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LONG COVID: BIOMARKER AND THERAPEUTIC DISCOVERY

1180 EAST ELLSWORTH ROAD · ANN ARBOR, MI 48108 · (800) 364-9897 · WWW.CAYMANCHEM.COM

In It for the Long Haul: Research Tools for Long COVID Syndrome

Olivia L. May, Ph.D., Cayman Chemical

Novel coronavirus (COVID-19) symptoms can persist in an estimated 10% of patients long past recovering from the worst impacts of acute infection and testing negative for SARS-CoV-2. Inconsistencies in symptoms, patient phenotypes, and risk factors make it difficult to pinpoint the exact cause of Long COVID, otherwise known as post-COVID syndrome (PCS) or post-acute sequelae of SARS-CoV-2 infection (PASC). Large-scale research projects and population studies are now looking at the reported symptoms to define Long COVID and to understand its long-term effects and how it can be treated. The U.S. National Institutes of Health is investing $1.15 billion towards Long COVID research to generate basic understanding of the underlying causes of these prolonged symptoms that could lead to effective prevention and treatment of the syndrome.

Multi-System Disease

The constellation of symptoms associated with Long COVID range from serious sequelae to nonspecific clinical manifestations that require a whole-patient perspective. The most common symptoms involve the pulmonary, cardiovascular, and nervous systems and can be grouped into three types of complaints: exercise intolerance, autonomic dysfunction, and cognitive impairment. But many additional symptoms and disease associations have been cataloged in nearly every organ and regulatory system.

Mental Health
- Anxiety
- Depression
- Sleep problems
- Substance abuse

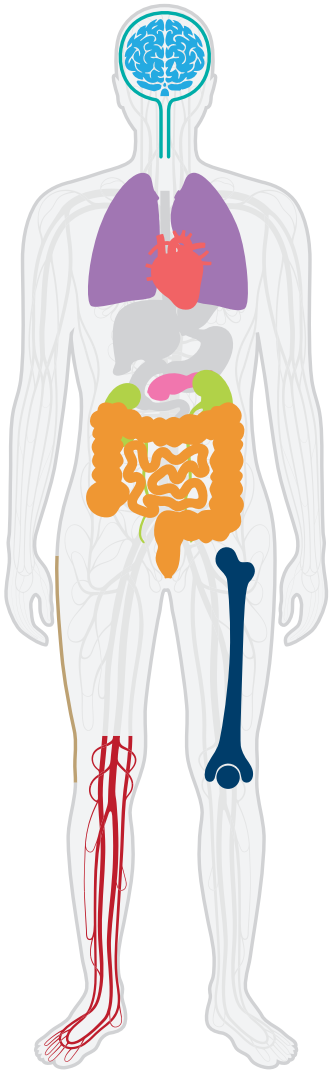
Respiratory System
- Cough
- Low blood oxygen
- Shortness of breath

Kidney
- Acute kidney injury
- Chronic kidney disease

Gastrointestinal
- Diarrhea
- Acid reflux
- Constipation

Skin Disorders
- Rash
- Hair loss

Blood Disorders
- Anemia
- Blood clots



Nervous System
- Stroke
- Headaches
- Memory problems
- Loss of smell and taste

Cardiovascular
- Arrhythmia
- Palpitations
- Heart failure
- Acute coronary disease

Metabolic/Endocrine
- Obesity
- Diabetes
- High cholesterol

Musculoskeletal
- Joint pain
- Muscle weakness

General
- Fatigue
- Malaise
- Mitochondrial dysfunction

An umbrella of symptoms and comorbidities have been documented in relation to Long COVID. Cayman carries thousands of products to support scientists examining this disease. Browse them all by viewing the full PDF at www.caymanchem.com/LongCOVIDimpact.

Cues from POTS and ME/CFS

Because this disease is so new, specific tests for lasting coronavirus symptoms are lacking, but a road map for treatment options has begun to be drawn from the current understanding of other disabling and complex health conditions with overlapping symptoms that can arise suddenly post viral infection. In fact, the new-found prevalence of Long COVID has brought fresh awareness to these other conditions along with new diagnosed cases.

