits do not block vasculature resulting in tissue hypoxia. They did, however, find evidence of decreased mitochondrial respiration.[24]

DISEASE MANAGEMENT WORKSHOP

The second workshop, "Disease Management" (Chairs: **Rasika Karnik** and **Rebecca Hamlin**), explored clinical approaches to diagnose and potentially treat Long COVID.

Vashi Negi of Purdue University presented “A BSL-2 Chimeric System to Screen SARS-CoV-2 E Protein Ion channel Inhibitors” for efficient high-throughput screening of potential antivirals against this conserved viroporin target.

James Baraniuk of Georgetown University presented “Naltrex-one Restores Impaired Transient Receptor Potential Melastatin 3 Ion Channel Function in NK Cells From Post COVID-19 Condition Patients,” highlighting how naltrexone can restore impaired TRPM3 function in natural killer cells of ME/CFS and Long COVID patients.[25]

Rebecca Skalsky of Oregon Health & Science University presented “Virus-Encoded MicroRNAs as Potential Biomarkers Of SARS-CoV-2 Infection States,” reporting the prevalence of EBV microRNAs in the blood of patients with moderate to severe SARS-CoV-2 infection. This suggests that EBV reactivation could contribute to acute or long-term symptoms.

Rebecca Hamlin of Stanford University presented “Investigating the Immunologic Basis of Long COVID in a Prospective Clinical Cohort,” showing specific immune transcription modules and inflammatory pathways that were associated with Long COVID symptoms.

Tongcui Ma of the Gladstone Institutes presented “Post-acute Immunological and Behavioral Sequelae in Mice after Omicron Infection.” Using a novel mouse model of Long COVID, Ma’s team found profound immune perturbations in the lung after the resolution of acute Omicron infection. Notably, these outcomes could be prevented with a COVID vaccine prior to infection.

Brian Walitt, of the National Institute of Neurologic Diseases and Stroke, reported on “Premorbid Conditions Predict Onset of New Neurological Manifestations and Long-term Disability Following SARS-COV-2 Infection,” indicating that prior neurologic but not psychiatric disease impacts the severity of neuro-PASC syndromes when compared to persons without any premorbid conditions.

Monika Haack, of Harvard Medical School, presented “Pain in PASC—The Role of Sleep Disturbances.” She reported that people with PASC have poor sleep quality, difficulties with sleep onset, insomnia, and increased time in wake and light sleep, which may impact inflammatory pathways that contribute to greater pain facilitation and decreased pain-inhibition, particularly in females.

Finally, **Sindhu Mohandas**, of the University of Southern California, presented “Exploring the Clinical Spectrum of Pediatric Long COVID and the Impact of COVID-19 Vaccines on Disease Course,” reporting that Long COVID symptoms are also seen in children of all ages, particularly adolescents, and vaccination was associated with improvement of symptoms in 43% of the patients.

Together, these talks highlighted biological pathways on the spectrum between acute and Long COVID, which might be targeted therapeutically to prevent or reverse Long COVID. Repurposing already existing and FDA-approved drugs that target these pathways would vastly accelerate the path to patients but need to be investigated.

DIAGNOSTICS, MANAGEMENT, AND THERAPEUTICS

The session on “Diagnostics, Management, and Therapeutics” (Chairs: **Matthew Durstenfeld** and **Lisa Gralinksi**) looked at translating mechanistic insights into clinical advances for patients.

Lisa Gralinski, of the University of North Carolina at Chapel Hill, presented “Complement Signaling Mediates Both Acute and Chronic Coronavirus-induced Lung Injury.” She noted that complement 3 knockout mice are protected against pulmonary fibrosis, as are mice treated with antibodies blocking the complement pathway (vilobelimab), which may provide an early intervention that would prevent long-term lung damage in patients.[26]

Harlan Krumholz, of Yale University, presented “Novel, Digital, Participant-centric Approaches to Long-COVID Research.” These include strategies to implement digital, decentralized, and democratized approaches to clinical research involving partnership with trial participants to engage them as equals and give them a voice in the process. Features include virtual townhall meetings between patients and clinical researchers for open dialogs and questions, which yield improvements in study design that benefit both the science and the patient experience. Along with convenient access to multi-modal, near real-time data, this creates value for study participants and provides a meaningful, respectful, and open relationship with investigators that overall improves the efficiency of the clinical trial process. He presented data from their digital observational study, LISTEN, and from their digital, decentralized platform trial of Paxlovid (Yale Pax LC Trial).

