Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: July 10, 2023

Citation: Walker WOM, Peppercorn K, et al., 2023. An understanding of the immune dysfunction in susceptible people who develop the post-viral fatigue syndromes Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome and Long COVID, Medical Research Archives, [online] 11(7). https://doi.org/10.18103/mra.v11i7.1.4083

DOI
https://doi.org/10.18103/mra.v11i7.1.4083

ISSN: 2375-1924

RESEARCH ARTICLE

An understanding of the immune dysfunction in susceptible people who develop the post-viral fatigue syndromes Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Long COVID

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ABSTRACT

Viral infection in most people results in a transient immune/inflammatory response resulting in elimination of the virus and recovery where the immune system returns to that of the pre-infectious state. In susceptible people by contrast there is a transition from an acute immune response to a chronic state that can lead to an ongoing lifelong complex post-viral illness, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. This susceptibility is proposed to be genetic or be primed by prior health history. Complex abnormalities occur in immune cell functions, immune cell metabolism and energy production, and in cytokine immune modulator regulation. The immune system of the brain/central nervous system becomes activated leading to dysfunction in regulation of body physiology and the onset of many neurological symptoms.

A dysfunctional immune system is core to the development of the post-viral condition as shown with diverse strategies of immune profiling. Many studies have shown changes in numbers and activity of immune cells of different phenotypes and their metabolism. Immune regulating cytokines show complex altered patterns and vary with the stage of the disease, and there are elements of associated autoimmunity. These complex changes are accompanied by an altered molecular homeostasis with immune cell transcripts and proteins no longer produced in a tightly regulated manner, reflected in the instability of the epigenetic code that controls gene expression. Potential key elements of the altered immune function in this disease needing further exploration are changes to the gut-brain-immune axis as a result of changes in the microbiome of the gut, and viral reactivation from latent elements of the triggering virus or from a prior viral infection. Long COVID, an Myalgic Encephalomyelitis/Chronic Fatigue Syndrome-like illness, is the post-viral condition that has arisen in large numbers solely from the pandemic virus Severe Acute Respiratory Syndrome Coronovirus-2. With over 760 million cases worldwide, an estimated ~100 million cases of Long COVID have occurred within a short period. This now provides an unprecedented opportunity to understand the progression of these post-viral diseases, and to progress from a research phase mainly documenting the immune changes to considering potential immunotherapies that might improve the overall symptom profile of affected patients, and provide them with a better quality of life.

Introduction

Viral illnesses result in transient immune/inflammatory responses that in most people rapidly resolve as the immune system returns to the pre-infection state. In susceptible people by contrast a transition from this acute immune response to a chronic state can lead to a complex ongoing and lifelong post viral illness. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is the 'umbrella' term used for these illnesses that arise in response to a major stressor, predominantly, but not exclusively a virus [1]. This trigger promotes a typical initial immune/inflammatory response but then in susceptible people the disease phenotype of ME/CFS develops, formally diagnosed if it persists for 6 months [2-4]. In New Zealand ~20% of new cases arise from the single endemic Epstein-Barr virus that causes glandular fever. Long COVID (LC) is a post viral illness linked solely to the pandemic virus, Severe Acute Respiratory Syndrome Coronovirus-2 (SARS-CoV-2) that has arisen in large numbers since 2020. Uniquely, for an ME/CFS-like illness, large numbers of Long COVID, estimated to be 100 million, have occurred within this short 3-year period of the pandemic, as a result of over 760 million cases of world-wide SARS-CoV-2 infections [5]. This scenario now provides opportunities to follow the pathophysiology of this immune dysfunction as the post viral disease progresses, utilizing longitudinal studies with large patient cohorts early from the onset of their post viral condition.

What determines susceptibility to chronic ME/CFS-like illnesses is still poorly understood. A key question is whether the people susceptible to developing the condition have a different immune profile from the majority who recover from the initial acute event. Jason and colleagues (2022) have provided a model for an ideal state where we could predict those who likely will develop the long-term post viral illnesses. Blood was taken from 4500 Northwestern University students in a longitudinal proactive study. Of these 238 had an Epstein-Barr virus infection giving rise to infectious mononucleosis (glandular fever) subsequently within the 5-year study period and further blood samples were taken from this subgroup. Eighteen of this subgroup developed severe ME/CFS. Blood samples were taken from the ME/CFS group, and from those who had recovered from the Epstein-Barr infection without developing a post-viral condition. Pre-illness blood samples of the ME/CFS group had detectable metabolic differences relevant to an immune and inflammatory response [6]. This study implies there may be a molecular signature within the complex immune system that would be a predictive for susceptibility to developing the post viral/stress conditions.

The complex immunological responses following exposure to the stressor that led to the post viral condition developing are challenging to understand. The body's response seems to reflect an unresolved interoception of continuing danger from the initial stress assault [7]. The diversity of the initiating stressors, whether it be a virus, another infectious agent, an environmental toxin, or simply a serious stress event in their life add to the complexity. Key unresolved questions have been whether prior health history and exposure to priming events are important for succumbing to the syndromes or a consequence of a genetic susceptibility, or a combination of both.

To investigate susceptibility factors in ME/CFS and LC, we have conducted a quantitative survey with 160 ME/CFS patients and 57 LC patients in New Zealand to infer whether their family genetic history and/or prior personal health history were significant predictors of developing the syndrome [8]. The phenotypic overlap found between LC and ME/CFS participants in terms of symptomology, severity of symptoms and capacity for activity provided further support for the suggestion that LC is a closely related ME/CFS-like illness as hypothesised by published literature [9-13], albeit with features specific to the SARS-CoV-2 virus.

Our study revealed that in the ME/CFS and LC patients there is commonly a history of frequent illness during childhood that required significant time to recover, and which can lead to underlying health conditions. We infer this can be a primer for contracting post-viral syndromes following subsequent exposure to the initiating stressor, for example, the Epstein-Barr virus. Additionally, environmental effects such as a vaccination and the immune response to it [14], or a previous viral infection [15], or stress from a life crisis [16] could be such a priming event. There was a significant relationship between the participants' ongoing fatigue illness and having a family member with similar chronic conditions, and a relationship between the participants' fatigue illnesses (i.e., ME/CFS or LC) and having a family member who developed LC after an initial COVID-19 infection. This indicated a likely genetic component [8].

Traditional Genome Wide Association studies (GWAS) to date have revealed linkage of ME/CFS to very few genetic risk loci at the accepted significance [17-20]. One very recent study used a combinatorial analysis of individual base variations

from such a GWAS and identified clusters of single nucleotide polymorphisms that were connected to 14 genes and could identify 91% of the individual patient samples from the UK biobank selected for the study [21]. Seven of these genes could be linked to autoimmune processes and inflammation. This is the strongest evidence yet that there are susceptible subgroups in the population who, because of their genetic profiles, and as well their environmental exposures are at risk of developing these diseases when they are subject to an external stressor whether it be viral infection or other stress event.

In this review we have attempted to integrate the many published papers connecting the immune system to the post-viral fatigue syndromes, ME/CFS and LC. The study design was to review the ME/CFS and LC literature and to provide as clear an understanding as is currently possible of the pathophysiology of the two conditions. We have focused on the following aims: (i) describing how immune cell profiling has been applied to profile changes in ME/CFS and LC; (ii) understanding the importance of chronic activation of the immune system and autoimmunity in the development of ME/CFS and LC; (iii) describing changes in the immune cells, their metabolism and their regulators in this chronic state; (iv) documenting the wide ranging changes in the molecular homeostasis of the immune cells; (v) briefly highlighting the likely importance of the role of the gut/brain/immune cell axis in these diseases, and the effects of viral reactivation on the immune cell dysfunction (vi) discussing a model for the role of the specific immune system of the brain and other aspects of the central nervous system (CNS) in maintaining the long-term diseases; and (vii) targeting areas of the holistic models of ME/CFS and LC where therapeutic options are worth exploring.

The overarching goal was to provide an integrated description of the central role the peripheral immune system and the immune system of the CNS play in the complex dysfunctional physiology of ME/CFS and LC, and how that manifests itself into such debilitating lifelong illnesses for most affected patients.

Immune profiling in Myalgic Encephalomyelitis /Chronic Fatigue Syndrome and Long COVID

As illustrated in Figure 1, we propose, a patient's genetic and environmental history predict and prime respectively a patient's susceptibility to entering a chronic immune state following an initial trigger that normally produces the initial transient immune/inflammatory response.