One such example is postural orthostatic tachycardia syndrome (POTS), a blood circulation disorder that presents as a type of dysautonomia with profound fatigue, brain fog, headaches, chest tightness, and rapid heartbeat, especially when standing up from a prone position.[1] Patients with POTS tend to have a lower-than-normal level of plasma and red blood cells. Medications that have proven to be effective at treating POTS include nervous system depressants, cholinesterase inhibitors, hyperpolarization-activated cyclic nucleotide-gated channel blockers and beta-blockers to reduce heart rate, α_1-adrenergic agonists and somatostatin mimics to stimulate vasoconstriction and increase venous return, α_2-adrenergic receptor agonists to reduce hypertension, antidiuretics and corticosteroids to increase blood volume, hormones to stimulate the production of red blood cells, and selective serotonin uptake inhibitors to control blood pressure and heart rate through central serotonin availability. Each of these must be tailored to an individual's needs since some may exacerbate a certain set of symptoms while relieving others.

Another example often triggered by viral infection is myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS).[2] ME/CFS is characterized by generalized fatigue that can lead to post-exertional malaise. Other symptoms include neuropathic pain, sleep abnormalities, cognitive dysfunction, orthostatic intolerance, and gastrointestinal problems. Current therapeutic options are largely limited to palliative care and cognitive management because the mechanistic basis of the disease is poorly understood. However, some symptoms can be treated or managed with antidepressants, psychostimulants, analgesics, sleep medications, and the drugs used to treat orthostatic intolerance mentioned above.[3]

Underlying Pathophysiology

For effective treatments to be developed for Long COVID, the molecular basis of the syndrome will first need to be understood. There are several leading theories for why COVID long haulers develop the syndrome.

Four Theories for Underlying Pathophysiology

Theory 1: A persistent latent SARS-CoV-2 infection

Theory 2: An autoimmune disorder evolved from the initial SARS-CoV-2 infection

Theory 3: An excessive immune response to SARS-CoV-2 infection with extreme inflammation

Theory 4: Impaired mitochondrial dynamics and energy production

Latent Infection

One theory is that the disease is a consequence of a persistent latent SARS-CoV-2 infection with failure to completely clear the pathogen. In cases of ME/CFS triggered by *Chlamydophila pneumoniae*, antibiotics helped improve symptoms.[4] However, ME/CFS observed after Epstein-Barr virus, dengue virus, Ebola virus, West Nile virus, or Chikungunya virus has not been successfully treated with existing antivirals for the most part.[5] One notable exception is the RNA polymerase inhibitor valganciclovir, which has been used to address elevated serum IgG levels for human herpesvirus 6 and Epstein-Barr virus in ME/CFS patients to improve fatigue and cognitive symptoms.[6]

As part of developing suitable antivirals designed to clear a latent virus to provide therapeutic relief in the case of Long COVID, researchers will also need to identify lingering, non-replicating SARS-CoV-2 products in biological samples and track neutralizing antibody levels over time in response to infection. More work will also be needed to establish the expression and localization of the angiotensin-converting enzyme 2 (ACE2) receptor throughout the body, as this is where SARS-CoV-2 enters host cells. Strategies to clear the latent viral infection could also potentially be controlled through scheduled vaccination or the delivery of monoclonal antibodies.

Flip to pages 4-5 to find Cayman's targeted compound screening libraries to explore potential antiviral therapies, tools to identify SARS-CoV-2 viral products and neutralizing antibodies, and tools to explore the interaction of SARS-CoV-2 with the human ACE2 receptor.

Learn more about the tools Cayman offers for research on the delivery of vaccines or therapeutic drugs using lipid nanoparticles on page 6.

Autoimmune Response

An alternative hypothesis is that Long COVID is an autoimmune disorder evolved from a misdirected or aberrant immune reaction to the initial SARS-CoV-2 infection. Immunological changes could come in the form of errant macrophages, impaired natural killer (NK) cell function, abnormal B cell activation (*e.g.*, antiphospholipid autoantibodies), diminished T cell counts, a reduced type 1 interferon (IFN) response (*e.g.*, developing neutralizing autoantibodies against IFNs), altered cytokine levels, or a gut microbiome imbalance. Identifying biomarkers associated with Long COVID will be important for understanding how to develop therapeutics to address those key effectors (*e.g.*, immunosuppressants, stimulants of NK cell function, immune adsorption/IgG depletion to remove autoantibodies). In cases involving dysautonomia, the antibodies produced after a SARS-CoV-2 infection may attack the autonomic nervous system causing nerve damage and disrupting its ability to regulate blood flow to the brain and muscles.