Finally, **David Systrom**, of Harvard University, presented “Neurovascular Dysregulation During Exercise,” highlighting insights from invasive cardiopulmonary testing that indicate preload failure, low VO_2 max, and failure in vascular tone during exercise, in both ME/CFS and Long COVID patients.[27] Venous oxygen content remains high in these syndromes, indicating that oxygen that is present is not being utilized, potentially due to mitochondrial dysfunction. Neurovascular dysregulation and in particular autonomic neuropathy may play a role; treatment with pyridostigmine, which stokes autonomic nerve function, improves cardiac output and VO_2 max in people with ME/CFS. This may provide much-needed relief from exercise intolerance, a debilitating problem for those with Long COVID, ME/CFS, POTS, and other dysautonomia conditions.

CONCLUDING REMARKS FROM THE ORGANIZERS

At the close of the meeting, **Blish** and **Pretorius** highlighted the value and synergy of bringing together people from different fields, including basic scientists and clinicians across specialties spanning pulmonology, vascular biology, immunology, virology, metabolism, neurology, and more, to spark new insights and collectively unravel this complex, multifactorial, and heterogenous disease. In particular, they recognized the importance of subgrouping patients by primary symptoms to understand pathology and identify appropriate treatments that may not respond to a one-size-fits all approach. Blish also noted that the breadth of studies we have been introduced to is incredible, especially the correlations with other diseases and inclusion of pediatric Long COVID, citing diverse talks ranging from

vascular disease and endothelial dysfunction to fibrosis, mitochondrial function, adipose tissue, lipid profiles, neurological deficits, Lyme disease, and many other topics. They concluded the meeting by emphasizing that to move the field forward scientifically and clinically “we need to hold hands; we need to work together.” The organizers note that this is what the Keystone Symposia conference allowed the participants to do: to join forces as a community and to forge new cross-disciplinary collaborations that hopefully will impact not only Long COVID but also related diseases such as ME/CFS, which have long plagued patients and perplexed doctors. The inclusion of patient advocates and patient perspectives is essential in this process and was an invaluable addition to this meeting. The community formed at the conference was inspired to carry the torch forward to provide answers and treatments for those suffering the debilitating disease Long COVID.

DISCUSSION

The Keystone symposium “Long COVID and Post-Acute Sequelae of SARS-CoV-2 (PASC) Pathogenesis and Treatment” highlighted several key themes and discoveries that have advanced our understanding of Long COVID and PASC. The goal of the meeting was to help people with Long COVID and similar conditions like ME/CFS and chronic Lyme disease, which have been neglected in the past. The RECOVER Initiative offers the largest platform to study Long COVID, yet implementation takes time. These efforts will build on preliminary findings by many groups around the world studying various cohorts and aspects of PASC, many of whom presented their findings at this meeting. Several themes have emerged in the biology, with inflammation and other immune alterations being the most common focus (Figure 2). Other factors include viral persistence, latent virus reactivation, tissue damage, and dysfunction, including microclotting, endothelial dysfunction, mitochondrial dysfunction, metabolic changes, pulmonary function changes, hormonal changes, and autonomic dysregulation. Ongoing studies are needed to identify which of these processes may be initiated during acute infection and simply persist long-term with chronic consequences, or if they arise later after the initial infection subsides, which may dictate treatment and prevention strategies. Similarly, given the extensive array of dysfunctions being documented, on molecular to organ and system-wide levels, identifying which issues are the origins of disease and driving disease progression versus which are downstream effects will be critical to determine the best targets to treat and cure the disease, rather than just manage individual symptoms.

What critical next steps are needed to take these insights from the bench to the bedside? First, there are many discoveries demonstrated in single cohorts or by single research groups that need to be independently replicated to validate major findings (Table 2). Second, the development of cellular and animal models of Long COVID and PASC, which has already begun, will help accelerate discovery science, identification of contributing mechanisms, and testing of potential therapies. Third, there is no substitute for randomized, placebo-controlled clinical trials. Small, mechanism-oriented studies are urgently needed to test specific hypotheses about causative targets and potential treatments. There is much interest in repurposing already approved drugs that act on identified targets, which would vastly accelerate the timeline to reach patients. However, this pool of drug candidates will need to be narrowed significantly before larger trials are undertaken. Ultimately, adequately powered

clinical trials that can definitively test the efficacy and safety of potential treatments will be needed for regulatory approval.

There was a call for closer collaboration between people with lived experiences, including patients and their doctors, with basic scientists, clinical researchers, and other key stakeholders to fill the needs of the patient community with appropriately focused research, and to derive insights into identify treatments for Long COVID that might otherwise be overlooked. This disease provides a unique opportunity to forge these inclusive collaborations, and to model a new way of engaging with patient communities to accelerate biomedical research advances and impacts.

