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**Myalgic Encephalomyelitis/Chronic Fatigue Syndrome-
Metabolic Disease or Disturbed Homeostasis due to Focal
Inflammation in the Hypothalamus?**

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Number of text pages:

Number of tables: 5

Number of figures: 1

Numbers of references: 268

Number of words in the Abstract: 196

Number of words in the Introduction: 783

Number of words in the Manuscript: 5,632

Number of words in the Conclusion: 51

Abbreviations

ADP=adenosine diphosphate

AMPK=5' adenosine monophosphate-activated protein kinase

ApoE= Apolipoprotein E

AT=anaerobic threshold

ATP=adenosine-5'-triphosphate

ANS= autonomic nervous system

BMI=body-mass index

β -FGF= β -fibroblast growth factor

CGRP= calcitonin-gene related protein
CNS=central nervous system
CRH= corticotropin-releasing hormone
CSF= cerebrospinal fluid
CVD= cardiovascular disease
FAD=flavine adenine nucleotide
FMS= fibromyalgia syndrome
GWI= Gulf War Illness
HDL=high-density lipid (cholesterol)
HPA=hypothalamic-pituitary-adrenal axis
IBS=irritable bowel syndrome
IFN γ = interferon- γ
IL-1 β = interleukin 1-beta
IL-33= interleukin 33
IL-37= interleukin 37
LDL=low-density lipid (cholesterol)
MCAS=mast cell activation syndrome
MCP=monocyte chemoattractant protein
ME/CFS=myalgic encephalomyelitis/chronic fatigue syndrome
MetS=metabolic encephalomyelitis
MI= myocardial infarction
MIF=macrophage inflammatory factor
MiRNA= microRNA
MIP=macrophage inflammatory protein
mtDNA= mitochondrial DNA

NGF=nerve growth factor

NE=norepinephrine

PTH= parathyroid hormone

PDH=pyruvate dehydrogenase

PDGF=platelet-derived growth factor

PPS/IC=Pelvic pain syndrome/Interstitial cystitis

Poly (I:C)=polyinosinic:polycytidylic acid

POTS= Postural orthostatic tachycardia syndrome

PPAR=peroxisome proliferator-activated receptor

RANKL= Receptor activator of nuclear factor kappa-B ligand

ROS=reactive oxygen species

SCF=stem cell factor

SEID=systemic exertion intolerance disease

SP= substance P

TCA=tricarboxylic acid

T2DM=Type 2 Diabetes Mellitus

TGF β =transforming growth factor β

TNF= tumor necrosis factor

UCP2= uncoupling protein 2

VEGF=vascular endothelial growth factor

Recommended Section

Other

Abstract

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex disease characterized by debilitating fatigue, lasting for at least 6 months, with associated malaise, headaches, sleep disturbance and cognitive impairment, which severely impacts on quality of life. A significant percentage of ME/CFS patients remains undiagnosed, mainly due to the complexity of the disease and the lack of reliable objective biomarkers. ME/CFS patients display decreased metabolism and the severity of symptoms appears to be directly correlated to the degree of metabolic reduction that may be unique to each individual patient. However, the precise pathogenesis is still unknown preventing the development of effective treatments. The ME/CFS phenotype has been associated with abnormalities in energy metabolism, apparently due to mitochondrial dysfunction, in the absence of mitochondrial diseases, resulting in reduced oxidative metabolism, mitochondria may be further contributing to the ME/CSF symptomatology by extracellular secretion of mitochondrial DNA, which could act as an “innate” pathogen and create an auto-inflammatory state in the hypothalamus. We propose that stimulation of hypothalamic mast cells by environmental neuroimmune pathogenic and stress triggers activates microglia leading to focal inflammation in the brain and disturbed homeostasis. This process could be targeted for the development of novel effective treatments.

Introduction

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is defined by the original diagnostic criteria (Fukuda, et al., 1994), and by the Canadian Consensus Criteria (Carruthers, et al., 2003), (Carruthers, 2007) followed by an international consensus (Carruthers, et al., 2011) and newer clinical diagnostic criteria developed by an NIH pathways to prevention workshop (Haney, et al., 2015) and the Institute of Medicine (Germain, et al., 2017). ME/CFS has also been known by other names (Unger, et al., 2016), most recently as Systemic Exertion Intolerance Disease (SEID), (Monro and Puri, 2018)

ME/CFS is a complex disease that involves the muscular, nervous, hormonal and immune systems (Natelson, 2001), (Georgiades, et al., 2003), (Brurberg, et al., 2014), (Brigden, et al., 2017), (Scheibenbogen, et al., 2017). As the name implies, ME/CFS is characterized by debilitating fatigue lasting for at least 6 months, with severe impairment of daily functioning and associated symptoms, such as sleep disturbances, muscle aches, flu-like malaise, gastrointestinal symptoms, orthostatic intolerance, chronic or intermittent pain, as well as cognitive impairment reflected as memory and concentration difficulties (Natelson, et al., 2007), {25039}, (Yancey and Thomas, 2012), (Ganiats, 2015), (Komaroff, 2015), (Scheibenbogen, et al., 2017).

The intensity of symptoms appears to be significantly affected by exertion (Rowe, et al., 2016). Anxiety and increased vulnerability to stress are also common in ME/CFS patients, including children affected by the disease (Smith, et al., 2003), (Crawley, et al., 2009). Abnormal hypothalamic-pituitary-adrenal (HPA) axis activity has been observed in many patients (Cleare, et al., 2001), thus suggesting an association between ME/CFS and disturbed neuro-endocrine mechanisms. Interestingly, ME/CFS patients are more likely to have migraine headaches than normal controls (Ravindran, et al., 2011). ME/CFS is often comorbid with disorders (Table 1) that are characterized by central nervous system (CNS) dysfunction, (Martinez-Martinez, et al., 2014) and which are also negatively affected by stress (Theoharides and Cochrane, 2004), (Theoharides, 2013): Gulf War Illness (GWI) (Gwini, et al., 2016),

Pelvic Pain Syndrome/Interstitial Cystitis (PPS/IC) (Whitmore and Theoharides, 2011), Fibromyalgia Syndrome (FMS) (Theoharides, et al., 2015c), and Mastocytosis (Theoharides, et al., 2015d) or Mast Cell activation syndrome (MCAS) (Petra, et al., 2015), (Akin, 2014). However, there are distinct differences between these other diseases such as between ME/CFS and FMS (Abbi and Natelson, 2013), (Pejovic, et al., 2015).