The figure illustrates that genetic factors inferred from family histories [8] and now from clusters of Single Nucleotide Polymorphisms (SNPs) in a GWAS analysis [21] both indicate there is a susceptible immune system in some people for developing ME/CFS. As well, it is proposed that environmental events like previous health issues, exposure to a virus bacteria or toxin, or a major physical, emotional, or psychological stress event can prime the immune system to react abnormally when exposed to a trigger event subsequently. Then there is transition to a chronic immune state that subsequently leads to ME/CFS. We believe development of LC occurs similarly in people with similar genetic factors or who have had similar priming exposures, following the SARS-CoV-2 trigger.

As we know that the immune system differs in ME/CFS patients compared to healthy patients, immune profiles can potentially identify vulnerable groups in a population [22]. Immune profile screening may become an essential clinical tool to help identify those at risk of developing post viral/stressor syndromes. This may help future clinicians give appropriate advice to vulnerable patients on the best way to recover from viral infections and/or major life stresses to lower risk of developing post-stressor conditions. Then therapeutic intervention for the patient when they are exposed to potential triggers with prophylactic courses of for example, antioxidants, and perhaps anti histamines to prevent mast cell activation, might be appropriate.

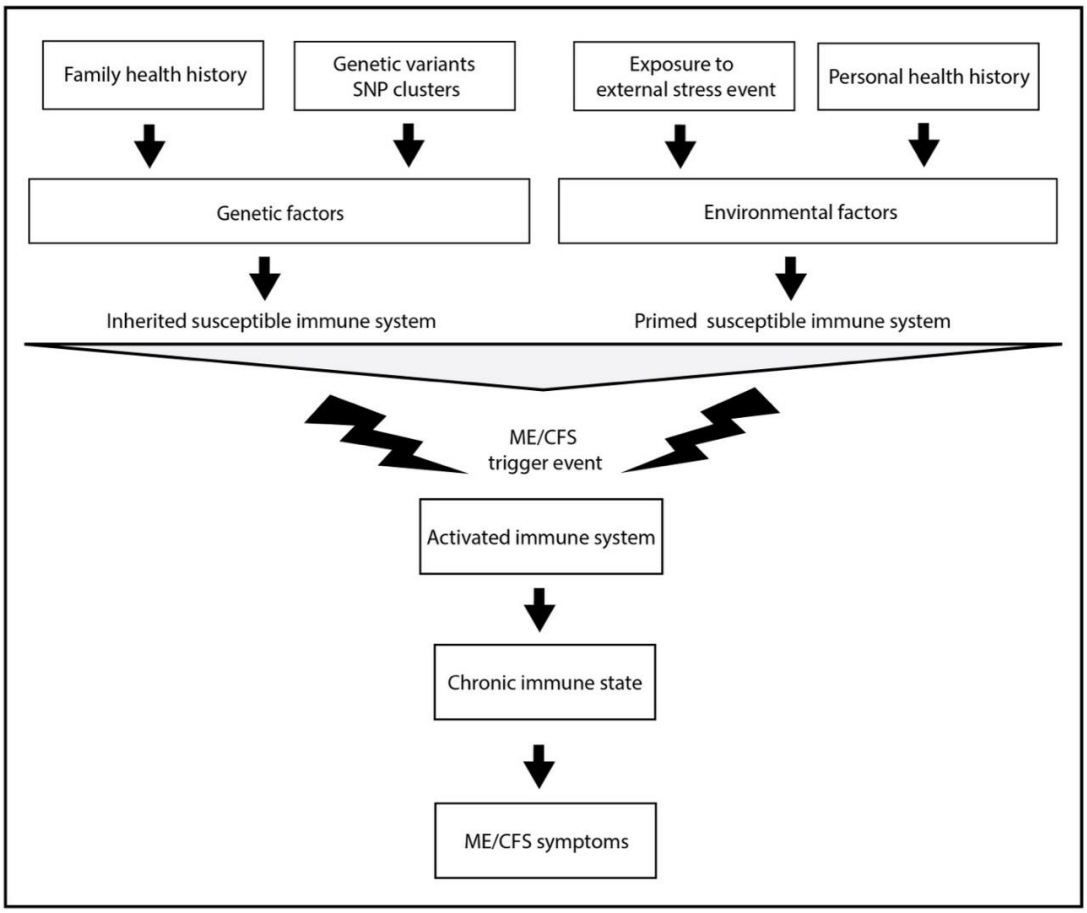

Figure 1. Proposal of how a genetically determined immune system or a primed immune system from a prior environmental exposure leads to ME/CFS following a major triggering event.

Critical technology for routine immune profiling, however, would need to be more accessible at the community level. Currently, it is only possible to do detailed immune profiling post-disease onset, and as part of research studies.

Immune profiling has already been trialed in LC patients. An exploratory, cross-sectional study of 215 LC patients used multi-dimensional immune phenotyping in conjunction with machine learning for immune profiling to identify key immunological features distinguishing LC from matched controls. Differences were found in specific circulating myeloid and lymphocyte populations, in circulating immune mediators, and cortisol was lower in those with LC [23].

A study with a different approach for immune profiling used multiple "omics" assays and serology to characterize global and SARS-CoV-2-specific immunity in patients with LC and non-LC clinical trajectories 8 months after infection [24]. The analysis focused on T cells and demonstrated that those with LC had systemic inflammation and immune dysregulation. The authors concluded that the normal crosstalk between the humoral and cellular arms of adaptive immunity had broken down in the LC group, resulting in immune dysregulation, inflammation, and onset of clinical symptoms associated with the ME/CFS-like illness.

A recent study also analysed the immune responses in blood at a transcriptional, cellular, and serological level up to 24 weeks post-COVID infection in 69 patients recovering from mild, moderate, severe, or critical COVID-19 in comparison to healthy uninfected controls, with >50% having ongoing symptoms more than 6 months post-infection. Immunophenotyping revealed significant differences in multiple innate (NK cells, LD neutrophils, CXCR3+ monocytes) and adaptive immune populations (T helper, T follicular helper, and regulatory T cells) in convalescent individuals compared to healthy controls, most evident at 12- and 16-weeks post infection. Significant changes to gene expression were evident at 6 months post-infection [25].

These studies show the value of immune profiling with onset of the post viral/stressor diseases, but this

value would be enhanced if immune profiling data for the individual patients were available prior to onset of disease. Then 'prior' data could be compared with that of other healthy controls to see if the susceptible subgroup had a different immune profile.

Immune system is in a chronic state in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Long COVID

Chronic immune dysregulation has commonly been cited in the past as an explanation for many of the symptoms seen in ME/CFS [26-28]. Long COVID as described above is also thought to be partly due to an abnormally functioning immune system [29]. We have proposed in an holistic model of ME/CFS and LC diseases that a key element of the development of syndromes is the failure of the immune/inflammatory response to resolve transiently so the immune system becomes chronically activated/dysregulated long term, where inflammatory and anti-inflammatory molecules and cells remain out of balance leading to many of the symptoms seen in Long Covid and ME/CFS [1]. Many early studies highlighted changes in the immune system of ME/CFS patients [26,30,31]. Recently, a large epidemiological study with 1.2 million cancer cases and 100,000 healthy controls found that ME/CFS patients have a significantly increased risk of non-hodgkins lymphoma [32]. ME/CFS patients may have a chronically activated immune system that pre-disposes patients to developing cancer.

(a) Regulation of immune cell subclasses in the chronic state

Many studies now have shown changes to both immune cell concentration and function in ME/CFS. Most commonly cited is the reduction in natural killer (NK) cell and CD8+ T cell toxicity, which have almost become a diagnostic feature of ME/CFS [31,33-36]. The reduction in NK cell and CD8+ T cell cytotoxicity may be due to reduced concentrations of perforin, an important glycoprotein forming pores in cell membranes of target cells, destroying cell membrane integrity and leading to lysis of cell. This reduction in perforin has been documented in NK cells in ME/CFS [37]. Dysfunctional mitogen-activated protein kinase (MAPK) cell signalling between NK cells has been found in ME/CFS [38] and that may contribute to the reduced NK cytotoxicity reported by other studies. Another theory for reduced NK cell cytotoxicity centres around defects in the Transient Receptor Potential Melastatin subfamily 3 ion channel (TRPM3) that is discussed in detail below.

With reduced NK cell cytoxicity being now a well established feature in ME/CFS what does this mean clinically? Natural Killer cells are involved in control of microbial infections, tumour control and regulation of immune cells [39]. The role of NK cells in shaping immune responses is widely recognised and their reduced cytotoxicity may play a role in the transition from the transient to the chronic state of the dysregulated immune system found in ME/CFS [40]. The change in NK cell cytotoxicity may also contribute to cardiovascular abnormalities found in ME/CFS patients, as lowered cytotoxicity is associated with increased atherosclerosis [41-43]. Not surprisingly, NK cell cytotoxicity has also been promoted as a potential biomarker/diagnostic technique for ME/CFS [44].