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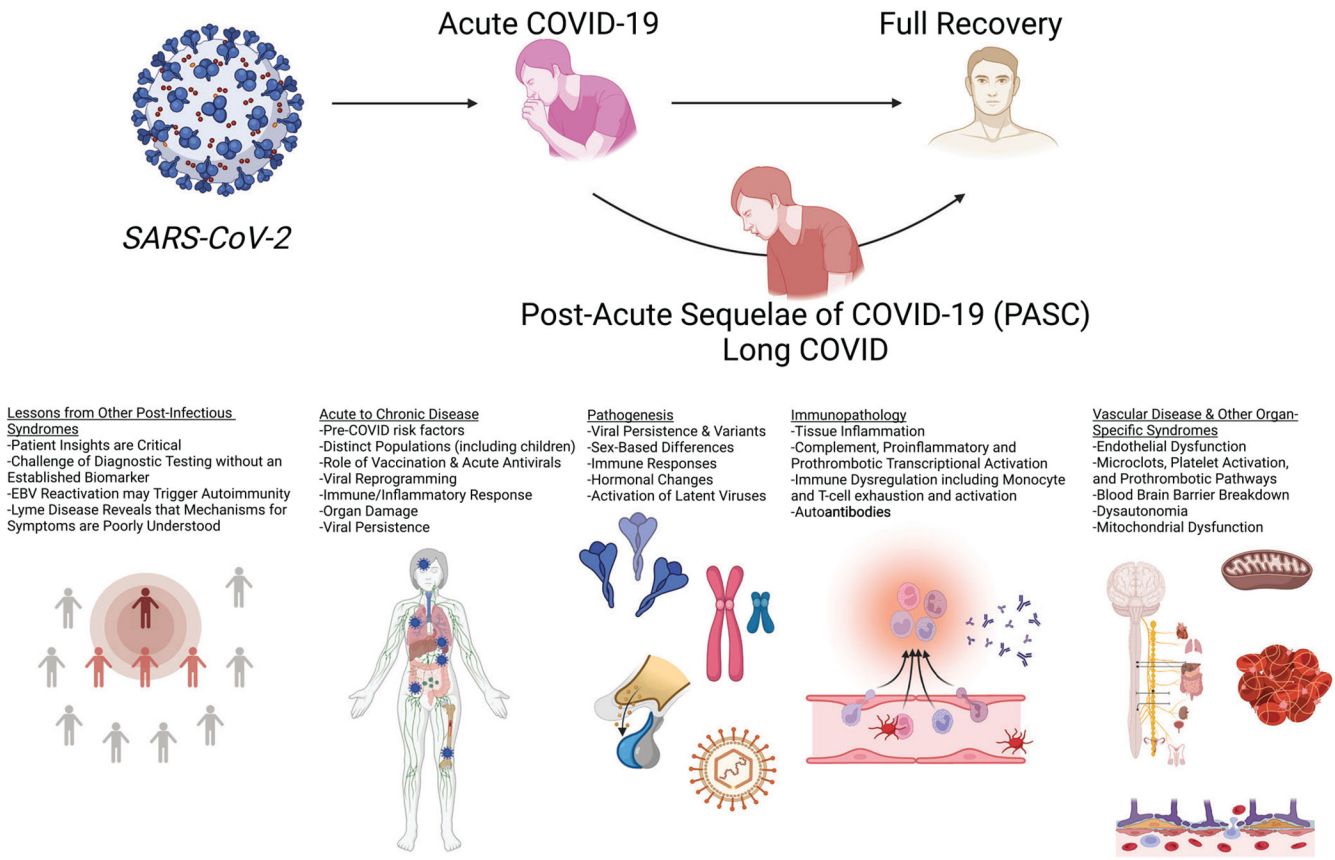

FIGURE 1.

Key themes from the Keystone Symposium on Long COVID and PASC pathogenesis and treatment. Figure made with Biorender.com. Adapted with permission from M. J. Peluso and S. G. Deeks (see Ref. 28). Early clues regarding the pathogenesis of Long COVID.

