About the Patient-Generated Hypotheses Journal

Welcome to the Patient-Led Research Collaborative's Patient-Generated Hypotheses Journal. The Journal compiles hypotheses to hasten the discovery of mechanisms and treatments for Long COVID and infection-associated chronic conditions like myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), postural orthostatic tachycardia syndrome (POTS), and mast cell activation syndrome (MCAS). People with Long COVID and associated conditions and caregivers of people with these conditions develop, write, select, and edit this Journal.

Historically, biomedical research prioritizes hypotheses developed by researchers without lived experience of the conditions they study. People with lived experience who have hypotheses about the mechanisms of their respective conditions generally do not have a platform to share their theories to direct future research outside of patient communities. The Journal aims to connect patients' hypotheses directly with the research and scientific communities.

Centering Patient Expertise

At Patient-Led Research Collaborative (PLRC), we have put patients in the driver's seat of research since April 2020. We center patients in sourcing hypotheses for Long COVID and associated conditions in the development of our own surveys,[1] in choosing what research to fund,[2] and in setting a new baseline for meaningful patient engagement.[3] Patients are the foremost experts on their own bodies, and often their research focuses on areas that the medical community has yet to adequately explore.

The Journal authors are patients and caregivers with diverse professional and academic backgrounds, ranging from PhDs to self-taught. The Journal highlights the immense talent pool of our patient network – from their backgrounds, their lived experience of being a patient or caregiver, and from their ability to synthesize existing research – and aligns their expertise with their own hypotheses. Additionally, patients and caregivers communicate amongst themselves, online and offline, to study their health, symptoms, lab results, medication reactions, and experiences, which provide a wealth of knowledge and data to build upon. This pattern recognition is invaluable and regularly results in patients identifying discoveries well before the public and medical community.

Structure and Aims of the Journal

Patient-led research is more effective, timely, accessible, and representative than research without input from lived experiences, and helps prioritize topics that are most important to the patient community. With this in mind, PLRC developed the Patient-Generated Hypotheses Journal. We formed a panel of patient-researchers with lived experience of Long COVID, ME/CFS, and other infection-associated chronic conditions.[4] Next, we created the process outlined in Figure 1, which includes determining criteria and format of hypotheses; crowdsourcing submissions; selecting based on strength of evidence; reviewing and editing chosen hypotheses; assembling chosen hypotheses into this publication; and submitting to an open science journal.

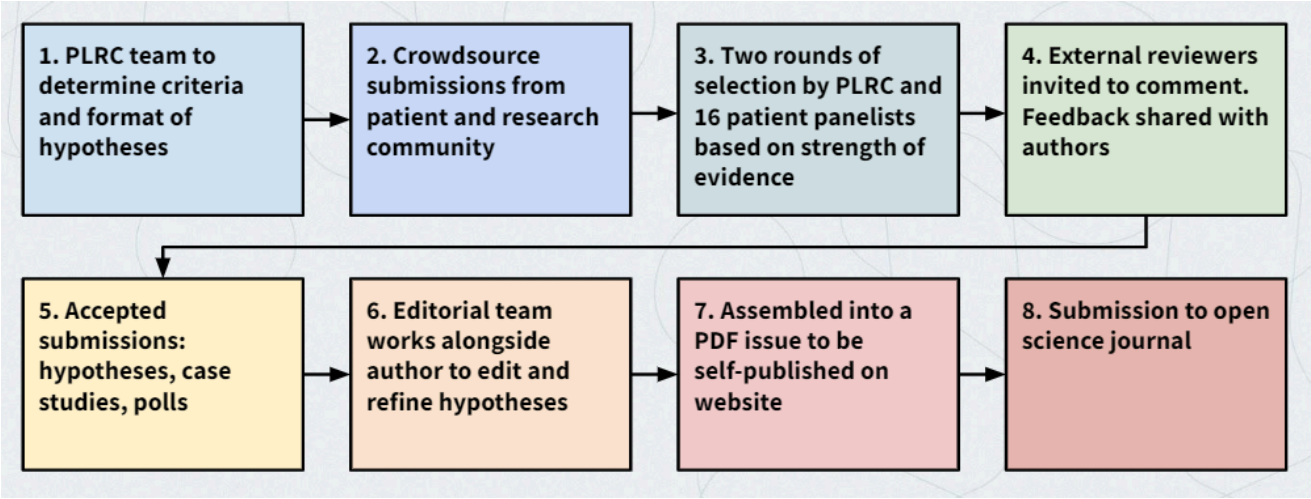

Figure 1. *Patient-Generated Hypotheses Process*

Each article includes an easy-to-read abstract and an in-depth hypothesis with supporting evidence. All highlight unanswered questions and areas for further research, and some provide instructions on how to test the hypotheses.