ME/CFS is estimated to affect as many as 2.5 million people in the US, which corresponds to about 1% of the total US population. (Vincent, et al., 2012), (Komaroff, 2015), (Ganiats, 2015) Other studies (Jason, et al., 2009), including Minnesota (Vincent, et al., 2012), as well as from the UK (Nacul, et al., 2011), (Collin, et al., 2017), Norway (Bakken, et al., 2014) and Italy (Capelli, et al., 2015) report a lower incidence. Women are apparently more susceptible than men, with an estimated ratio of 4:1 (Germain, et al., 2017). The disease predominantly affects adults, even though symptoms may appear in childhood and adolescence (Crawley, 2014), (Nijhof, et al., 2011) ,(Jason, et al., 2006). Unfortunately, a significant number of suspected ME/CFS patients remain undiagnosed (Jason, et al., 2006) mainly due to the complexity of the disease and the lack of reliable diagnostic biomarkers (Klimas, et al., 2012). Multisystem diseases such as ME/CFS are often very timely and expensive to diagnose, and most patients go through years of searching and agony, as well as significant financial expenditures and impairment of their quality of life (Germain, et al., 2017). The economic health burden for ME/CFS in the USA was estimated to be \$24 billion in 2018. (Jason, et al., 2008) . This makes imperative the need for the development of objective diagnostic biomarkers that will not only assist in the critical identification of patients with ME/CFS, but will also provide essential information on the pathophysiological mechanisms involved.

A number of mechanisms and molecules have been implicated in the pathogenesis of ME/CFS (Gerwyn and Maes, 2017). Autoimmune (Sotzny, et al., 2018) and metabolic (Tomas and Newton, 2018) pathways appear to play key roles in the pathophysiology of ME/CFS (Theoharides, et al., 2004b), (Maes, et al., 2011), (Booth, et al., 2012). Neuroimmune and neuroendocrine processes might

also be involved, but are still largely unknown (Dietert and Dietert, 2008), (Bower, 2012). Clinical and subclinical viral infections have been suspected, but never confirmed, as a possible risk factor for the development of ME/CFS (Katz, et al., 2009), (Fremont, et al., 2009). The involvement of neuroinflammation of the brain has recently been suggested without any specific pathogenetic mechanism. (Glassford, 2017), (Tomas and Newton, 2018), (Morris, et al., 2018) Here we give an overview of the current understanding of the associations between ME/CFS and metabolic disease, and propose that focal inflammation in the hypothalamus due to local activation of mast cell and microglia, may alter homeostasis and provide a target for novel treatment approaches.

Metabolic Irregularities

ME/CFS has been found to involve irregularities in the metabolism, energy, amino acid, nucleotide, nitrogen, hormone, and oxidative stress metabolism (Armstrong, et al., 2014), (Germain, et al., 2017). In particular, it has been proposed that the severe and prolonged fatigue experienced by ME/CFS patients may be a consequence of abnormalities in bioenergetic function (Tomas, et al., 2017). Much evidence suggests that the pathophysiology of ME/CFS is highly associated with alterations in normal energy metabolic processes (Fluge, et al., 2016) and abnormalities in cellular bioenergetics (Fluge, et al., 2016;Hornig, et al., 2015), (Fluge, et al., 2016), (Tomas, et al., 2017). There is also evidence to suggest that patients with ME/CFS might be at an increased risk for developing metabolic syndrome-associated diseases, such as diabetes, cardiovascular disease and thyroid disease (Maloney, et al., 2009).

Apparently, systemic exertion intolerance in repeated cardio-pulmonary exercise tests was demonstrated in ME/CFS patients present as compared to healthy controls suggesting insufficient metabolic adaptation to incremental exercise (Vermeulen and Vermeulen, I, 2014), (Keller, et al., 2014). It should be noted, that the Vermeulen and Vermeulen study including controls, which were not matched to ME/CFS in terms of fitness, while the Keller et al study had no controls. McCully et al

published a number of papers showing that when matched for aerobic fitness, cardiorespiratory responses to exercise in patients with ME/CFS only and ME/CFS plus FM were not different from those in sedentary healthy controls (Cook, et al., 2006).

Such intolerance, if real, may involve a switch to anaerobic glycolysis, i.e. a reduction in oxidative metabolism, and an increase in lactate production (Murrough, et al., 2010), (Shungu, et al., 2012b), which constitute the most common metabolic alterations observed in patients with ME/CFS. These characteristics have mainly been attributed to deconditioning, a state characterized by loss of muscle tone and power from prolonged lack of use (Bains, 2008). However, even though increased lactate production was originally noted, possibly related to the reduction of post-exercise oxygen delivery (McCully, et al., 2004), the same effect could not be substantiated suggesting a possible decrease in oxygen delivery perhaps due to reduced blood flow (McCully and Natelson, 1999). In particular, there was elevated ventricular lactate, but no significant difference in high energy phosphatase metabolites in patients with ME/CFS as compared to patients with major depressive disorder or healthy volunteers (Shungu, et al., 2012a). In some cases, alterations in glucose utilization and lactate production were evident only after physical exercise of ME/CFS patients (Fluge, et al., 2016). ME/CFS plasma and serum metabolomics point in the direction of a hypometabolic state (Naviaux, et al., 2016), (Fluge, et al., 2016), (Germain, et al., 2017), (Nagy-Szakal, et al., 2018).