As with other immune cells studied in ME/CFS and Long Covid patients, the results are highly variable [45]. The numbers of NK and T cells have been found not to differ between ME/CFS and healthy controls [22,31,46]. However, other studies have consistently found reduced subclasses of NK cells [35,36].

Other lymphocytes have been found to be at abnormal levels in ME/CFS patients. Reductions in CD16+, CD56+ lymphocytes, CD8+ lymph node homing T-cells, plasmablasts, CD3+, CD56+ lymphocytes and effector CD8+ T cells have all been found in ME/CFS patients compared to healthy controls [45,47,48]. On the contrary increases in CD2+, CD26+, CD26+ surface markers, T effector memory, T helper effector, cytotoxic effector T cells, naive B cells, transitional B cells, regulatory T cell subtypes CD25+, FOXP3+ and CD4+ have been found in ME/CFS patients compared to healthy controls [35,45,47,48]. Other studies have been contradictory and have shown no changes to cells in ME/CFS patients compared to healthy controls. No changes were seen in B cell populations or in Th17 T regulatory cells [45,47].

While conflicting results complicate definitive conclusions on the holistic pattern of change in the chronic immune system in ME/CFS, there may be individual differences dependent upon the initial stressor or simply among the individual patients themselves.

We have studied healthy controls and age gender matched ME/CFS patients with similar disease courses, level of functionality, and symptom profiles, in a number of multi-omic and molecular studies. At this molecular level each patient shows individual differences over several of the studies [49,50]. Most relevant to the immune system, the response of this

homogeneous group to COVID19 RNA vaccination varied markedly, ranging from a mild effect on their health for a day or two to requiring two-three weeks of bedrest to recover, and in one case hospitalisation for two weeks to stablise their physiology and homeostatic mechanisms. This suggests the immune systems of the individual patients are reacting quite differently to the reactive vaccine.

In LC, white blood cell levels associated with anti-inflammatory responses, viral response and vascular homeostasis in peripheral blood mononuclear cells were significantly different from the profiles isolated from healthy controls [23]. Molecules from Type 1 conventional dendritic cell (cDC1) populations involved in cross-presentation with CD8+ T cells were also significantly decreased reducing their ability to respond to viral infection. Significant decreases in T helper cells were seen in LC patients.

Recently, LC patients were shown to have reduced CD4+ and CD8+ effector memory cell numbers compared to healthy controls and programmed cell death protein 1 (PD1) was significantly increased in both CD4+ and CD8+ central memory cells [51]. Programmed cell death protein 1 (PD1) down regulates T cell activity during immune responses and can be permanently expressed by cells following chronic infection. It therefore may play a role in reduced numbers of T cells seen in the body, or be a result of chronic SARS-CoV-2 infection [52].

(b) Immune cell metabolism

In an effort to understand why cells function differently in ME/CFS patients, studies on metabolism of immune cells have been carried out to determine how changes can alter the function of immune cells and lead to disease [53]. Increases in immune cell metabolism can lead to inflammation whereas decreases in metabolism can lead to progression of chronic infection or cancer.

The mitochondrion is a common source of study. Neutrophils were found to have mitochondrial dysfunction in ME/CFS patients and the degree of dysfunction is related to the severity of symptoms [54,55]. For example, respiratory burst from neutrophils involving the release of reactive oxygen species to kill pathogens was reduced in ME/CFS patients compared to healthy controls [36]. This highlighted an important aspect of the immune cell dysfunction in ME/CFS patients.

Mitochondrial dysfunction may be the metabolic cause, or the effect of the reduction in cytotoxicity of NK cells in ME/CFS patients. These cells were shown to have dysregulated oxidative phosphorylation and glycolytic pathways [56,57]. Peripheral Blood Mononuclear Cells (PBMCs) also exhibit dysregulated energy producing pathways including oxidative phosphorylation and glycolysis. Mitochondrial respiration was found to have a lower maximal threshold in ME/CFS patients PBMCs compared to controls [56]. This might explain the frequent relapsing in ME/CFS with the inability for patients to increase energy production when exposed to normal day to day stresses [58].

There is altered metabolism in T cells in ME/CFS patients. Mitochondrial metabolism and glycolysis in activated CD4+ T cells were shown to be not significantly different, but activated CD8+ T cells in ME/CFS patients had reduced mitochondrial metabolism, ATP production and reduced mitochondrial membrane potential, all indicators of lowered mitochondrial efficiency. Glycolysis was found to be reduced in both quiescent CD4+ and CD8+ T cells in ME/CFS patients compared to healthy controls [53].

Transmission electron microscopy has been used to show stimulated T cells exhibit increased apoptosis and necrotic cell death in ME/CFS patients and this correlated with disease severity. Mitochondria were swollen and morphologically abnormal in the ME/CFS patients' T cells potentially explaining the significant increases in apoptosis seen [59].

(c) Altered cytokine profiles

Fluctuations in cytokine levels could explain many of the symptoms seen in ME/CFS including fever, myalgia, cognitive impairment and fatigue [60]. While some studies are contradictary, one critical study showed cytokine levels can vary across the stages of the illness [61]. Because of their variability, they have been suggested to be unsuitable as biomarkers for these syndromes [62]. Each cytokine has many complex interactions with other cytokines and immune cells, some of which are still not fully understood. Therefore it is hard to know if cytokines are causal or simply a result of a dysregulated immune system or both in ME/CFS patients.

The published studies are summarised below:
Significant increases in many cytokines (TGF-beta, IL-10, IFN-y, TNF-α, IL-12, IL-21, IL-22, IL-27 and TNF-α) were observed in ME/CFS patients compared to healthy controls [36,47,61,63]. These cytokines all play complex roles within the immune system. While these cytokines when studied individually have clear roles, when examined

collectively the interpretation is confusing due to each having intersecting functions. For example, some inhibit B-cell function, whilst others activate B cells, whilst also promoting cytokine production [47]. Hence it is difficult to understand the clinical significance of these results as a whole when looking at each cytokine individually [64-68].

From a clinical point of view with this plethora of intersecting functions it is hard to determine how each cytokine may be important to the disease progression of ME/CFS, considering the complexity of the immune system. The cytokines found to be elevated also appear to contradict one another with activation and inhibition of certain cells confusing the interpretation of what the overall impact on the immune system would look like.

It should be noted that when cytokines are studied in the context of other measures, clinical significance is easier to extrapolate. One study showed that cytokine levels are significantly correlated with T cell metabolism in ME/CFS patients. Resting CD8+ T basal glycolysis significantly decreased when cytokines (IL-2, IL-8, IL-10, IL-12, IL-9) and stem cell growth factor beta (SCGF-β) were increased in ME/CFS patients compared to healthy controls. Quiescent CD8+ compensatory glycolysis was significantly decreased when cytokine TNF-α and macrophage colony stimulating factor (M-CSF) were increased in ME/CFS patients compared to healthy controls [53]. One cytokine, IL-17 showed significant correlation with increases in CD4+ basal respiration and maximal respiration, whereas IL-9 showed negative correlation with cytokine increases leading to decreases in activated CD4+ T basal respiration in ME/CFS patients compared to healthy controls.

Another study on cytokines compared them to clinically-relevant measures. A study of 10 ME/CFS patients and 10 healthy controls showed a significant direct correlation between levels of fatigue and the hormone leptin in ME/CFS patients [69], although there was no direct correlation between fatigue and the other 51 cytokines analysed. While leptin is an adipokine, primarily a hormone that modulates metabolism, it also has a role in modulating immune cell function [70].

Considering disease severity and disease duration may illuminate why many studies do not arrive at the same conclusions. A study on ME/CFS patients compared to healthy controls found 17 cytokines with a statistically significant difference when patients were grouped as having mild, moderate and severe disease [61]. This showed evidence for a correlation of increased levels of cytokines with disease severity. Levels of cytokines were inversely correlated with fatigue duration. Patients earlier in their disease had higher cytokine levels than both controls or long-duration cases. This indicates that cytokines may be involved in the initial ME/CFS disease progression but not necessarily essential for sustaining chronic disease. Such a finding is also very important because most studies do not group according to disease duration and this may explain the variation and contradictory results seen from the multiple cytokine studies [71]

A tabulated summary of the complex changes in immune cells and cytokines in ME/CFS has been well documented [72].