Activation of IFN signaling, which is mediated by the STING pathway and signaling through certain toll-like receptors (TLRs), is crucial for innate defense against viral infections. In ME/CFS patients, the TLR3 agonist rintatolimod is specifically being developed to increase NK cell function and is in an expanded access clinical trial program to treat fatigue-like symptoms of COVID long-haulers.[7,8] Other immune modulating compounds that have been investigated for the treatment of ME/CSF and may have benefit for Long COVID include γ-globulin, the anti-IL-6 antibody anakinra, the anti-CD20 B cell-depleting antibody rituximab, and the T regulatory cell-eliminating/IFN-inducing agent cyclophosphamide.[3]

Cayman offers a suite of innate immunity research tools to study the STING pathway and other pattern recognition receptor signaling pathways such as TLRs that can be viewed on pages 7-8.

Excessive Inflammation

Part of an excessive immune response to SARS-CoV-2 infection involves a disproportionate release of cytokines and extreme inflammation. The cytokine storm, characterized by increased levels of IL-2, IL-7, GM-CSF, CXCL10, CXCL20, CCL2/MCP-1, and TNF-α experienced by many COVID-19 patients, could be mediating a pattern of hyperinflammation that is associated with tissue damage or loss of cell function (*e.g.*, vasculitis, coagulopathy, endothelial dysfunction, neurological abnormalities) that could cause Long COVID symptoms.[2, 9-10] Inflammation and coagulation are two processes with considerable cross-talk, each driving the other towards detrimental pro-thrombotic and/or pro-inflammatory activation. Cytokine-mediated neuroinflammation could also be playing a role in causing fatigue and other neurological symptoms.

Inflammation is thought to be cleared by an active biochemical process that stimulates macrophage phagocytosis and efferocytosis and counters pro-inflammatory cytokine production through specialized pro-resolving lipid mediators (SPMs), such as resolvins.[11] Epoxyeicosatrienoic acids (EETs) can also stimulate the resolution of inflammation by promoting the production of pro-resolution mediators, such as lipoxins, and activating anti-inflammatory processes. EETs are rapidly metabolized by soluble epoxide hydrolase (sEH), but their levels can be stabilized with the use of sEH inhibitors. Both resolvins and EETs are known to diminish thrombosis and stimulate cytokine clearance and cellular repair. Thus, sEH inhibitors and resolvins may have a therapeutic role in alleviating symptoms of Long COVID.

Extensive infiltration of neutrophils, which extrude neutrophil extracellular traps (NETs) into the pulmonary capillaries of COVID-19 patients, is associated with fibrin deposition and vascular lesions and can serve as a scaffold for thrombogenesis that could lead to multiple system dysfunctions that are related to Long COVID symptoms.[12] The use of recombinant human DNase-I to degrade extracellular DNA associated with NETs is under investigation for improved blood flow and outcomes after experimental stroke, traumatic brain injury, and COVID-19-induced acute respiratory distress syndrome and may help address some of the deficits associated with Long COVID.[13,14]

The research tools Cayman offers to aid researchers in exploring the role of inflammation and thrombosis in Long COVID can be found on pages 9-13.

Mitochondrial Dysfunction

Increasing evidence suggests that SARS-CoV-2 takes over immune cell mitochondria, replicates within mitochondrial structures, and impairs mitochondrial dynamics leading to problems with energy production and normal cell death.[15] Mitochondria participate in an immune response to viral infection by engaging IFN signaling *via* retinoic acid-inducible gene I-like receptors (RLRs) and the mitochondrial antiviral-signaling protein (MAVS). Viruses can alter mitochondrial structure through fission and fusion to manipulate the IFN response or to prevent apoptosis, both of which benefit viral survival. These alterations can lead to poorer mitochondrial energy

production and oxidative stress. Metabolic disruption, increased mitochondrial damage, reductions in ATP production, and impaired oxidative phosphorylation are all associated with ME/CFS and are likely the case for Long COVID-19 patients as well.[3]