ME/CFS association with metabolic disease

Metabolic syndrome (MetS) is a disorder characterized by an imbalance between energy expenditure and storage, and is diagnosed by the simultaneous presence of three of the following five conditions: (a) central type (or abdominal), (b) obesity, (c) increased blood pressure, elevated fasting glucose levels, (d) high levels of serum triglycerides, and (e) decreased high-density lipid (HDL) cholesterol levels (Mottillo, et al., 2010), (Kaur, 2014). MetS is also linked to insulin resistance, a condition in which, despite normal insulin secretion by pancreatic β -cells and hyperinsulinemia, can lead to

hyperglycaemia and the development of Type II diabetes mellitus (T2DM) (Petersen and Shulman, 2006). In addition, high blood pressure and high cholesterol levels are closely linked to increased oxidative stress and endothelial dysfunction, thus enhancing the pro-inflammatory nature of microvascular atherosclerotic disease (Li, et al., 2007). In other words, subjects with MetS are at an increased risk of developing cardiovascular disease (CVD) and T2DM (Isomaa, et al., 2001), (Dekker, et al., 2005), (Petersen and Shulman, 2006).

Approximately half of patients with ME/CFS also appear to have a previously undiagnosed medical condition, most often diabetes, CVD and thyroid diseases (Maloney, et al., 2009). Few studies have investigated the possible associations between MetS and ME/CFS (Maloney, et al., 2009), (Naviaux, et al., 2016), (Germain, et al., 2017), (Bozzini, et al., 2018). It was first suggested that patients with ME/CFS were twice as likely to have MetS, as compared to controls, after adjusting for body-mass index (BMI), waist circumference, triglycerides and glucose levels (Maloney, et al., 2009). MetS components in the ME/CFS group were significantly correlated with worse fatigue, but not with worse physical or mental functioning, contrary to previous observations (Tsai, et al., 2008), (Maloney, et al., 2009). A correlation of MetS with fatigue has also been observed in patients with FMS, a condition clinically similar to ME/CFS in which muscle pain and fatigue are the main symptoms; specifically, MetS components [low-density lipoprotein (LDL) cholesterol, as well as urinary norepinephrine (NE)/epinephrine and NE/cortisol ratios], were significantly higher in women with FMS, as compared to healthy controls (Loevinger, et al., 2007).

Some studies have reported abnormal findings concerning the cardiovascular system, but one study was in patients with small hearts (Miwa and Fujita, 2009;Azevedo, et al., 2007) and the other was in adolescents (Wyller, et al., 2008),and autonomic nervous system (ANS) dysfunction (Meus, et al., 2013). Low blood pressure was noted in certain ambulatory cases of patients with ME/CFS (Newton, et al., 2009), (Wyller, et al., 2011), (Frith, et al., 2012). However, when patients with

ME/CFS were matched to healthy controls by V02 max there were no differences in cardiovascular parameters (Cook, et al., 2006).

Dysautonomia including Postural orthostatic tachycardia syndrome (POTS) may be present in many patients with ME/CFS (Hollingsworth, et al., 2010) and could also explain other ME/CFS symptoms, such as fatigue, vertigo, decreased concentration, tremors and nausea (Bozzini, et al., 2018). Interestingly, the low systolic blood pressure observed in ME/CFS patients is usually accompanied by exaggerated diurnal variation, which is inversely correlated with increasing fatigue (Davis, et al., 2000), (Newton, et al., 2009).

Overall, it appears that metabolic disease components show significant correlations with the fatigue in ME/CFS patients and not with the disease itself. For example, blood pressure, as well as insulin resistance, are probably secondary to fatigue, and most probably reflect the lack of physical activity and prolonged lack of muscle use in ME/CFS patients. This makes sense if one considers that low blood pressure could give rise to fatigue through brain/or muscle hypoperfusion (Newton, et al., 2009), and that insulin sensitivity is highly dependent on the oxidative capacity of the muscle (Canto and Auwerx, 2009).

Metabolomics, small-molecule metabolite profiling (Daviss B., 2005), has provided relevant information that could distinguish ME/CFS patients (Naviaux, et al., 2016). Several studies have performed metabolite analysis of various biological fluids, [urine, blood, serum and cerebrospinal fluid (CSF)] from ME/CFS patients (Georgiades, et al., 2003), (Jones, et al., 2005), (Niblett, et al., 2007), (Suarez, et al., 2010), (Armstrong, et al., 2012), (Armstrong CW, et al., 2015), (Hornig, et al., 2016). However, despite confirming disturbances in energy, amino acid, nucleotide, nitrogen, hormone and oxidative stress metabolomics, they have not been able to determine a distinct, reproducible metabolic profile for ME/CFS (Germain, et al., 2017). Nevertheless, one study identified nine biochemical disturbances that were common to both male and female patients with ME/CFS, but not healthy controls (Naviaux, et al., 2016). Overall, there were marked decreases in sphingolipid,

glycosphingolipid, phospholipid, purine, microbiome aromatic amino acid and branch chain amino acid metabolites, as well as in flavine adenine nucleotide (FAD) and lathosterol, which identified hypometabolic profile for ME/CFS. These changes correlated with disease severity and had an apparent diagnostic accuracy that exceeded 90% (Naviaux, et al., 2016). Interestingly, the metabolic abnormalities found in ME/CFS patients, were opposite (i.e. decreased instead of being increased), to those observed in MetS suggesting that ME/CFS patients could be more resistant to hypertension, dyslipidaemia, obesity and insulin resistance even though previous studies discussed above had reported an increased association between ME/CFS and metabolic syndrome.