What is the cytokine profile in patients with LC? Significant increases in IL-2 IL-6, IL-4, IFN-β, IFN-λ1, IFN-γ, CXCL9, CXCL10, IL-8, IL-1β, TNF-α, IL-17 have been found in LC patients compared to healthy controls [73-75]. This is partially conflicting with cytokines that have been found to be reduced in other trials. Interferon-gamma (IFN-γ), IL-8, IL-6, IL-2, IL-17, IL-13, IL-4 have all been found to be significantly reduced in LC patients compared to healthy controls. A reduction in IL-8 is consistent with a post-viral cytokine picture; IL-8 recruits NK cells and neutrophils to sites of inflammation so with reduced IL-8 there would also be reduced action of NK cells and neutrophils for fighting off inflammation/infection. A significant reduction in IFN-γ suggests reduced immune function as IFN- γ is secreted by NK cells, CD4+ Th1 and CD8+ cytotoxic lymphocytes[76].

There were significantly increased levels of IL-1β, IL-6 and TNF-α shown in a 2022 study in patients who did not recover from initial SARS-CoV-2 infection indicating this triad of cytokines may play a role in LC [74] while the result with IL-6 appears in conflict with the first study. Similarly, another contemporary study had apparent conflicts with significantly raised levels of IL-2, and IL-17 and TNF-a when compared to patients with acute SARS-CoV-2 infection, and no significant differences between levels of IFN-γ, IL-10 and IL-4 [75]. The newly evolving data awaits further studies to provide clarity. However, cytokine levels generally in the post-viral syndromes have proved too sensitive to time of disease onset and severity to be simple molecular biomarkers for diagnosis and responsiveness to potential therapies.

A very recent comprehensive review by Low & Akrami proposes how the symptoms of LC and its pathophysiology can be explained by a dysregulated immune inflammatory response involving enhanced cytokine cascades and activated microglia in the brain/CNS [77].

(d) Trained immunity – innate memory cells and inflammation

Trained immunity refers to the ability for innate immune cells to develop memory that aids in protecting against secondary infections [78]. Trained immunity is mediated by metabolic and epigenetic changes that result in reprogramming of innate immune cells following primary infection. A study of the innate immune system of ME/CFS patients compared to both patients with non ME/CFS related fatigue and to healthy controls measured white blood cell count and high sensitivity-C reactive protein (hs-CRP). High sensitivity-CRP is essentially measurements of CRP within a far smaller range so as to detect trace levels in blood. Plasma concentrations of hs-CRP as well as WBC concentrations were significantly higher in both fatigue sets of patients compared to healthy controls [79].

(e) Autoimmunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Long Covid

Elevated antibodies against muscarinic acetylcholine receptors (AchR) and adrenergic receptors (AdR) have been correlated with cardiovascular disease, postural hypotension, fatigue, and Sjögren's syndrome [80-82] and in some cases they have led to similar symptoms to those seen in ME/CFS. Antibodies against M3 and M4 AchR and beta-2 AdR were significantly elevated in ME/CFS compared to controls [83]. These autoantibodies could well play a role in the pathogenesis of the disease as there was a significant correlation of levels of beta-2 AdR and muscarinic AchR antibodies with T cell activation, immunoglobulin levels, antinuclear antibodies and thyroid peroxidase antibodies [83] consistent with findings in other autoimmune diseases [84]. Interestingly, one study found that memory CD8 T cells express significantly higher levels of of b-2 AdR and therefore are more sensitive to noradrenaline [85]. This receptor is also the primary mediator responsible for producing inflammatory cytokines that are increased in ME/CFS patients and this may explain the increased inflammation seen in ME/CFS patients [83]. Antibodies to herpesvirus and SARS-CoV-2 antigens were significantly increased in LC patients. Non classical monocytes, involved in anti-inflammatory processes such as vascular homeostasis, were increased in this study [23]. Increases in CD5+CD19+ B cells in ME/CFS patients produce IgAs that have had been associated with autoimmune disease [46].

(f) Molecular changes in peripheral immune cells

(i) Transcriptome: To gain insight into the molecular changes arising from changes in gene expression in immune cells in the chronic state of ME/CFS, transcriptomes of PBMCs (containing monocytes, B cells, T cells and NK cells) were examined by RNA-seq analysis in a small well-characterized patient group (10 patients) with age/gender-matched healthy controls (10 control subjects). Twenty-seven gene transcripts were increased 1.5- to 6-fold and six decreased 3-to 6-fold in the patient group ($P < 0.01$). The most enhanced gene transcripts were functionally related to inflammation. A functional network analysis identified interactions between the products related to inflammation, circadian clock function, metabolic dysregulation, cellular stress responses and mitochondrial function. Ingenuity pathway analysis ($P < 0.05$) highlighted stress and inflammation pathways [86].

Single cell transcriptomics has been used to assess the changes in individual cell types. Dysregulation of monocytes was a significant feature between ME/CFS patients and controls consistent with a heterogeneous population of cells in the ME/CFS group, the extent of which varied among individual patients. Monocyte signalling to T and NK cells was predicted to be elevated [87].

Transcriptome changes were examined in a female ME/CFS cohort following exercise to highlight pathways that were uniquely altered in an ME/CFS group. During recovery, dysregulated immune signalling was found in pathways related to stress in the disease group consistent with the core symptom of postexertional malaise, but not in the controls. There was no change during the exercise in the ME/CFS group, but surprisingly the controls exhibited significant changes related to lymphocyte differentiation, signalling and fate [88].

(ii) Proteome: The proteome of PBMCs was analysed by SWATH-MS analysis in a small well-characterised group of ME/CFS patients and matched controls. A principal component analysis (PCA) was used to stratify groups based on protein abundance patterns, and it segregated the ME/CFS patients from the controls. A total of 99 differentially regulated proteins were identified in the ME/CFS patients ($P < 0.01$, Log 10 (Fold Change) > 0.2 and < -0.2). Many were related to

mitochondrial function and energy production, and a significant number in the immune inflammatory response, and involved in DNA methylation, apoptosis, and proteasome activation [86].

We have carried out a pilot proteomic study of PBMCs from LC patients compared with healthy controls as shown in Figure 2. Immune cell proteins were also analysed by SWATH-MS with a small cohort of patients. In a principal component analysis the LC group (red) were well separated from the healthy controls (blue) as shown in Figure 2A. An enrichment analysis of the differentially regulated proteins ($p<0.01$, FC >1.5) in the LC group compared to the healthy controls showed that around one third (99/347) (red circles) are involved with the immune process. This is similar to the results of previous transcriptomic, and proteomic studies of the PBMCs from ME/CFS patients compared with controls[86,89].