The purpose of this Journal is to inspire biomedical researchers to use these hypotheses in their research, to partner with the authors and other patients/caregivers in testing hypotheses, and to uplift patient-generated hypotheses as credible sources of research generation.

By giving patient communities a platform and voice in the research process, we anticipate faster discoveries and better outcomes for people with chronic illnesses.

Journal Issues

Our first issue contained six hypotheses authored by patients and caregivers on topics including low-dose doxycycline, astrocyte dysregulation, and poll results. Our second issue features four hypotheses involving carbonic anhydrase activity, elevations of *Streptococcaceae,* chronic inflammation, and BCG vaccination.

[1] McCorkell, L., Assaf, G. S., Davis, H. M., Wei, H., & Akrami, A. (2021). Patient-Led Research Collaborative: Embedding patients in the Long COVID narrative. *Pain Reports*, *6*(1), e913. https://doi.org/10.1097/pr9.0000000000000913

[2] Patient-Led Research Collaborative. (2022). *Patient-Led Research Fund*. https://patientresearchcovid19.com/projects/patient-led-research-fund/

[3] Council of Medical Specialty Societies. (2023, February 15). *The Promise of Patient-Led Research Integration into Clinical Registries and Research - CMSS*. CMSS. https://cmss.org/patient-led-research-integration/

[4] Patient-Led Research Collaborative. (2022). *Patient-Generated Research Hypotheses*. https://patientresearchcovid19.com/projects/patient-generated-research-hypotheses/

Hypothesis

Elevated abundance of the *Streptococcaceae* family within the intestinal microbiome is a consistent and relevant finding for ME/CFS

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Abstract

Alterations of the fecal microbiome have been reported in numerous studies of ME/CFS patients. However, only two sizable studies to date include quantifications of less abundant bacterial families which favor oxygenated environments, particularly *Enterococcaceae* and *Streptococcaceae*. Both studies contain data showing significant elevations of *Streptococcaceae* in ME/CFS patients compared to controls, although the more recent study does not remark upon it. This previously unnoticed replication suggests *Streptococcaceae* abundance may be useful as an ME/CFS biomarker, and may point towards a pathological role for *Streptococcaceae* in ME/CFS.

Hypothesis

The gut microbiome has gained growing attention in the last decades for its roles in human health and chronic disease.[1,2,3] Such attention has not escaped the field of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) research. Recently, multiple groups have published reviews of several studies, finding altered gut microbiome composition in ME/CFS, as assessed via fecal samples.[4,5,6]

The present hypothesis draws attention to a potentially important common finding between an early study by Sheedy et al.[7] and a recent study by Lupo et al.[8] – elevated abundance of gut microbiota from the *Streptococcaceae* family in ME/CFS patients versus healthy controls. This finding appears to have been incidental, as Sheedy et al. is not cited in the study by Lupo et al.

This unintended replication gives rise to the present hypothesis: elevated abundance of *Streptococcaceae* in the gut microbiota is a potential marker and possible driver of pathophysiology for some cases of ME/CFS.

Sheedy et al. is an earlier study of the fecal microbiome which reports culturing ~1000x more *Streptococcaceae* colony-forming units (CFUs) in their ME/CFS patient cohort (n=108) versus healthy controls (n=177).[7] The recent study by Lupo et al. looks at the gut microbiome of ME/CFS patients using 16S rRNA next-generation sequencing (NGS) technology.[8] This study reports predominantly on the altered composition of the most abundant genera of

gut microbiota, e.g., noting reduced and increased abundances of the *Lachnospiraceae* family and *Bacteroides* genera, respectively. Although the authors focus on the most abundant families and their genera, the study's technology has sensitivity to less abundant families, including *Streptococcaceae*. Close examination of family data in Supplementary Figure 3 reveals that *Streptococcaceae* is clearly elevated in ME/CFS patients, in comparison both to healthy relatives and unrelated healthy controls.

The following sections address three important questions: How strongly does the data from these two studies support the hypothesis? What are the mechanisms by which *Streptococcaceae* might be associated with ME/CFS? What further studies should be done to test this hypothesis?

How strongly does the data support the hypothesis?

Of the numerous fecal microbiome studies, Sheedy et al. and Lupo et al. are the only two known to date which speak to *Streptococcaceae* abundance in ME/CFS cases vs controls. Both studies have sizable cohorts and exhibit methodological strengths, making the replication of elevated *Streptococcaceae* noteworthy.