The mitochondrial modulating combination of NADH and coenzyme-Q_{10} (ubiquinol) can improve fatigue in ME/CSF patients.[16] Supplementing methylphenidate with various mitochondrial metabolites and antioxidants including acetyl-L-carnitine, α-lipoic acid, and N-acetyl-L-cysteine has been used to treat fatigue in severe cases of ME/CFS and may show benefit for similar symptoms associated with Long COVID.[17] Because a dysregulated pyruvate dehydrogenase complex may lead to mitochondrial deficits in these patients, investigators are exploring the use of pyruvate dehydrogenase kinase (PDHK) inhibitors to decrease expression of PDHKs that negatively regulate the complex and promote the conversion of pyruvate to lactate.[18] AMP-activated protein kinase (AMPK), which plays a key role in controlling metabolism, may also be impaired in these patients. Small molecule activators of AMPK, including the thiazolidinedione peroxisome proliferator-activated receptor (PPAR) agonists, stimulate mitochondrial biogenesis and have been explored as treatments for a variety of neurological diseases.[19] The oxidative stress created by dysfunctional mitochondria could be reversed through the use of antioxidants, which have also been shown to lessen fatigue.[20]

A wealth of tools that Cayman offers to study mitochondrial biology can be found on page 14.

Conclusion

While there is still a lot to learn about COVID-19 and its long-term effects, researchers' understanding is evolving by the day. Cayman aims to support the basic and drug discovery research needed to provide avenues for therapies and hope for people living with long-term COVID-19 effects.

Article References

1. Goldstein, D.S., Eldadah, B., Holmes, C., *et al. Circulation* **111(7)**, 839-845 (2005).
2. Islam, M.F., Cotler, J., and Jason, L.A. *Fatigue: Biomed. Health Behav.* **8(2)**, 61-69 (2020).
3. Toogood, P.L., Clauw, D.J., Phadke, S., *et al. Pharmacol. Res.* **165**, 105465 (2021).
4. Chia, J.K. and Chia, L.Y. *Clin. Infect. Dis.* **29(2)**, 452-453 (1999).
5. Richman, S., Morris, M.C., Broderick, G., *et al. Clin. Ther.* **41(5)**, 798-805 (2019).
6. Watt, T., Oberfoell, S., Balise, R., *et al. J. Med. Virol.* **84(12)**, 1967-1974 (2012).
7. Mitchell, W.M. *Expert Rev. Clin. Pharmacol.* **9(6)**, 755-770 (2016).
8. Clinical Trials Arena Company News (7 January 2021). Available from: https://www.clinicaltrialsarena.com/news/company-news/aim-doses-first-patient/
9. Mehta, P., McAuley, D.F., Brown, M., *et al. Lancet* **395(10229)**, 1033-1034 (2020).
10. Jarrahi, A., Ahluwalia, M., Khodadadi, H., *et al. J. Neuroinflammation* **17(1)**, 286 (2020).
11. Panigrahy, D., Gilligan, M.M., Huang, S., *et al. Cancer Metastasis Rev.* **39(2)**, 337-340 (2020).
12. Barnes, B.J., Adrover, J.M., Baxter-Stoltzfus, A., *et al. J. Exp. Med.* **217(6)**, e20200652 (2020).
13. Vaibhav, K., Braun, M., Alverson, K., *et al. Sci. Adv.* **6(22)**, eaax8847 (2020).
14. Earhart, A.P., Holliday, Z.M., Hofmann, H.V., *et al. New Microbes New Infect.* **35**, 100689 (2020).
15. Ganji, R. and Reddy, P.H. *Front. Aging Neurosci.* **12**, 614650 (2021).
16. Castro-Marrero, J., Cordero, M.D., Segundo, M.J., *et al. Antioxid. Redox Signal.* **22(8)**, 679-685 (2015).
17. Kaiser, J.D. *Int. J. Clin. Exp. Med.* **8(7)**, 11064-11074 (2015).
18. Rutherford, G., Manning, P., and Newton, J.L. *J. Aging Res.* 2497348 (2016).
19. Corona, J.C. and Duchen, M.R. *Free Radic. Biol. Med.* **100**, 153-163 (2016).
20. Davis, J.M., Murphy, E.A., Carmichael, M.D., *et al. Am. J. Physiol. Regul. Integr. Comp. Physiol.* **296(4)**, R1071-R1077 (2009).