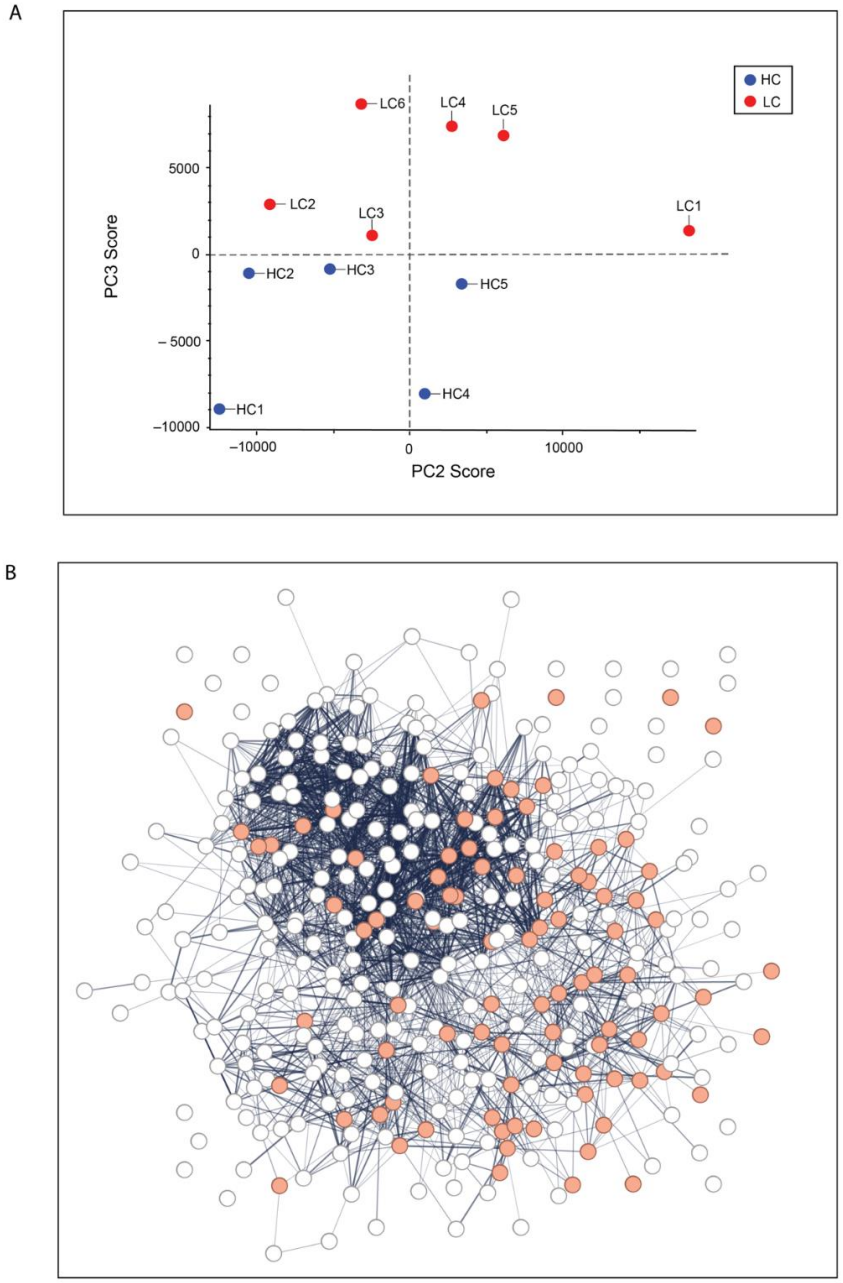

Figure 2. A. Principal component analysis of SWATH-MS proteomics data from Long COVID patients (LC, red circles) against healthy controls (HC, blue circles). B. Differentially regulated proteins ($p \leq 0.01$, minimum

1.5 fold change) are represented as circles. The red circles highlight those proteins which are associated with the Gene Ontology term 'immune system process'.

These reports of significant changes in concentration in the mitochondrial related proteins in ME/CFS was re-enforced with a study of mitochondrial metabolic parameters in PBMCs and plasma in a clinically well-characterised cohort of six ME/CFS patients compared to age- and gender-matched controls. It was followed by quantitative mass spectrometry-based proteomics. The PBMCs from ME/CFS patients showed significantly lower mitochondrial coupling efficiency, and proteome changes indicative of altered mitochondrial metabolism, leading to a decreased capacity to provide adequate intracellular ATP levels [90].

An aptamer-based technology has been applied to a pilot study of 20 ME/CFS female patients and controls using plasma. Nineteen proteins were significantly different in abundance between patients and controls, and they related to the extracellular matrix, the immune system and cell-cell communication. Pathway and cluster analyses highlighted cell-cell signaling of the ephrin pathway which is involved in regulation of immune response as well as axon guidance, angiogenesis, and epithelial cell migration [91].

(iii) DNA methylome: DNA from PBMCs of a well-characterised cohort of 10 ME/CFS patients was used to generate reduced genome-scale DNA methylation maps using reduced representation bisulphite sequencing (RRBS). The data were analysed utilising the differential methylation analysis package (DMAP) analysis pipeline to identify differentially methylated fragments, and the Methylkit pipeline to quantify methylation differences at individual CpG sites. The DMAP identified differentially methylated fragments and Methylkit identified 394 differentially methylated cytosines that included both hyper- and hypo-methylation. Clusters were identified where differentially methylated DNA fragments overlapped with or were within proximity to multiple differentially methylated individual cytosines. These clusters identified regulatory regions for 17 protein encoding genes related to metabolic and immune activity. Analysis of gene bodies (exons/introns) with changed methylation identified 122 unique genes [92]. Comparison with earlier array studies [93-98] on PBMCs from ME/CFS patients and controls showed 59% of the genes identified in this study were also found in one or more of the earlier studies. Functional pathway enrichment analysis identified 30 associated pathways included immune, metabolic and, given the analysis was of immune cell genomes, perhaps surprisingly neurological-related functions were differentially regulated in ME/CFS patients compared to the matched healthy controls. Within the enriched functional immune, metabolic and neurological pathways, the enriched neurotransmitter and neuropeptide reactome pathways highlighted the disturbed neurological pathophysiology was reflected in the immune cells as well as likely being present in the cells of the brain and CNS [92].

A longitudinal precision medicine study mapped genomic changes in two selected ME/CFS patients through a period that contained a severe relapse. DNA was isolated from the patients and a healthy age/gender matched control at regular intervals. This sampling captured a patient relapse in each case. Reduced representation DNA methylation sequencing profiles were obtained from DNA samples from times spanning the relapse recovery cycle. Both patients showed a methylome with significantly higher variability (10- 20-fold) through the whole period of sampling compared with the control. During the relapse, unique single base changes in the methylome profiles of the two patients were detected in regulatory-active region of the genome that were associated, respectively, with 157 and 127 downstream genes, indicating disturbed metabolic, immune and inflammatory functions as well as mitochondrial function and energy production [49].

(g) Transient receptor potential melastatin subfamily 3 ion channel

Transient receptor potential melastatin subfamily 3 (TRPM3) is an ion channel involved in calcium signalling. Studies aimed at uncovering why NK cells have reduced cytotoxicity in ME/CFS patients have examined this ion channel [99]. Most TRP channels are permeable to calcium and are essential for molecular and cellular processes throughout the body [100]. Nociception, temperature sensation and secretory processes are involved with TRPM3 [100] but it has recently also been associated with pathologies of the central nervous system (CNS). These channels are stimulated by tissue injury, inflammation, temperature changes and exogenously by pregnenolone sulfate (PregS) [101,102].

The TRPM3 channels have abnormal function in ME/CFS suggesting that they may play some role in its pathophysiology [99,103,104]. One study on unstimulated CD56brightCD16dim/- NK cells showed significantly reduced numbers of TRPM3

receptors, which may be evidence for why NK cytotoxicity is reduced in ME/CFS patients [99]. Surface expression of TRPM3 on CD56 NK and CD19 B lymphocytes was reduced and this correlated with a significant reduction in intracellular calcium ion concentration [103]. A study investigating genetic abnormalities in ME/CFS patients found their DNA had five single nucleotide polymorphisms (SNPs) that affected TRPM3 in the patients, indicating another potential cause for decreased NK cell function [105].

Naltrexone, an anti-opioid drug has been shown at low doses to have a modulatory effect on TRPM3 by blocking mu-opioid receptors that normally inhibit these channels [102,106]. Naltrexone is commonly used to help with opioid and alcohol cessation disorders due to its antagonistic action on this mu-opioid receptor [107]. At lower doses it has shown efficacy as a therapeutic in several conditions such as Crohn's disease, fibromyalgia, multiple sclerosis and potentially now ME/CFS [108-111]. The clinical relevance here is a potential anti-inflammatory influence [112]. Naltrexone has been used specifically to target the known deficit in NK cell cytotoxicity found in ME/CFS patients [102]. The study used IL-2 stimulated NK cells from ME/CFS patients to determine its efficacy. The TRPM3 channels on the stimulated NK cells had reduced activity following pregnalone sulphate applications that would normally exhibit enhanced TRPM3 activity. Naltrexone (200mM) was then applied to stimulated IL-2 NK, which then restored TRPM3 channel activity. This indicated that if the deficit in TRPM3 function in ME/CFS is significantly involved in the pathogenesis, low doses of naltrexone may be of clinical importance when treating this patient population.

(h) Molecular subtypes/phenotypes

Molecular studies imply that there is variation among individual patients that can influence their cellular immune profiles. Where there is a heterogeneous population of functional and dysfunctional cells in a patient this adds to the complexity of therapeutically targeting the immune dysfunction. Individual targets may have to be characterized for individual patients, rather as is being done for individual cancer patients and their specific tumours.

Several research publications have concluded there are multiple subtypes in ME/CFS as discussed by Tate et al 2023 [50]. They might arise in ME/CFS because of the nature of the different initiators of the disease among the patient cohorts under study. LC patients suffering from the post viral fatigue syndrome have a single originating stressor, the SARS-CoV-2 virus and they have provided a unique opportunity to evaluate whether subtypes might arise from different initiators. However, most of the many symptoms ascribed to ME/CFS have also been associated with Long COVID. It seems more likely that there is a continuous spectrum of pathophysiological responses within all patients because of their genetic profile/health history that determine their disease course [50].

Current experience is that some patients can show benefit from a particular treatment, others no change and yet a third group have their symptoms made worse. The same physiological changes of pregnancy can also cause quite disparate effects in ME/CFS affected women to suggest there are at least three subgroups—those that 'improve', experience 'no change', or 'relapse'. This same phenomenon of benefit/no change/harm is also found not uncommonly with promising medications or supplements. While informative publications studying different aspects of ME/CFS have concluded there are subtypes for that feature, varying in numbers from study to study, there is a danger of defining a plethora of different subtypes but not with utility to provide benefit for every patient within the subtype. Subtypes or phenotype characterisation, however, does seem a useful way to help progress understanding and beneficial treatment strategies for ME/CFS patients including for immune modulation.