The 2018 review by Du Preez et al. rates Sheedy et al. highly for study methodology, standing out as the largest study by a significant margin (over 2x).[6,7] Among the culture-based studies, it also stands out as the only one to specifically utilize aerobic culture methods required to quantify the abundance of more aerophilic families, including both *Enterococcaceae* and *Streptococcaceae.* These reside at low levels in the oxygen-poor intestinal environment.

More recently, studies are gravitating towards NGS approaches, including *metataxonomic* studies (based on 16S rRNA sequencing) surveying genera and later *metagenomic* studies (based on whole-genome sequencing) capable of drilling down to species and even strains.[9,10] This focus on quantifying the numerous comparisons among genera and species from highly speciated families often obscures less abundant families. Even when highly elevated, relatively aerophilic bacteria such as *Streptococcaceae* remain far less abundant in the intestinal environment. One should note, however, that the raw data of all NGS studies completed to date may yet contain unreported information about less abundant families such as *Streptococcaceae.* So far, Lupo et al. stands out as the one known NGS study which reports family-level data, albeit in the supplementary data.[8] While not very large, it is sizable and carefully designed, including three cohorts of n=35, cases, close relatives, and unrelated controls.

While the pool of sizable studies speaking to *Streptococcaceae* abundance is currently limited to only two studies, it is noteworthy that a newly published but small metagenomic study (n=10) also reports higher *Streptococcaceae* in ME/CFS patients for 4 of 5 case-control matched pairs.[11]

The two larger studies also report on *Enterococcaceae*, another relatively aerobic family, describing either a much smaller elevation[7] or no difference[8] in the ME/CFS population compared to the healthy controls. Thus, *Streptococcaceae* appears to stand out among more aerophilic families of the human fecal microbiome.

How might Streptococcaceae be associated with ME/CFS?

If further replicated, elevated *Streptococcaceae* in ME/CFS patients could imply one of three scenarios:

- a pathogenic role for one or more species in the *Streptococcaceae* family;
- a pathway by which ME/CFS pathology drives elevated *Streptococcaceae*; or
- a common factor driving both *Streptococcaceae* elevation and ME/CFS pathology.

The latter two scenarios are made plausible by the strongly aerobic nature of *Streptococcaceae* relative to other gut-resident microbes, i.e., any pathological increase in gut oxygenation could drive higher *Streptococcaceae* abundance. However, the two studies anchoring this hypothesis hint at a more direct role for *Streptococcaceae*, as both show a much stronger elevation of *Streptococcaceae* compared to *Enterococcaceae*, which is another rather aerobic family in the human gut microbiome.

It is plausible that *Streptococcaceae* plays a pathogenic role based on several lines of evidence. First, several of its species are known pathobionts (adaptively pathogenic) and capable of intracellular persistence.[12] Secondly, the largest genus of *Streptococcaceae*, *Streptococcus,* has been implicated as a trigger for autoimmune-like cardiovascular disease through molecular mimicry, including cross-reactivity of antibodies against *Streptococcus* with endothelial cell epitopes.[13] Thirdly, *Streptococcus* molecular mimicry appears to directly contribute to a documented innate immune evasion pathway from platelet cell defense against *Streptococcus*.[14] Finally, some *Streptococcus* immune mechanism findings potentially comport with recent reports of endothelial dysfunction and platelet hyperactivation in ME/CFS.[15,16]

Overall, there are several reasons to further investigate if *Streptococcus* is playing a driving role in the pathobiology of a subset of ME/CFS patients.

How to test this hypothesis?

Recent comparisons suggest NGS technology can accurately recapitulate culture-based assays.[17] This bolsters the comparison of the two disparate studies which form the basis of the present hypothesis. Furthermore, it points to an immediate next step: re-analyzing raw data from the several existing NGS studies using different computational processing pipelines designed to consider relative abundance comparisons across a broad range of known gut microbiome families including facultative anaerobes, such as *Streptococcaceae* and *Enterococcaceae.* This screening of facultative anaerobes should ideally also include known opportunistic gut pathogens, such as *Staphylococcaceae* and *Enterobacterioaceae*, as another reference point to further determine how specific the sign or role of *Streptococcaceae* may be.

For new studies seeking to replicate the elevated *Streptococcaceae* findings with new data, quantitative PCR (qPCR) may be the most robust technique. It is increasingly validated against both older culture techniques and NGS for microbial quantification.[20,21] As with metagenomic NGS sequencing, qPCR is typically based on amplifying and reading 16S rRNA sequences which contain both highly conserved and variable regions. However, it quantifies targeted species of interest instead of sequencing against a database of known taxa.