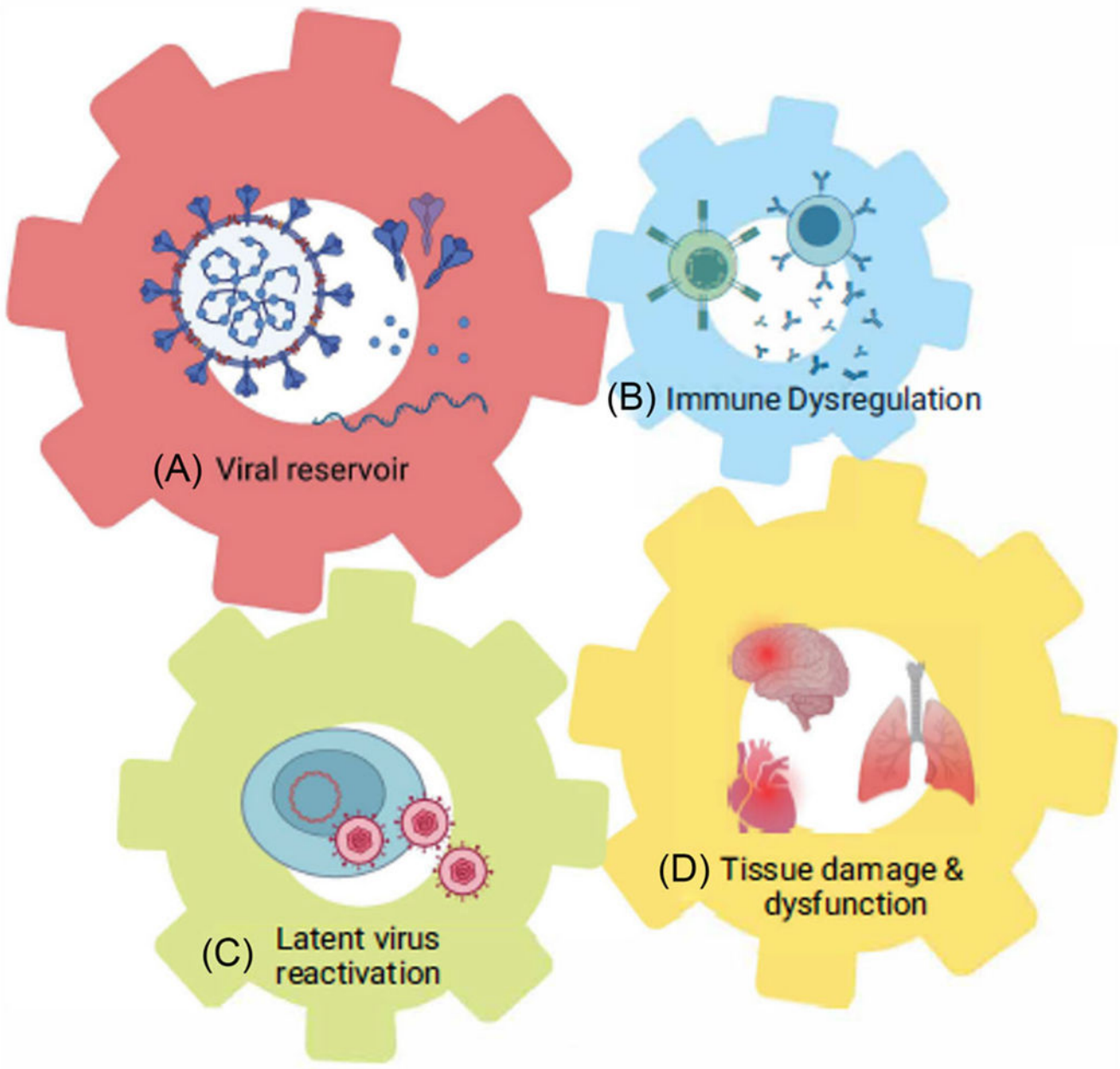

FIGURE 2.

Potential pathogenic mechanisms of PASC/Long COVID. Key pathogenic themes from the Keystone Symposium. Adapted with permission from A. Iwasaki and D. Putrino (see Ref. 29). Why we need a deeper understanding of the pathophysiology of Long COVID.

TABLE 1

Aims of the Keystone Symposium on Long COVID and post-acute sequelae of SARS-CoV-2 (PASC): pathogenesis and treatment.

	Description
1	To present and discuss the most current and cutting-edge results regarding the pathogenesis and treatment of Long COVID and post-acute sequelae of SARS-CoV-2 (PASC).
2	To address and resolve current controversies that may slow progress in this field of research and development.
3	To consider the future directions of the field, including the identification of the most pressing unanswered questions, the areas needing more focus, and those that would benefit from new approaches and methodologies.
4	To stimulate collaborations by providing a setting conducive to focused, intense discussion among scientists with broad interests at the interface of viral infection and human health and disease, including engagement with industrial partners interested in targeting new cells and molecular pathways.
5	To provide a forum for trainees and new investigators including underrepresented members of this community to learn about current work in the field, to present their own work, and to network with established investigators.

TABLE 2

Key priorities to identify treatments for people with Long COVID.

Replication of major mechanistic findings including in the RECOVER Initiative
Disentangling pathogenic, targetable causes of disease versus downstream effects
Development of cellular and animal models
Randomized, placebo-controlled clinical trials • Test specific mechanistic hypotheses and identify candidates for larger trials • Definitively test the efficacy and safety of potential treatments for regulatory approval
Closer collaboration between people with lived experience, clinicians, basic scientists, clinical researchers, and other stakeholders