The Central Nervous System immune response in Myalgic Encephalomyelitis /Chronic Fatigue Syndrome and Long Covid

Viruses and other infectious agents are less likely to reach the brain directly and other parts of the central nervous system (CNS), given the innate and adaptive immune defense mechanisms of the peripheral nervous system. As well, the CNS resides behind specific blood-brain barriers that restrict entry of both infectious agents and immune cells. Under normal physiological conditions, meningeal, endothelial, epithelial, and glial brain barriers protect the CNS from the dynamic events of the immune system in the periphery [113]. The CNS immunity then exists in a segregated state, with a partition occurring between the brain parenchyma and meningeal spaces. The brain parenchyma is protected by perivascular macrophages and microglia, whereas the meningeal spaces are supplied with a diverse immune repertoire. The brain's tissue-resident macrophages, the microglia, fulfil many functions including, clearing tissue

damage, and developmental clearing of neural progenitors and synaptic material [114].

How do microglia become involved in the prolongation of the ME/CFS and LC syndromes into long lived chronic conditions? Microglia activation is observed in both these diseases and is important for coordinating the CNS immune system during associated neuroinflammation. Although there is a paucity of imaging studies, neuroinflammation has been deduced to occur through evidence of activation of microglia in both ME/CFS [115], and LC [116]. The non-invasive imaging technique, Positron Emission Tomography (PET) coupled with Magnetic Resonance Spectroscopy (MRS) detected a radioactive ligand for a translocator protein expressed only in activated glial cells as a marker of neuroinflammation [115]. In the case of ME/CFS, the degree of activation measured by binding of the ligand to the microglia in their activated state was proportional to the severity of the symptoms of the condition and was occurring largely in the limbic system (cingulate cortex, hippocampus, amygdala, and thalamus), a region between the brainstem and the upper regions of the brain. The nearby midbrain and pons region of the brainstem were also potentially affected. It has been noted ME/CFS symptoms can be linked to vital life functions of the brain stem as "the hub relaying information back and forward between the cerebral cortex and various parts of the body" [117]. It has also been proposed that persistent brain stem dysfunction occurs in Long COVID to explain the long-lasting nature of this post-viral illness [118].

Although the initiating mechanism for chronic microglial activation in ME/CFS and LC is unclear, the PET/MRS evidence clearly shows that a peripheral body event can cause this in the CNS leading to chronic neuroinflammation as a precursor to all CNS mediated symptoms of ME/CFS [119]. The chronic activation of microglia may through the release of cytotoxic molecules such as proinflammatory cytokines, and reactive oxygen intermediates, as well as proteinases and complement proteins be the precursor to the dominant neurological symptoms of the diseases. These M1 polarized microglia have a high concentration IL-12 and IL-23, and a low IL-10 phenotype, and are associated with the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-12, IFN-γ, and TNF-α), as well as chemokines (CCL-2, CCL-20), CXCL-10, cytotoxic substances (ROS, RNS, NO, EAA), and prostaglandin E2 [120-122].

How can a systemic immune/inflammatory response to an infection or severe stress event, transmit signals or molecules into the CNS to involve the brain's immune system? This is possible through known neurovascular pathways or through a disrupted blood brain barrier (BBB) with an intensity that is sufficient to initiate the ME/CFS neurological phenotype, firstly with the changes in the brain/CNS and then through signaling back to the periphery. The afferent arc of the inflammatory reflex is made up of sensory neurons that can relay signals to the brain from the organ systems, whereas the efferent motor neurons can signal back to the organs to alter their function [123]. If this efferent signaling becomes non physiological because of chronic brain dysfunction, precipitated by fluctuating neuroinflammation, then it provides a mechanism by which both ME/CFS and LC could be sustained as long-term illnesses. We have proposed neuroinflammation is a key component of maintaining these diseases [124,125], and through its fluctuation precipitating relapses [1].

Increased blood brain barrier permeability (BBBP) has also been proposed as a possible mechanism in patients with ME/CFS to lead to many of the symptoms they experience [126], because immune cells and neurotoxic molecules might enter the brain inappropriately activating microglia and causing neuroinflammation. Systemic inflammation alone has indeed been shown to lead to increases in BBBP [131].

The gateway reflex is another proposed pathway through which immune cells can enter the CNS. The "gateway" is in the form of a specific blood vessel [127]. A potential site through which pathogenic Th17 cells can move into the CNS is via L5 dorsal vessels in rodents [128]. In the early stages of encephalomyelitis, the chemokine CCL20 attracted pathogenic Th17 cells that were subsequently released attracting further T-helper cells. It has been shown that increased levels of Th17 levels in the brain contribute to neuroinflammation and neurodegeneration [129]. An easy gateway like this through which CNS entry is possible could explain many of the chronic symptoms experienced by ME/CFS and LC patients.

The gut brain axis and viral reactivation as potentiators of immune dysfunction

The links between the gut, brain and the immune system are showing that this axis could play a larger role in the development of diseases like ME/CFS and LC [130-132]. A reduction in microbial diversity (dysbiosis) in the human microbiome is a key feature of many neurological diseases such as multiple sclerosis and Parkinson's disease [130,131]. What is not clear is whether the dysbiosis is present

before onset of the disease, or because of it. Since the microbiota can be manipulated in patients this is an exciting potential area where a real difference could be made by such treatments. There is increasing evidence that reactivation of Epstein-Barr Virus (EBV) in an intermediate abortive lytic replication state [133] and that expression of the EBV-encoded protein, dUTPase, can have a profound influence on the BBB integrity [134], and can promote pro-inflammatory cytokines known to disrupt the BBB.

Figure 3 summarises the immune cell dysfunctional activities in ME/CFS and LC of the cells of the peripheral immune system in the chronically activated state and their immunomodulating cytokines. It illustrates the consequential interactions with the immune system of the CNS and the gut microbiome that are important to sustain ongoing illnesses.

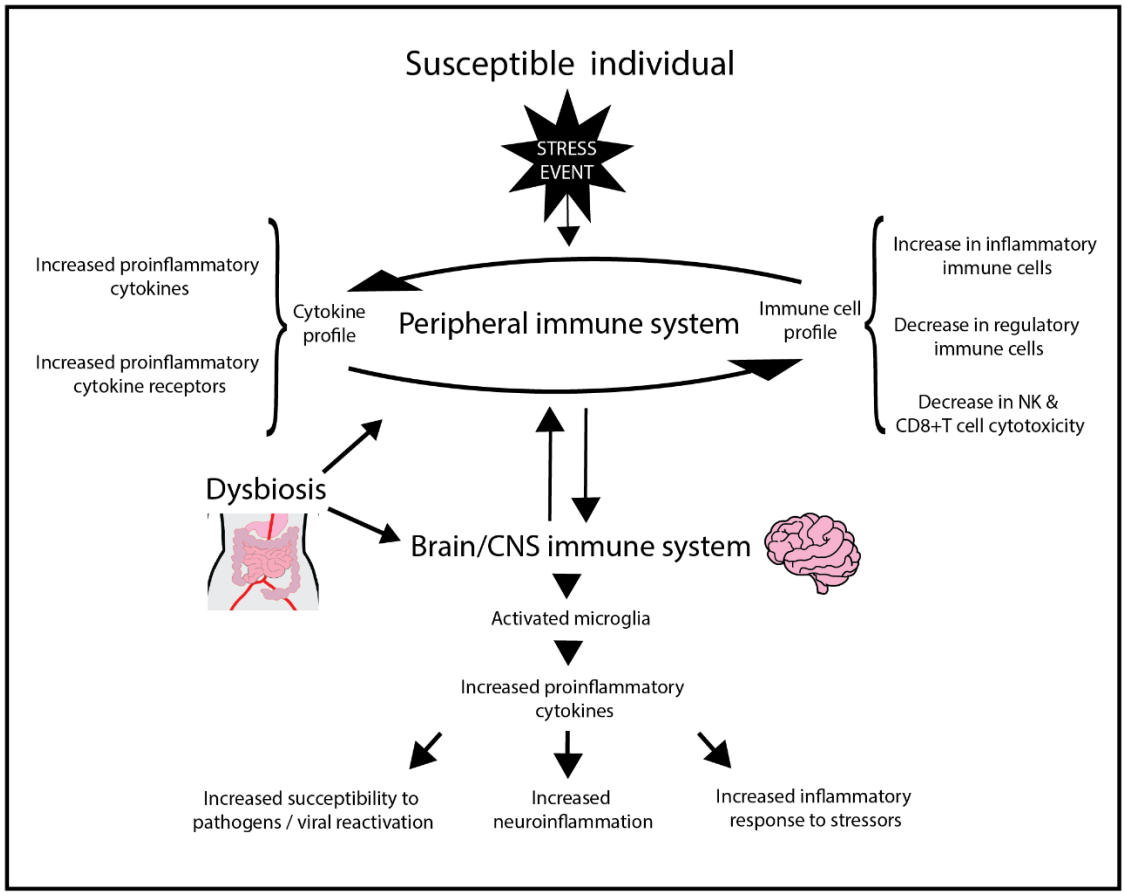

Figure 3. Dysregulation of the peripheral immune system in the chronic state in ME/CFS and LC and subsequent interactions with the CNS and gut microbiome.