Another study that used targeted plasma metabolomics reported a similar trend of hypometabolic state in ME/CFS patients (Germain, et al., 2017). Even though the metabolite compounds were not all identical to the ones studied by Naviaux et al., both agreed on the presence of disturbances in lipid and fatty acid metabolism (Germain, et al., 2017). These findings are also in agreement with reported deficiencies in the urea and the TCA cycles, (ornithine/citrulline and pyruvate/isocitrate ratios), which ultimately result in reduced levels of ATP production in patients with ME/CFS (Yamano, et al., 2016). Other studies revealed that ME/CFS have reduced substrates that enter oxidation downstream of pyruvate dehydrogenase (PDH), such as glutamine, glutamate and phenylalanine, thus suggesting impaired pyruvate catabolism, which ultimately results in increased utilization of acetyl-CoA-producing amino acids as alternative substrates for fuelling aerobic metabolism via the TCA cycle (Armstrong, et al., 2012), (Armstrong CW, et al., 2015), (Fluge, et al., 2016). Reduced concentrations of amino acids that maintain TCA cycle capacity were detected in patients with ME/CFS (Fluge, et al., 2016), suggesting impaired fuelling of the TCA cycle by pyruvate. This finding is in line with the results of other studies where TCA cycle intermediates were also found to be reduced in both urine (Niblett, et al., 2007) and plasma (Yamano, et al., 2016) samples from ME/CFS patients.

Mitochondrial dysfunction

Overall, the ME/CFS phenotype has been associated with mitochondrial dysfunction, 5' adenosine monophosphate-activated protein kinase (AMPK) impairment, oxidative stress and skeletal muscle cell acidosis (Myhill, et al., 2009), (Kennedy, et al., 2005), (Brown, et al., 2015), (Tomas, et al., 2017). The main ME/CFS symptoms, such as fatigue, exercise intolerance and myalgia, are also shared by patients diagnosed with primary mitochondrial disorders (Filler, et al., 2014), (Gorman, et al., 2015). However, unlike the mitochondrial dysfunction observed in mitochondrial disorders is known to be caused by mutations in either nuclear or mitochondrial DNA (mtDNA) (Tomas, et al., 2017), these mutations in patients with ME/CFS are extremely rare (Billing-Ross, et al., 2016), (Schoeman, et al., 2017). In addition, certain mitochondrial enzymes have been found to discriminate between mitochondrial disorders and ME/CFS. Notably respiratory chain complex (RCC) I, III and IV activity (Smits, et al., 2011) appears to be significantly higher in ME/CFS patients. Instead, ATP production rate was found to be within the normal range in ME/CFS patients, but significantly decreased in approximately three quarters of the patients with mitochondrial disease, and was therefore regarded as the most reliable discrimination test (Smits, et al., 2011).

Muscle biopsies from ME/CFS patients have shown mitochondrial degeneration, atrophy of type II fibers and fusion of mitochondrial cristae, decreased mitochondrial membrane permeability, severe deletions in mtDNA genes that are involved in cellular energy processes, as well as oxidative damage from increased production of free radicals (Myhill, et al., 2009), (Morris and Maes, 2013). Mitochondrial dysfunction has also been observed in peripheral mononuclear blood cells (PMBC) of ME/CFS patients, even though it has not yet been elucidated if they constitute the cause of the disease (Myhill, et al., 2009), (Myhill, et al., 2013), (Tomas, et al., 2017). Notably, a significant correlation has been observed between the extent of mitochondrial dysfunction and the degree of ME/CFS severity, thus suggesting that mitochondrial dysfunction might be a contributing factor in ME/CFS pathology, at least in a subset of patients (Myhill, et al., 2009), (Booth, et al., 2012). However, it is

difficult to assess mitochondrial dysfunction that is usually done by measuring the levels of lactate and pyruvate in the serum, best done by serial serum sampling from an arm after a brief period of exercise.

When limited amounts of oxygen are available, as is usually the case with intense exercise, anaerobic glycolysis, or otherwise called the lactic acid system, provides an effective means of energy production. During this process, glucose is catabolized via the glycolytic pathway, resulting in pyruvate being converted to lactate by lactate dehydrogenase. This process lasts 10-30 seconds during maximal effort and produces about 5% of the glucose energy potential in the form of adenosine-5'-triphosphate (ATP) molecules (2 molecules of ATP for every molecule of glucose). ATP synthesis can be estimated by measuring the anaerobic threshold (AT), i.e. the rate of oxygen consumption at work rate when blood lactic acid begins to accumulate, and the maximal work rate (Morris and Maes, 2014). The AT indicates a switch during which ATP synthesis stops being produced by mitochondria and occurs via the anaerobic route (Morris and Maes, 2012), whereas anaerobic threshold and recovery time following exercise depends on lactate production and clearance rates (Fluge, et al., 2016). When aerobic conditions are normal, pyruvate is transported into mitochondria and converted to acetyl-CoA by either PDH or via degradation of fatty acids and ketogenic amino acids. In either case, acetyl-CoA is further oxidized in the tri-carboxylic acid (TCA) cycle, producing some ATP, and the electron transport chain (respiratory chain), which generates ATP from ADP by oxidative phosphorylation (ox-phos). Acetyl-CoA thereby serves to fuel mitochondrial respiration and ATP production by oxidative phosphorylation (Fluge, et al., 2016) for essential tissue functions (Myhill, et al., 2009).

Reduced ATP production is associated with increased levels of reactive oxygen species (ROS), which may ultimately lead to mitochondrial damage and the hypometabolic profile of ME/CFS (Naviaux, et al., 2016), (Armstrong CW, et al., 2015). Severely reduced or impaired mitochondrial oxidative phosphorylation in ME/CFS patients is highly correlated with significantly increased intracellular lactate levels, even in the recovery phase of a mild exercise where ATP synthesis is extremely low (Vermeulen, et al., 2010), (Morris and Maes, 2014).