For further robustness, opportunities to collect cecal samples (vs fecal) should be sought, e.g., concurrent with colonoscopy examinations. The cecal region has the highest oxygen concentration in the colon, giving *Streptococcaceae* a relative advantage. Thus, the elevation effect, if the hypothesis holds, is likely strongest there.

Should any or all of these approaches (i.e., re-computation, qPCR, further NGS) confirm the elevation of *Streptococcaceae* in ME/CFS, then further new data/analyses should be sought that would drill deeper into specific *Streptococcus* species. While family or genus level studies are the fastest path to replication, any bacterially-driven pathology is apt to arise from specific species.

In tandem with these single time point population studies, clinical treatment studies that longitudinally track both specific symptoms and *Streptococcaceae* abundance can be considered utilizing antibiotics with efficacy against *Streptococcaceae*. To date, two open-label studies of ME/CFS patients with confirmed elevations in *Streptococcus* have reported reductions of both specific ME/CFS symptoms and *Streptococcus* abundance.[22,23]

Conclusion

The present hypothesis, that elevated *Streptococcaceae* is a promising marker and potential driving mechanism for ME/CFS, is supported by 1) two studies of patient and healthy cohorts with distinct microbiome quantification techniques, and 2) a range of mechanistic studies revealing *Streptococcus*-associated pathology potentially consistent with a key role in ME/CFS.

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Hypothesis

Chronic inflammation in Long COVID reduces dopamine levels, and contributes to fatigue and brain fog

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Abstract

Some of the most debilitating symptoms of Long COVID include brain fog and extreme fatigue. Cytokine storms and chronic inflammation are well-documented in SARS-COV-2 infections. Chronic inflammation, in turn, may impact central dopamine signaling. Lowered dopamine and/or dopamine dysfunction could be the underlying mechanism behind the brain fog and fatigue associated with Long COVID.

Introduction

Dopamine is a natural substance found in the body. It is part of a group of chemical messengers called catecholamines, which act as signaling molecules both within and outside the nervous system. In the central nervous system, dopamine signaling plays a crucial role in several important processes such as motor coordination, attention, and motivation. Dopamine dysfunction can cause numerous symptoms, many of which are seen in Long COVID. These symptoms include: fatigue, motor difficulties, executive dysfunction, and cognitive dysfunction.[1] One cause of dopamine dysfunction is chronic inflammation.

Both chronic inflammatory diseases and central nervous system (CNS) disorders are associated with (1) dopamine dysfunction, and (2) a post-viral onset. Parkinson's Disease (PD) is a neurodegenerative disorder in which the progressive loss of dopamine neurons in the substantia nigra drives motor and cognitive difficulties. Post-viral PD and Parkinsonism have been documented in historic epidemics, such as the 1918 flu epidemic and more recent flu outbreaks,[2,3] as well as in some cases of Long COVID.[4,5]

Multiple sclerosis (MS) is another neurodegenerative condition that causes fatigue and cognitive dysfunction similar in presentation to Long COVID. Notably, recent research suggests MS may be a long-term complication of Epstein-Barr virus infections.[6] The "dopamine imbalance hypothesis" has also been proposed as an underlying mechanism of fatigue in MS.[7]

To date, there have been at least 20 documented cases of "post-COVID-19 Parkinsonism."[4] Although these numbers are currently low, it is possible that the inflammatory damage from acute COVID infection may constitute a neurological "hit and run" that leads to a subsequent increased incidence of PD and/or Parkinsonism.[5]

Hypothesis

SARS-CoV-2-induced neuroinflammation may impact dopamine signaling in the nervous system. Impaired dopamine signaling may contribute to the debilitating cognitive symptoms some Long COVID patients experience.

How inflammation affects dopamine

Acute inflammation can have profound effects on dopamine signaling, with studies demonstrating that vaccination or interferon-alpha treatment increase dopamine activity.[8,9] In the acute phase of an infection, this response may promote rest and recovery (i.e., “sickness behaviors”).[10] However, chronic inflammation and exposure to inflammatory cytokines can cause pathological impairment of dopamine neurons and dopamine signaling via a number of mechanisms.[11,12]

In mouse models, inflammatory stimuli can cause dopamine neuron degeneration and behavioral phenotypes similar to what is seen in PD.[13,14] Dopamine neurons are particularly sensitive to metabolic stress and can express major histocompatibility complex class I (MHC-I) in response to inflammation, which in turn can directly recruit cytotoxic T-cells to attack these neurons.[15] As dopamine signaling itself suppresses inflammation, the death of dopamine neurons can further feed into systemic inflammation.[16]