Therapeutic options and prospects

(a) Approaches to therapies

Since research first began on ME/CFS and now LC the end goal has and will always be to find and employ a safe and efficacious treatment to help those suffering with these illnesses. To date, no successful treatments have been identified. Initially there was much controversy about the aetiology of ME/CFS and a large focus developed on treating ME/CFS as a psychological disorder by using cognitive behavioural therapy (CBT) and graded exercise therapy (GET) [135]. We now know that these are not efficacious and can be harmful [136-138]. Some patients have claimed daily CBT according to a prescribed programme can incrementally alleviate symptoms over time and therefore the potential use of these treatments should not be discounted fully [50]. Our own unpublished studies on post exertional malaise following a prescribed exercise session have shown some patients respond cardio physiologically to suggest even mild exercise is potentially harmful whereas other patients might get benefit on a on a 3-day cycle of mild exercise allowing time to recover between sessions [139].

In search for a therapy for ME/CFS, researchers have targeted those parts of ME/CFS patients biochemical physiology that are known to be dysfunctional in an effort to alleviate symptoms. There has been some promise in certain areas but

targeting only one part of what is a complex immune and biochemical disorder can only have limited success.

Table 1 Potential therapeutics for ME/CFS and CFS with their targets

Therapeutic	Target	Ref
Cyclophosphamide	DNA (alkylating agent)	140
Naltrexone	Opioid receptors (indirectly on TRPM3 receptors/NK cells)	141
Alpha Interferon	NK cells	30,142
IgG	Immune system modulation	143-146
Laennec (human placenta extract)	Non-specific	147,148
Valganciclovir	EBV, HHV-6	149
Loratadine, Fexofenadine, Famotidine, Nizatidine	Histamine Receptors	51
Idebenone, MitoQ	Mitochondria	150,151
Cytoflavin	Mitochondria	152
Dimethyl fumarate	Nrf2 Pathway	153
Edaravone	Free Radicals	154
Anhydrous Enol-oxaloacetate	Mitochondria	155
Probiotics	Microbiome	156-159
Faecal microbiota transplant (FMT)	Microbiome	160,161
Rituximab	CD20 on B lymphocytes	162,163

As already discussed, NK cells have significantly reduced function in ME/CFS patients compared to healthy controls. Therefore, an obvious therapeutic target would be one that sought to increase their function. One study using alpha interferon which is known to activate NK cells improved quality of life in patients with reduced NK cell function [30,142].

As discussed above low dose naltrexone has shown promise as a drug that may improve symptoms in ME/CFS [141]. It has shown efficacy as a drug that can improve NK cell cytotoxicity in ME/CFS patients but there has not been a large-scale clinical trial [102]. Itanercept is an inhibitor of tumour necrosis factor, a soluble inflammatory cytokine, and it may be effective against neuroinflammation.

Valganciclovir, an antiviral, has been trialed on a subset of ME/CFS patients to evaluate safety and efficacy of the drug. Patients with suspected viral onset ME/CFS and increased antibody levels to either Epstein Barr virus (EBV) or human herpesvirus 6 (HHV-6) were eligible for this study. General, physical and mental fatigue, reduced motivation and reduced activity were all assessed in this study along with monocytes, neutrophils, cytokines and IgG antibodies against EBV and HHV-6. Mental fatigue, and cognitive function showed significant improvement whilst physical and general fatigue showed improvement, but it was not statistically significant. Monocyte count was decreased in patients on valganciclovir compared to placebo. Th1-type cytokines were increased as well as TNF-α and IL-17F in patients on valganciclovir [149]. Valganciclovir therefore could potentially be used as a therapeutic in a specific subset of ME/CFS patients, but further study would be needed to evaluate if there were any real clinical improvement in their overall condition. Once there is a clearer understanding of ME/CFS subgroups/phenotypes studies like this would help to reduce conflicting results when evaluating the efficacies of potential treatments.

Based on the theory that mast cells overreact during SARS-CoV-2 infection in some patients, LC patients were treated with a combination of antihistamines and had improvement in their symptoms compared to those that were not treated. The antihistamine regime was a combination of loratadine 10 mg b.d or fexofenadine 180 mg b.d with either famotidine 40 mg o.d or nizatidine 300 mg o.d [51].

Human placental extract (Laennec) has been used to reduce fatigue in ME/CFS patients. Laennec was given as an IV infusion of 4ml twice weekly for 5 weeks. Patients had a significant reduction in in fatigue and a corresponding decrease in depression and anxiety [147]. Subcutaneous injections of Laennec showed similar improvement in symptoms [148,164].

(b) Immunotherapies

Immunotherapy has progressed considerably over the last two decades. Immunotherapies are now far more specific and can target specific cell types to

treat disease whilst causing less harm/side effects to the patient. Traditional broad-spectrum immunotherapies, such as glucocorticoids, whilst still useful lack the specificity of the newer monoclonal antibodies being used daily to modulate disease. Immunotherapy took a huge leap forward when tumour necrosis factor (TNF) inhibitors were first used to treat rheumatoid arthritis in the late 1990s. Subsequently, more small-molecule-based-therapeutics targeting other cytokines, inflammatory cells, receptors and inflammatory pathways were developed changing the ability to control many conditions.

Although immunotherapies are disease modifying, they are not curative. They are also more effective when used early in the disease before there is damage caused by chronic inflammation [165]. One form of immunotherapy that has been trialed in ME/CFS patients is intravenous immunoglobulin G (IgG) [143-146]. Clinical trials performed in the 1990s were conflicting with two claiming significant benefit and the other two claiming no benefit at all. This led to a period of disinterest in the therapy, but it is now being revisited [166]. Immunoglobulin G is the most common antibody found in the blood [167]. Four subclasses of IgG (IgG1-4), have different roles in the immune system as they uniquely bind to their own antigens and activate immune cells and activate the complement system specifically by binding to Fcγ receptors on cells [168,169].

Pathogens tagged by IgG or IgG immune complexes will therefore be recognised by cells with Fcγ receptors leading to a phagocytic response. Fcγ family receptors are found on monocytes, macrophages, neutrophils, eosinophils, natural killer cells, dendritic cells and B cells. Subclasses of this family are expressed differently on each cell and have inhibitory and stimulatory rolls essential to correct function of the immune system. Intravenous IgG therapy was therefore hypothesised to help ME/CFS patients because their immune system was dysregulated and unable to deal with viral reactivation and other pathogens [166]. The IgG4 subclass was inferred to be beneficial as an immunotherapy due to its ability to activate T-regulatory cells leading to the production of IL-10 and TGF-β (anti-inflammatory cytokine) [170].

B cells have been shown to be dysregulated in ME/CFS patients [48]. As B cells are the only cell type that can give rise to antibody secreting cells their overactivity can lead to autoimmunity [171]. B cell depleting drugs such as Rituximab have shown significant reduction in disease progression/ symptoms in diseases like multiple sclerosis (MS) and rheumathoid arthritis [172,173]. As ME/CFS has been suggested to have an autoimmune component contributing to disease, a monoclonal antibody like rituximab was a promising therapeutic [174]. Some efficacy in reducing symptoms of ME/CFS in fifteen patients was shown compared to patients taking placebo. In a blinded study, two infusions two weeks apart of 500 mg/m2 rituximab were given to patients or placebo in the treatment group. After 12 months of follow up 67% of ME/CFS patients receiving rituximab had significantly improved symptoms compared to only 13% in the placebo group [162]. These results indicated that B cells may have a significant role in the pathogenesis of ME/CFS. A phase III trial of the drug enrolled 151 patients, was double blinded and randomised. This study disappointingly however, found no difference between ME/CFS patients on rituximab and ME/CFS patients on placebo [163].

Similar to rituximab, cyclophosphamide was thought to potentially be linked to ME/CFS patients when oncologists noted that patients on cyclophosphamide as part of a chemotherapy plan had remission of ME/CFS symptoms. A phase II trial has been recently done showing improvement in ME/CFS symptoms but this was not in comparison to controls so should be interpreted accordingly [140].