Among the factors that may contribute to mitochondrial dysfunction, the most prominent ones appear to be increased levels of pro-inflammatory cytokines, such as interleukin-1beta (IL-1 β) and tumor necrosis factor (TNF), which directly inhibit mitochondrial respiration by increasing mitochondrial membrane permeability, which ultimately leads to membrane depolarization and an increased production of ROS (Morris and Maes, 2013). However, even though TNF is elevated in the serum of patients with FMS, (Theoharides, et al., 2010c) it was not consistently elevated in ME/CFS (Brenu, et al., 2011), but was apparently associated only with increased IL-4 (Hanson, et al., 2001). There was also no significant difference in serum cytokine levels across the night (Nakamura, et al., 2010) or post exercise (Nakamura, et al., 2013). There is some evidence of stronger correlation of cytokines alterations early in the course of illness rather than severity (Hornig, et al., 2015). It has been proposed that “cytokine co-expression networks” may be more predictive of ME/CFS phenotype (Klimas, et al., 2012), (Hornig, et al., 2016), but looking for such biomarkers in the periphery would not reflect inflammation in the brain. One study reported that of 27 cytokines studied in CSF from ME/CFS patients, only IL-10 was significantly reduced {26107}. Another paper using network analysis of CSF cytokine levels reported an inverse relationship with interleukin 1 receptor antagonist only in classical, but not in atypical ME/CFS (Hornig, et al., 2017).

Certain microRNAs (miRNAs) may turn out to be distinct or differentially expressed in ME/CFS. Recently, miRNAs have been implicated in the hypothalamic control of energy homeostasis (Najam, et al., 2018). However, the available studies in patients with ME/CFS did not report any consistent pattern whether pre- or post-exercise, plasma,(Brenu, et al., 2014) NK cells (Petty, et al., 2016) or CD8⁺ cells (Brenu, et al., 2012). One recent important study showed exercise induced changes in CSF fluid from patients with ME/CFS, Gulf War Illness and sedentary controls found twelve diminished miRNAs after exercise (Baraniuk and Shivapurkar, 2017), (Baraniuk and Shivapurkar, 2018).

Focal Inflammation in the Diencephalon and Dysfunctional HPA axis

Neuroinflammation (Nakatomi, et al., 2014), (Glassford, 2017), (Tomas and Newton, 2018), (Morris, et al., 2018) and immune dysfunction (Morris, et al., 2014), (Nijs, et al., 2014), (Trivedi, et al., 2018) have been suggested as being involved in the pathogenesis of ME/CFS, but serum levels of proinflammatory cytokines have not been confirmed as discussed later. Considerable evidence indicates that ME/CFS is characterized by dysfunction of the HPA axis, (Theoharides, et al., 2010b), (Morris, et al., 2016) and symptoms are known to worsen by stress (Smith, et al., 2003)), (Theoharides and Cochrane, 2004), ((Crawley, et al., 2009;Theoharides and Cochrane, 2004;Theoharides, 2013). Stress can also worsen or precipitate obesity and cardiovascular events (Theoharides, et al., 2008), (Theoharides, et al., 2011), (Alevizos, et al., 2013), (Sismanopoulos, et al., 2013), through local inflammation (Matusik, et al., 2012;Libby, et al., 2002).

Corticotropin-releasing hormone (CRH) is secreted from the hypothalamus under stress and stimulates the HPA axis via activation of two main types of G protein-coupled receptors, CRHR-1 and CRHR-2 (Chrousos, 1995). CRH secreted under acute stress, has been implicated in the pathophysiology of neuroinflammatory disorders and myocardial infarction (MI) (Jiang, et al., 1996;Krantz, et al., 2000;O'Kane, et al., 2006;Slominski, 2009).

We propose that stimulation of hypothalamic mast cells by environment, neural, immune pathogenic (Lyme, mycotoxins) or stress triggers (CRH, somatostatin) activates microglia leading to focal inflammation and disturbed homeostasis (Figure 1). Mast cell and/or microglia triggers may derive from the nasal cavity, or may reach the brain area through a disrupted BBB or through the lymphatics. Stimulated mast cells could secrete molecules that can alter homeostasis directly (via secretion of CRH, urocortin) or activate microglia (via secretion of histamine, tryptase and mtDNA). Microglia then release more inflammatory molecules (IL-1 β , IL-6, and CCL2) that further disrupt homeostasis, causes mitochondrial dysfunction and contribute to fatigue both centrally and peripherally. In fact, activated microglia have been reported to contribute to the pathophysiology of

sleep disorders (Nadjar, et al., 2017). The involvement of more than one trigger can lead to a significantly heightened response and lower the triggering threshold of both mast cells and microglia leading to chronic symptoms.

Mast cells are unique tissue immune cells involved in allergic reactions (Theoharides, et al., 2015d), but also act as sensors of environmental and psychological stress (Theoharides, 2017). Even though we invoke stimulation of mast cells in the hypothalamus, it does not necessarily mean that mast cells should necessarily be stimulated outside the CNS. Nevertheless, there have been reports of an association between ME/CFS and acute rhinitis including significantly higher TNF and CXCL8 levels in nasal lavage fluid (Repka-Ramirez, et al., 2002). In addition, chronic rhinosinusitis symptoms were significantly higher in patients with ME/CFS (Chester, 2003), apparently due to non-allergic rhinitis (Baraniuk and Ho, 2007). It is well known that both allergic and perennial rhinitis involve activation of mast cells (Bachert, et al., 2018). More recently, it was reported that the incidence of ME/CFS was higher in patients with a history of atopy (Yang, et al., 2015). Moreover, circulating blood mast cell precursors were found to be higher in ME/CFS patients (Nguyen, et al., 2017).

Mast cells are located perivascularly in the hypothalamus, thalamus and third ventricle of the diencephalon (Edvinsson, et al., 1977), (Pang, et al., 1996). CRH could stimulate MC in the hypothalamus since CRHR-1 gene is expressed on human cultured mast cells, activation of which induces production of vascular endothelial growth factor (VEGF), (Cao, et al., 2005) which could increase permeability of the blood-brain barrier (BBB) (Theoharides and Konstantinidou, 2007), (Theoharides, 1990), (Esposito, et al., 2002) leading to inflammation of the brain (Theoharides, et al., 2004a). Moreover, CRH is synthesized by mast cells (Kempuraj, et al., 2004) implying it could have autocrine effects. Interestingly, even somatostatin stimulates mast cells (Theoharides, et al., 1990). Mast cells are also found in the pineal, the pituitary and the thyroid glands (Theoharides, 2017) further extending their contribution to the symptoms of ME/CFS such as sleep disturbances dysfunctional HPA axis and fatigue due to thyroid dysfunction. Mast cells are well-known for their role in allergic

reactions, (Beaven, 2009) but mast cells are now considered important in innate and acquired immunity, (Galli, et al., 2008) antigen presentation, (Gong, et al., 2010) and inflammation (Theoharides, et al., 2010a).