Adapting to brain hypometabolism

Metabolic changes in the brain may occur after an acute inflammatory event.[17] Brain hypometabolism refers to a localized decrease in glucose consumption, typically inferred by decreased blood supply. Brain hypometabolism occurs in several CNS disorders, such as Alzheimer's and PD.[18] Notably, regional brain hypometabolism has also been documented in Long COVID via positron emission tomography (PET) imaging techniques.[19] Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), which can present similarly to Long COVID, is also associated with a hypometabolic state.[20] Dopamine dysfunction could be an adaptation to metabolic changes frequently seen in Long COVID; alternatively, neuroinflammation or damage to dopamine neurons could precipitate a hypometabolic state in limbic structures.[21]

Pro-inflammatory immune signaling

Additional inflammatory processes can also affect dopamine synthesis. Cytokines are chemical messengers in the immune system that regulate immune functions such as inflammation.[22] Pro-inflammatory cytokines induced by infections like SARS-COV-2 and influenza, can impact brain dopamine levels in a number of ways. For example, inflammation can prevent dopamine synthesis by limiting the availability of tetrahydrobiopterin (BH4), an enzyme cofactor required to convert tyrosine into dopamine.[11]

Microglia are the brain's resident immune cells with several functions. Under baseline conditions, microglia quietly patrol the nervous system for signs of trouble. Once activated, microglia undergo a dramatic shift in behavior and begin secreting proinflammatory cytokines, which further fuels the neuroinflammation process.[23] Inflammation can cause microglia to become activated and engage in pro-inflammatory signaling. Additionally, inflammation can block dopamine receptors via striatal adenosine A24 receptors.[24] This immune signaling may be an effort to decrease the brain's energy demands.

Long COVID symptoms such as brain fog and fatigue could be caused by dopamine dysfunction driven by chronic inflammation. After establishing whether dopamine dysfunction is present in Long COVID, further research could establish precise pathways and treatment options.

How to test the hypothesis

Researchers have used PET scans and dopaminergic radioligands such as Carbon-11-FLB 457 to study dopamine in vivo in PD.[25] PET scans of Long COVID patients and healthy controls could provide insight into dopamine dysfunction in Long COVID.

Testing recommendations

1. Use screening questions and clinical measures to identify Long COVID patients who have symptoms consistent with dopamine dysfunction. In addition to self-reported symptoms, consider clinical measures such as the Mini Mental Status Exam, Mini Cog, Computerized Continuous Performance Test, and basic neurological and motor exams.[26, 27,28]
2. Account for medications or comorbidities known to affect dopamine levels. Since many people with Long COVID take these medications and have relevant comorbidities, excluding them may not be feasible. Consider asking participants to discontinue dopaminergic medications prior to testing.
3. Select participants who live close to the testing facility and use a quiet, low-light waiting room to limit external variables and minimize post-exertional malaise (PEM).[29]
4. Consider testing two groups of Long COVID patients (in addition to a control group) based on the presence or absence of symptoms potentially related to dopamine dysfunction.
5. Perform all PET scans at the same time of day because circadian rhythm can affect dopamine levels.[30]

Unanswered questions

1. Is chronic inflammation the cause of dopamine dysfunction in Long COVID?
2. Is dopamine dysfunction in Long COVID a way to protect the brain during metabolic stress?
3. How does PEM affect dopamine synthesis in Long COVID?
4. Can dopaminergic medications used to treat PD, such as levodopa/carbidopa or pramipexole, reduce brain fog and fatigue in people with Long COVID?[31]
5. Cholamine-O-methyltransferase (COMT) inhibitors are sometimes used to increase dopamine levels in patients with PD.[32] Could drinking natural COMT inhibitors, such as those

found in green tea,[33] reduce cognitive symptoms of Long COVID?
6. Green tea contains polyphenols that may be neuroprotective against PD.[33] Does drinking green tea during acute COVID infection have a neuroprotective effect against Long COVID?[34]
7. How does gut microbiome dysbiosis affect dopamine levels in Long COVID?
8. Dopamine synthesis requires adequate intake and absorption of dopamine cofactors and precursors (e.g., vitamin B6, L-tyrosine, L-DOPA). Many biopsychosocial aspects of LC impact dietary intake and absorption, ranging from anosmia to food insecurity. Could supplemental L-tyrosine, L-DOPA, and/or vitamin B6 help alleviate symptoms of dopamine dysfunction in Long COVID?