(c) Energy systems: immune cell mitochondria and oxidative stress

Mitochondrial function has been found to be impaired in ME/CFS patients [175,176]. As mitochondria are responsible for producing much of the energy needed for cellular function, restoring mitochondrial activity back to normal would in theory help alleviate some ME/CFS symptoms. One study has sought to improve mitochondrial function by giving exogenous coenzyme Q10 (CoQ_{10}). CoQ is responsible for carrying electrons between complex I and complex II in the mitochondria and has been shown to be reduced in ME/CFS patients [177,178]. This reduction has also been shown to lead to a decrease in energy production and an increase in Reactive Oxygen Species (ROS) [179,180]. Therefore, targeting CoQ_{10} therapeutically should improve mitochondrial function and reduce symptoms.

The study used a combination of oral CoQ_{10} with selenium daily for eight weeks leading to a significant improvement in reported fatigue and quality of life. There was also a reduction in pain, inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, TNF-α) and an improvement in lipid profiles [179]. Idebenone (a CoQ_{10} analogue) also has promise as

a potential treatment. Idebenone has better bioavailability and improved absorption and has been shown to improve chronic fatigue in multiple sclerosis meaning it could potentially be used for ME/CFS and LC patients [151]. MitoQ is another CoQ_{10} analogue that shows promise in improving quality of life in ME/CFS patients [150].

Patients with ME/CFS and LC have also been noted to have increased oxidative stress (an imbalance between reactive oxygen species and antioxidants) [150,181]. Cytoflavin (a formulation of succinic acid, inosine, riboflavin and nicotinamide) has been used on patients recovering from SARS-CoV-2 infection. Cytoflavin has an antioxidant effect and is targeted at reversing mitochondrial dysfunction. In this study on recovering Covid patients their quality of life was improved with reduced weakness, fatigue and breathlessness compared to the control group [152]. Some other antioxidant drugs approved for use in neurological disease have shown efficacy in reducing fatigue. Dimethyl fumarate (DMF) a nuclear factor erythroid 2-related factor 2 (NRF2) activator is used to reduce fatigue in patients with multiple sclerosis [153]. Edaravone is a free radical scavenger used in amyotrophic lateral sclerosis (ALS) however it has been known to exacerbate fatigue [154]. N-acetylcysteine (NAC) can also be taken by patients to increase levels of glutathione (natural antioxidant) with the hope of mitigating oxidative stress [182].

Another recent study on ME/CFS and LC patients involving the mitochondria has shown that the metabolic intermediate oxaloacetate can reduce both mental and physical fatigue in both illnesses [155]. Anhydrous Enol-oxaloacetate was given in varying doses twice-three times a day for 6 weeks in both patient groups. The reduction in fatigue was not only significant but was dose dependent with larger doses resulting in greater reduction in fatigue.

(d) Microbiota

As discussed above the gut brain axis may play a part in the pathogenesis of ME/CFS and LC. Dysbiosis is thought to contribute to neurological diseases such as multiple sclerosis and Parkinson's. Although there is limited clinical research on LC patients with regards to the gut brain axis a recent hypothesis paper has stated the need for more research to establish whether there is significant link [183]. Therefore, if this does play a role in the pathogenesis of ME/CFS and LC patients what treatment options could target the microbiota in patients to reduce symptoms?

As of yet there has been very little study on therapeutics with relations to the gut brain axis for LC patients. However, there has been a variety of trials on ME/CFS of varying quality. Many studies have looked at the efficacy of probiotics in ME/CFS patients with mixed results. One study showed increases in lactobacillus and bifidobacterial in ME/CFS patients on probiotics compared to those on placebo [156]. Another study showed reduction in anxiety of ME/CFS when given probiotics [157]. Inflammatory markers such as TNF-α and CRP have also been reduced in ME/CFS on probiotics [158,159]. Another treatment often used in relation to dysbiosis in the GI tract is faecal microbiota transplantation (FMT). It is now used regularly for patients with recurrent clostridium difficile colitis [184]. The use of FMT has shown quite promising results in a subgroup of ME/CFS patients with IBS. 70% of patients had an improvement in symptoms with 58% of patients having prolonged remission [160]. Another study on ME/CFS patients, again many with IBS, showed marked improvement in symptoms for those that underwent FMT [161]. Currently two double-blind randomised controlled trials are investigating whether FMT is a viable therapeutic in ME/CFS patients (ClinicalTrials.gov identifier (NCT number): NCT04158427 and NCT03691987). The second study currently has 80 participants enrolled which once completed should give further clarification on the true efficacy of FMT in ME/CFS patients and give a clearer picture on what true relation there is between ME/CFS symptoms and dysbiosis.

Figure 4 illustrates the potential immune modulating therapeutics and their tissue targets in the body.

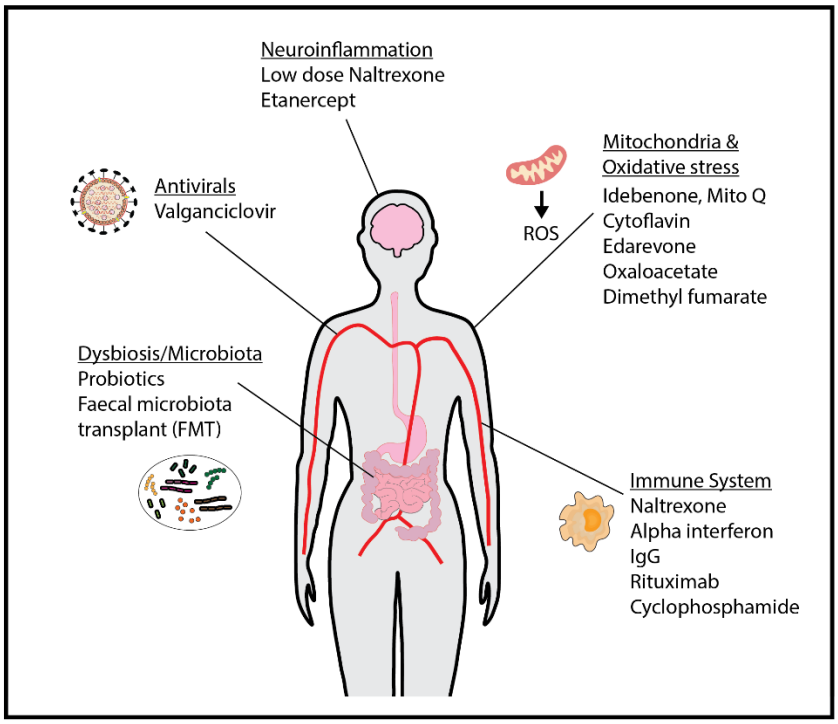

Figure 4. Potential therapeutics and their target body systems for modulation of the dysfunctional immune system in ME/CFS and LC for patient benefit

Conclusion:

The peripheral immune system is a critical pivot point for susceptible people to develop long lasting post viral or post stressor debilitating illnesses. One study suggests the susceptible group might have features of their immune defence system that are perhaps subtly different from those who manage an external threat posed by infection or stress transiently and returning to a quiescent state without damage to normal healthy physiology. Rather in the susceptible people the immune response becomes chronic with a cascade of spreading changes that reach the brain and CNS and lead to the chronic illness state where body physiology is poorly regulated by a dysfunctional brain and CNS. ME/CFS and LC are names given to reflect specific external triggers; ME/CFS, an umbrella term for a number of these different initiators, and LC when the trigger is solely the SARS-CoV-2 virus. The complexity of both the peripheral immune system and that of the CNS with their multiple different cell types with specific functions, and their many immune regulators, the cytokines, a broad category of small proteins important in signaling between the cells, is clearly demonstrated in these illnesses. Multiple papers have documented changes in both peripheral cell numbers and activity, and up or down regulation of cytokines. Complexity is compounded as regulation can be in either direction according to the state and severity of the illness and may vary markedly among individually affected patients. The evolving knowledge of the connection between the immune system and the microbiome and the CNS adds to this complexity. Here we have attempted to provide some coherence and integration to the myriad of changes, and insight into what opportunities there are for immune modulation in patients. This would aim to reverse the cascade of events that have led many to a severely debilitated state of health with no effective therapies. Therapeutic strategies to target immune mediated inflammatory disease like ME/CFS and LC have progressed from broad specificity approaches to highly specific targeting of cytokines and their receptors and to small molecule drugs targeting the inflammatory pathways. Restoring the immune homeostasis in diseases like ME/CFS and LC might not be possible because of the network of changes in multiple components of the system. Improving the quality of life for patients, however, at least might be possible if the patient-to-patient variability in immune dysfunction is not too diverse.

Conflicts of Interest: The authors have no conflicts of interest to declare.

Funding statement: The authors are grateful to the Associated New Zealand Myalgic Encephalomyelitis Society (ANZMES) for support, and for generous personal donations from ME/CFS families.

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