Mast cells can be stimulated by neurons, hormones, environmental, neuroimmune, pathogenic and stress triggers. (Table 3), (Theoharides, et al., 2015d), (Theoharides, 2017). Reactive oxygen species (ROS) can also stimulate mast cells (Swindle and Metcalfe, 2007). (Robuffo, et al., 2017), (Toniato, et al., 2017) Mast cells also secrete leptin that could contribute to cachexia and fatigue (Taideman, et al., 2009). Mast cells secrete as many as 100 different mediators (Table 4) (Mukai, et al., 2018), (Theoharides and Kalogeromitros, 2006) (Wernersson and Pejler, 2014) often selectively without degranulation (Theoharides, et al., 2007), utilizing different secretory pathways (Xu, et al., 2018). Mast cells can also secrete danger signals, (Theoharides, 2016), including many chemokines and cytokines (Conti, et al., 2017),(Mukai, et al., 2018) especially mitochondrial DNA (mtDNA), (Zhang, et al., 2012) which could act as an “innate pathogen” (Zhang, et al., 2011) leading to a localized brain auto-inflammatory response (Collins, et al., 2004;Marques, et al., 2012;Sun, et al., 2013;Theoharides, et al., 2013). Extracellular mtDNA could either be secreted directly in the diencephalon or could reach the brain through lymphatics (Louveau, et al., 2015). We had reported that mtDNA is increased in the serum of children with autism spectrum disorder (ASD) (Zhang B, et al., 2010). Mast cell-derived mediators can then stimulate microglia (Zhang, et al., 2016), (Patel, et al., 2016) to secrete additional pro-inflammatory and homeostasis-disrupting molecules (Table 5) contributing to fatigue and neuropsychiatric symptoms (Theoharides TC., et al., 2016). It is interesting that peptide Y was found to be elevated in plasma of patients with ME/CFS and correlated significantly with stress (Fletcher, et al., 2010), as this peptide is known to stimulate mast cells (Mousli and Landry, 1994).

An important part is that combination of triggers is likely to play a more important pathogenetic role than individual ones. For instance, we reported that combination of CRH and NT have synergistic

action in stimulating VEGF secretion without tryptase from human mast cells (Donelan, et al., 2006), as well as induce the expression of each other's receptors on human mast cells (Alysandratos, et al., 2012). More recently, we showed that the combination of SP and IL-33 has synergistic action in stimulating TNF secretion without tryptase from human cultured mast cells (Taracanova, et al., 2017c).

CRH is often released together with another peptide, neurotensin (NT), which is vasoactive (Leeman and Carraway, 1982) and has also been implicated in inflammation (Mustain, et al., 2011) and neurological diseases (Caceda, et al., 2006). NT is increased in the skin following acute stress (Theoharides, et al., 1998) and increases vascular permeability, an effect synergistic with CRH (Crompton, et al., 2003), (Donelan, et al., 2006).

Mast cells are also stimulated by the peptide Substance P (SP), (Church, et al., 1991;Theoharides, et al., 2010d;Taracanova, et al., 2017a) initially characterized by Leeman and colleagues, (Chang and Leeman, 1970;Carraway and Leeman, 1973) and shown to participate in inflammatory processes (Mashaghi, et al., 2016;O'Connor, et al., 2004;Hokfelt, et al., 2001;Douglas and Leeman, 2011). IL-33 is a member of the IL-1 family of cytokines and has emerged as an early warning sign (dubbed "alarmin") (Moulin, et al., 2007) in autoimmune or inflammatory process (Saluja, et al., 2015;Theoharides, et al., 2015a;Theoharides, 2016). IL-33 is secreted by fibroblasts and endothelial cells, (Liew, et al., 2010) but also from mast cells. (Tung, et al., 2014) IL-33 augments the effect of IgE on secretion of histamine from mast cells and basophils (Moulin, et al., 2007), (Silver, et al., 2010), but the effect of IL-33 when used by itself or in combination with SP on secretion of IL-1 β from human mast cells has not been reported. Substance P stimulated secretion of VEGF, an action augmented by IL-33 (Theoharides, et al., 2010e).

We recently showed that stimulation of human mast cells by SP given together with IL-33 markedly increases secretion and gene expression of the pro-inflammatory cytokine, TNF (Taracanova, et al., 2017b). Interestingly, chronic rhinosinusitis, which is quite common in patients with ME/CFS as

discussed earlier, has been associated with high levels of nasal IL-33 (Ozturan, et al., 2017), which could reach the hypothalamus through the cribriform plexus.

Does any treatment modality work?

There are currently no FDA approved drugs for the treatment of ME/CFS and the available psychological, physical and pharmacological interventions do not appear to be effective (Bains, 2008;Pae, et al., 2009;Morris and Maes, 2014;Loades, et al., 2016;Collatz, et al., 2016;Castro-Marrero, et al., 2017;Brigden, et al., 2017). Mitochondria appear as one appealing drug target for the treatment of ME/CFS, but other papers reported no apparent alteration in ATP production (Shungu, et al., 2012b). Chemokines and cytokines have been proposed as targets for neuroinflammatory disorders (Pranzatelli, 2018), but such have not been tried in ME/CFS