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Hypothesis

Carbonic anhydrase activity is increased in myalgic encephalomyelitis/chronic fatigue syndrome

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Abstract

Recent research has shown reduced levels of carbon dioxide in people with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and Long COVID. This may be explained by an increase in the activity of carbonic anhydrase, which is one of the enzymes responsible for the conversion and transport of carbon dioxide and for pH regulation. An increase in carbonic anhydrase activity could alter the redox metabolism, increase mast cell-mediated inflammation, and produce neurological symptoms by triggering vasoconstriction in the brain. If proven right, testing for increased carbonic anhydrase activity may be used as a diagnostic and/or surrogate outcome biomarker, while carbonic anhydrase inhibitors may be of benefit in the treatment of ME/CFS and Long COVID.

Hypothesis

There are many anecdotal reports on social media of people with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) presenting with hypocapnia/low blood carbon dioxide (CO2). It is assumed that in the absence of cardiac or pulmonary pathology, hypocapnia is due to hyperventilation.[1] Recently, Natelson et al. utilized capnography to measure end tidal CO2 in 32 ME/CFS patients and found the majority to be hypocapnic.[2] Tachypnea (rapid breathing) was not present and the authors concluded that hypercapnia was the result of hyperpnea (deep breathing), but the volume of breath was not measured.

It seems possible there could be other explanations for hypocapnia could be possible, such as impaired cellular respiration leading to lowered CO2 production, as in the itaconate shunt hypothesis of ME/CFS proposed by Phair,[3] or increased carbonic anhydrase activity.

Carbonic anhydrase (CA) is a metalloenzyme that catalyzes the interconversion of CO2 and bicarbonate; the direction of the reaction depends on pH.[4] Humans possess 15 isoforms of CA found in a variety of tissues.[5,6] CA II, present in erythrocytes and responsible for CO2 transport, is notable for having an extraordinarily high turnover number.[7] CA II associates with the CO2 metabolon, which facilitates rapid gas exchange across the red blood

cell membrane.[8] If CA II activity is increased, then hypocapnia may be present, as CO2 may be more readily taken up by erythrocytes, where it is held as bicarbonate, carbaminohemoglobin, and other carbamino compounds.[9,10]

In addition to its roles in CO2 transport and pH regulation,[9,10] CA is involved in diverse processes including bone resorption,[11] hepatic gluconeogenesis,[7,12] mast cell- mediated inflammation,[13] olfaction and gustation,[14] and diet preferences.[15,16]

CA impacts neurological function directly by altering the pH of the brain.[17] Another mechanism by which CA produces neurological symptoms is through a reduction in cerebral perfusion, as hypocapnia decreases the cerebral blood flow by decreasing the diameter of cerebral arterioles.[18] Acetazolamide, a non-specific carbonic anhydrase inhibitor, has been used in the treatment of intracranial hypertension in Ehlers-Danlos Syndrome;[19] hypermobility and intracranial hypertension are common in ME/CFS.[20] Lubell has proposed that high- dose thiamine may act as a CA inhibitor and suggested clinical trials for its efficacy in treating symptoms of ME/CFS, which include profound fatigue and reduced mental acuity or 'brain fog'.[21]

Increased CA activity may have links to other signs, symptoms, and comorbidities of ME/CFS besides hypocapnia. Increased CA activity can cause bone resorption which is inhibited by acetazolamide;[10] ME/CFS patients are at increased risk of fracture, but it is unclear if this is due to osteoporosis.[22] CA inhibition was found to limit mast cell- mediated inflammation;[12] individuals diagnosed with moderate and severe ME/CFS have increased levels of circulating naive mast cells, as well as aberrant expression of mast cell surface markers in severe cases.[23] Low dose acetazolamide has been used to treat premenstrual dysphoric disorder;[24] premenstrual mood lability and premenstrual syndrome are common in ME/CFS.[25,26]

ME/CFS is characterized by increased markers of oxidative and nitrosative stress.[27] CO2 is not merely a by-product of metabolism, but an important signaling molecule and modulator of redox reactions.[28] It is conceivable that increased CA activity could alter redox metabolism and signaling, leading to redox imbalance and increased oxidative stress.