The peroxisome proliferator-activated receptor (PPAR) agonist bezafibrate improves mitochondrial function by stimulating mitochondrial biogenesis and increasing the oxidative phosphorylation efficiency in a number of studies (Valero, 2014;Wang, et al., 2010;Johri, et al., 2012). It has also been suggested that, since fatigue is associated with hypotension in ME/CFS patients, increasing blood pressure might present an effective therapeutic approach to this symptom. Even though previous studies using the mineralcorticoid fludrocortisone failed to show any improvement (Peterson, et al., 1998), (Rowe, et al., 2016), use of the agonist midodrine to increase blood pressure has produced some improvement of the fatigue (Naschitz, et al., 2004). Interestingly, angiotensin II inhibitors have been shown to increase mitochondrial membrane potential, to improve mitochondrial function and to stimulate mitochondrial biogenesis (Morris and Maes, 2014), (de Cavanagh, et al., 2011). Indeed, blockade of angiotensin II has been shown to prevent the onset of T2DM in mice by increasing fat oxidation, decreasing muscle triglycerides and improving glucose tolerance (Mitsuishi, et al., 2009). The angiotensin receptor blocker telmisartan improves mitochondrial dysfunction by

enhancing mitochondrial biogenesis and protecting vascular and endothelial cell damage (Takeuchi, et al., 2013), (Kurokawa, et al., 2015). Similarly, the angiotensin receptor blocker losartan has been shown to improve mitochondrial respiratory chain function and coenzyme Q10 (CoQ10) content in hypertensive animals (Sumbalova, et al., 2010). However, given the blood pressure lowering effects of these agents it is unlikely they will be useful in ME/CFS, except maybe in select patients.

Several natural compounds may have a beneficial effect on mitochondrial function. Magnesium ions play critical roles in energy metabolism and in maintaining normal muscle function, by being positively active regulator of glycolysis and of all enzymatic reactions involving phosphate group transfer from ATP (Dominguez, et al., 2006), (Morris and Maes, 2014). Several studies have demonstrated that magnesium ion supplements significantly increase muscle strength and maintain optimal physical activity performance in humans (Brilla and Haley, 1992), (Newhouse and Finstad, 2000), (Kass and Poeira, 2015), (Zhang, et al., 2017). In experimental animals, this improvement in exercise performance seems to occur via enhancing glucose availability in the brain and muscle, and via reducing/delaying lactate accumulation (Zhang, et al., 2017). Magnesium sulphate may also improve mitochondrial respiratory function and prevent nitrous oxide production in the brain (Xu, et al., 2002), (Yang X, et al., 2007).

Coenzyme Q10 deficiency has been reported in patients with ME/CFS (Maes, et al., 2009), (Maes, et al., 2012), (Filler, et al., 2014). However, administration of CoQ10 to patients with ME/CFS have failed to show any benefit (Campagnolo, et al., 2017).

Naturally occurring flavonoids have potent anti-oxidant, anti-inflammatory and neuroprotective actions (Guo, et al., 2009; Middleton, et al., 2000; Xiao, et al., 2011) and are generally considered safe (Harwood, et al., 2007; Kawanishi, et al., 2005; Theoharides, et al., 2014; Theoharides, et al., 2014). The flavonoid genistein, attenuates muscle fatigue in humans by down-regulating oxidative stress and enhancing anti-oxidant enzyme activity (Ding and Liu, 2011). The flavonoids epigallocatechin, naringin and curcumin can ameliorate ME/CFS symptoms in experimental models

(Sachdeva, et al., 2009), (Vij, et al., 2009), (Gupta, et al., 2009), (Sachdeva, et al., 2011). Other reports have documented similar chronic fatigue attenuating effects for the Astragalus flavonoids (Kuo, et al., 2009) and of olive extract (Gupta, et al., 2010). The isoflavones genistein and daidzein, have been shown to reverse the effects of polyinosinic:polycytidylic acid (poly(I:C)) on mouse locomotor activity and brain inflammatory mediator expression in a mouse model of fatigue (Vasiadi, et al., 2014). Quercetin appears to increase exercise tolerance by attenuating oxidative stress in mouse brain, while at the same time conferring anti-oxidant and anti-inflammatory action (Kempuraj, et al., 2005), (Davis, et al., 2009), (Ishisaka, et al., 2011).

Luteolin suppresses adipocyte activation of macrophages and inflammation (Deqiu, et al., 2011;Ando, et al., 2009), while it increases insulin sensitivity of the endothelium (Deqiu, et al., 2011). Luteolin also inhibits mast cells (Asadi, et al., 2010;Weng, et al., 2015;Patel and Theoharides, 2017) and microglia (Jang, et al., 2008),(Patel, et al., 2016). In this context, it is interesting that luteolin improved symptoms of both ASD (Taliou, et al., 2013), (Tsilioni, et al., 2015), post-Lyme syndrome (Theoharides and Stewart, 2016) and brain fog (Theoharides, et al., 2015b) in open-label trials. We recently showed that tetramethoxyluteolin is more potent than luteolin in its ability to inhibit human cultured microglia (Patel, et al., 2016) and mast cells (Patel and Theoharides, 2017). Intranasal administration of select flavonoids may reduce inflammation in the hypothalamus and correct the central pathogenesis of ME/CFS. Novel treatment approaches are required to address the central pathogenic processes. For instance, intranasal administration of microvesicle-entrapped curcumin was shown to inhibit inflammation of the brain in a mouse model (Sun, et al., 2010).

Conclusions

Overall, the ME/CFS phenotype has been associated with apparent abnormalities in the metabolic profile, possibly due to local inflammation in the hypothalamus. Compounds that could inhibit

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inflammation in the brain, such as tetramethoxyluteolin or the anti-inflammatory cytokine IL-37 (Dinarello, et al., 2016), (Mastrangelo, et al., 2018), may be potential treatment options.

DISCLOSURES

TCT is the inventor of US patents No. 7,906,153; No. 8,268,365 and PCT application No. 13/722, 397 for the treatment of neuroinflammatory conditions.

CONFLICTS OF INTEREST

There is no conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Participated in searching the literature: EH, MA, IT, GD

Wrote or contributed to the writing of the manuscript: EH, MA, IT, TCT

Prepared the graphics: IT, TCT

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FOOTNOTES

Erifili Hatziagelaki, MD, PhD and Maria Adamaki, PhD are contributed equally.