While unfortunately it is not possible to test activities of all CA isoforms in all tissues, it is feasible to test CA activity in the blood and saliva of ME/CFS patients against that of healthy controls. If a difference is found, it could explain symptoms and signs as discussed above, and perhaps CA activity could be used as a diagnostic or surrogate outcome biomarker. A finding of no difference in CA activity between ME/CFS and healthy controls could indicate decreased CO2 production due to impaired cellular respiration, or possibly a difference in CO2 metabolon function in the ME/CFS cohort. The CO2 metabolon includes band 3; band 3 phosphorylation is known to affect erythrocyte deformability,[29] which is diminished in ME/CFS.[30]

Another feasible test is to In addition to healthy controls, it might be interesting to measure blood and salivary CA activity in Long COVID (LC) patients, since LC has been compared with ME/CFS in terms of similarities of symptoms and possible underlying disease mechanisms.[27,31] Mancini et al. found that 46% of people with LC met diagnostic criteria for ME/CFS.[32] Mancini et al. also reported hypocapnia at

rest as measured by end tidal CO2 in both LC and ME/CFS cohorts, and that the pooled average resting respiration rate was 15.6 (n=41, SD=5), which is within normal range.[32] Acute infection with some COVID-19 variants is associated with anosmia and dysgeusia.[33] Repetitive transcranial magnetic stimulation was found to improve the sense of smell and taste in a clinical study of 93 patients with non-COVID related phantogeusia and/or phantosmia, hyposmia, and hypogeusia. The improvement was accompanied by an increase in blood and salivary CA activity.[34] Anosmia in LC suggests reduced CA VI activity, but hypocapnia with normal respiratory rate at rest might suggest increased CA II activity. Anosmia in LC tends to resolve over time;[35] a longitudinal study of blood and salivary CA activity and LC symptoms could reveal if there are time-dependent changes in either marker and whether they correlate with specific symptoms.

*Author chose to use a pseudonym to protect themselves from possible discrimination at work and school. PLRC verified the author's identity, and the author is available for communication via their email. PLRC feels it is important to allow for the voices of patients and caregivers, to be elevated who would otherwise be silenced due to society's unfair treatment of people with chronic illnesses, must be elevated.

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Hypothesis

BCG vaccination as a treatment option for ME/CFS and Long COVID

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Abstract

The Bacillus Calmette-Guérin (BCG) vaccine has notable "trained immunity" effects. It has shown therapeutic effects in autoimmune diseases such as type 1 diabetes (T1D) and multiple sclerosis (MS). The BCG vaccine is the most commonly used vaccine worldwide. It may be a treatment option for Long COVID and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS).

Hypothesis

COVID-19, like many viral infections, can lead to myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS).[1] Although many biological abnormalities are described for ME/CFS, it is still unknown what ultimately drives the disease process. There is evidence that viral reactivation or its immunological consequences may play a central role.[2]

During viral reactivation, some of the viruses that all humans harbor within their bodies leave their latent (dormant) stage and begin to replicate. One of the viruses frequently implicated here is Epstein-Barr virus (EBV), a member of the family of the human herpesviruses.[3–5] In this respect, ME/CFS shows similarities to other immune-related disorders that have altered immune responses to EBV. These include systemic lupus erythematosus (SLE), Sjögren's syndrome, and multiple sclerosis (MS).[6,7] It has proven difficult to stop viral reactivation on a durable basis with classical antiviral medications.[8]

Immunomodulatory effects of the BCG vaccine

The BCG vaccine, used since 1921 to prevent tuberculosis, has several non-specific effects which all relate to its ability to modulate immune functions. These include anti-cancer effects[9] and preventive effects against infectious diseases such as respiratory

syncytial virus, human papillomavirus, herpes simplex virus,[10] and malaria.[11]

The BCG vaccine also has disease-modifying effects in a range of autoimmune diseases. For instance, in humans with early-onset type 1 diabetes (T1D), repeated BCG vaccinations (three shots within 2 years) were shown to induce long-term clinical remission in a double-blind, randomized controlled trial.[12,13] In early stages of MS, a single dose of BCG vaccine was shown to prevent progression to clinically definite disease when given after the first demyelinating event.[14] BCG vaccination was also shown to have preventive immune effects, including increasing resistance against childhood leukemia,[15] atopic dermatitis,[16] juvenile T1D,[17] and, in patients with T1D, against COVID-19.[18]

Hypothesis

From these clinical observations, it appears plausible that BCG vaccination could also have disease-modifying effects in ME/CFS and/or in the ME/CFS subtype of Long COVID. It is especially intriguing that BCG vaccination was shown to be therapeutically effective in other disorders where an aberrant immune response against reactivated EBV seems to play a central role, such as MS.[14]

Also notable are the documented antiviral effects of BCG vaccination on human herpesviruses. These effects suggest that BCG vaccination may be able to prevent reactivation of latent viruses, including human herpesviruses such as varicella zoster virus, cytomegalovirus, or EBV.[10] There is a considerable body of evidence both from animal research and from human trials that BCG vaccination may be effective against reactivations of human herpesvirus.[19] This has recently been confirmed in a subgroup analysis from a randomized controlled trial where BCG vaccination was tested for its ability to prevent COVID.[20] However, in the latter trial, benefits were restricted to males.

It may also be worth considering that a different vaccine—a staphylococcal vaccine no longer on the market—is among the few interventions that have been shown to be effective against ME/CFS in the past.[21,22]

Immune system benefits for patients with ME/CFS and/or Long COVID

The biological effects observed after BCG vaccination may also explain why the vaccine could be effective in ME/CFS and Long COVID. For one, BCG vaccination was shown to affect regulatory immune cells involved in the dampening and balancing of systemic inflammation.[23,24] This effect is thought to be based on the expansion of CD4+ T cells (Tregs). Indeed, BCG vaccination was associated with gradual demethylation (activation) of signature genes expressed in highly potent Tregs, including Foxp3, TNFRSF18, CD25, and IL2.[25]

These changes occur on the epigenetic/transcriptional level and apparently involve bone marrow stem cells.[26] That change process may explain the long latency between BCG vaccination and clinical effects in the T1D and MS trials.

The biological effects of BCG administration seem to also include metabolic changes in immune cells, with a shift of glucose metabolism from overactive

oxidative phosphorylation toward accelerated aerobic glycolysis. This shift may explain the BCG vaccine's effect on blood glucose levels in the T1D trials[27] and its possible benefits in autoimmune and nervous system diseases.[28] This metabolic reprogramming may be of benefit in ME/CFS, where CD4+ and CD8+ T cells were found to have reduced glycolysis at rest (while CD8+ T cells also had reduced glycolysis following activation).[29]

BCG vaccination was also shown to induce tumor necrosis factor alpha (TNFα) with subsequent reduction of cytotoxic (including autoreactive) T cells.[30] This could explain the effects of BCG vaccination in MS, with its pathogenetic background of autoreactivity (autoimmunity). A certain background of autoreactivity has also been found in ME/CFS and Long COVID.[31, 32,33]

BCG vaccinations as a trained immunity intervention

On a broader and more principal level, the effect of BCG might be in accordance with the "old friends hypothesis."[34] This hypothesis tries to explain why allergic and autoimmune diseases—such as T1D, MS, and ME/CFS—are on the rise in modern environments.

According to the "old friends hypothesis," diverse microbes play a key role in the immune system's development. Constant exposure to those microbes, found both in the environment and in the human body, can strengthen certain immune responses. Without exposure to those microbes, the immune system fails to develop properly, increasing the risk of autoimmune disease. The set of microbes living within the human body—the human endogenous microbiome—has included mycobacteria since the times of the Neanderthals. These mycobacteria may have helped to keep our endogenous microbiome in a good balance—including the many species of herpesviruses, which are also part of this internal microbial orchestra, and which were shown to confer health benefits if kept in a controlled (latent) stage.[35] It has long been known, for instance, that tuberculosis itself protects from both T1D and MS.[36]

From these considerations, it may be plausible to mimic these "old friends" with interventions now widely discussed as "trained immunity" interventions.[37,38] Here, BCG vaccinations may be the most powerful option.[39, 40, 41]

How to test the hypothesis

A feasible way to gather evidence on the effectiveness of BCG vaccination would be a large cohort study with random selection of Long COVID or ME/CFS patients to receive the BCG vaccine—which would be much easier and cheaper than a randomized controlled trial (RCT). Also, double-blind RCTs may be difficult in this context given the fact that BCG vaccine causes a typical skin reaction at the injection site. Faustman Laboratory at Massachusetts General Hospital has performed rigorous BCG vaccine studies, and their expertise should be tapped in any study plans with BCG vaccination.[12,18]

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About Patient-Led Research Collaborative

Patient-Led Research Collaborative drives patient-led and patient-involved research into Long Covid and associated conditions while following rigorous and sound research methodology, and to advocate for policies that enable patients, particularly the most marginalized, to survive and thrive worldwide. We ground our work in the principles of disability justice and participatory research methods, and in the knowledge that those who experience an illness are best able to identify research questions and solutions.

Patient-Generated Hypotheses Journal Publication Panel

Hannah Davis, Dr. Tess Falor, Malka Goldberg, Dr. Rochelle Joslyn, Anna Kern, Jerry Lin, Netia McCray, Lisa McCorkell, DVL Padma Priya, Laila Santana, Mihaela Suleap, Richelle Sepulveda, Hannah Wei.

For more information on the panelists, please visit:
patientledresearch.com/projects/patient-generated-research-hypotheses

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