Aspects of our work described above were funded in part by the Michael and Katherine Johnson Family Fnd and an Anonymous donation to TCT.

Legend to Figure 1.

Diagrammatic representation of the proposed mast cell-microglia interactions in the hypothalamus, contribute to the pathogenesis of ME/CFS, and could serve as targets for treatment.

Hypothalamic mast cells are stimulated by stress-associated triggers such as CRH, HK-1 and SP along with mtDNA and IL-33, some derived from nasal cavity, while others may reach the area through a disrupted blood-brain barrier or through lymphatics. Stimulated mast cells then secrete molecules such as CXCL8, NT, TNF, tryptase and mtDNA, (CXCL) which activate microglia to secrete more inflammatory molecules especially, IL-1 β , IL-6, and CXCL8 that further disrupt homeostasis, causes mitochondrial dysfunction and contribute to symptoms of ME/CFS. Luteolin could inhibit these processes at different steps as shown.

Table 1. Conditions Often Comorbid with ME/CFS

-
- Chronic inflammatory response syndrome (CIRS)
 - Fibromyalgia syndrome (FMS)
 - Ehlers-Danlos Syndrome (EDS)
 - Gulf War Illness (GWI)
 - Interstitial cystitis/bladder pain syndrome (IC/BPS)
 - Irritable bowel syndrome (IBS)
 - Mast cell activation syndrome (MCAS)
 - Multiple chemical sensitivity syndrome (MCSS)
 - Post-Lyme syndrome
 - Postural orthostatic tachycardia syndrome (POTS)
 - Post-traumatic stress disorder (PTSD)
 - Restless leg syndrome
-

Table 2. Dysregulated Molecules that May Contribute to the Pathogenesis of ME/CFS

- Cachexins
 - Calcineurin
 - Heavy metals
 - Herbicides
 - Inflammatory cytokines
 - Leptin
 - Melatonin
 - miRNAs
 - Mitochondrial enzymes
 - Neuroendocrine disruptors
 - Neuropeptides
 - Neurotransmitters
 - Reactive Oxygen Species
 - Toxins (mycotoxins, Borrelia toxins)
 - Uncoupling protein 2
 - Xenobiotics
-

Table 3. Mast Cell Triggers

<i>Stimulating degranulation</i>
Acetylcholine
Adenosine
Complement fragments
- C3 α , C4 α , C5 α
Drugs
- Local anesthetics, lactam antibiotics, neuromuscular junction blockers, vancomycin
Eosinophil granule proteins
IgE
IgG ₁
IgG ₄
Lysophosphatidylserine
Histamine
Serotonin
Lysophosphatidic acid
Peptides
- Adrenomedullin, CGRP, Endorphin, Endothelin, Hemokinin-1, Leptin, Mastoparan, Neurotensin, NGF, PTH, Somatostatin, SP, Thrombin, VIP
Tryptase
<i>Stimulating selective release of mediators without degranulation</i>
ATP
Borrelia burgdorferi (Lyme Disease)
CRH
Heavy metals
- Aluminum, cadmium, mercury
Herbicides
- Atrazine, glyphosate
IL-33
Mycotoxins
LPS
SCF
Viruses

Table 4. Mast Cell Mediators

Mediators	Pathophysiologic effect
<u>Prestored</u>	
<u>Biogenic Amines</u>	
Dopamine	Neurotransmission
Histamine	Vasodilation, angiogenesis, mitogenesis, pain
5-Hydroxytryptamine (5-HT, serotonin)	Vasoconstriction, pain
<u>Polyamines</u>	
Spermidine, spermine	Secretory granule stability, inhibition of secretion
<u>Chemokines</u>	
IL-8 (CXCL8), MCP-1 (CCL2), MCP-3 (CCL7), MCP-4, RANTES (CCL5), Eotaxin (CCL11)	Chemoattraction and tissue infiltration of leukocytes
<u>Cytokines</u>	
IL-4, IL-5, IL-6, IL-15, IL-17, IL-31, IL-33, TNF	Immune cell maturation, inflammation
<u>Enzymes</u>	
Arylsulfatases A	Lipid/proteoglycan hydrolysis
Beta-hexosaminidase	Degradation processes
Beta-glucuronidase	Degradation processes
Beta-glucosaminidase	Degradation processes
Beta-D-galactosidase	Degradation processes
Carboxypeptidase A	Peptide processing
Cathepsins B,C, D, E, L	Degradation processes
Chymase	Tissue damage, pain, angiotensin II synthesis
Grnzyme B	Inflammation and pre-apoptotic effects
Kinogenases	Synthesis of vasodilatory kinins, pain
Phospholipases	Arachidonic acid generation
Renin	Angiotensin II generation
Tryptase	Tissue damage, activation of PAR, inflammation, pain
Metalloproteinases (CPA3, MMP9, ADAMTSS)	Tissue damage, modification of cytokines/chemokines
<u>Growth factors</u>	
b-FGF	Neovascularization
NGF	Nerve growth, mast cell activation
SCF	Mast cell growth and activation
TGFβ	Anti-inflammatory, pro-fibrotic
VEGF	Neovascularization, vasodilation
<u>Peptides</u>	
ACTH	
Angiogenin	Neovascularization

Angiopoietin	Neovascularization
Corticotropin-releasing hormone	Inflammation, mast cell stimulus, vasodilation
Endorphins	Analgesia
Endothelin	Sepsis
Hemokinin-1	Inflammation, mast cell stimulus, pain, vasodilation
Kinins (bradykinin)	Inflammation, mast cell stimulus, pain, vasodilation
Leptin	Food intake regulator
Melatonin	Biologic clock regulator
Neurotensin	Inflammation, mast cell stimulus, vasodilation
RANKL	Osteoclast differentiation and activation
Somatostatin	Mast cell stimulant, anti-secretory
Substance P	Inflammation, mast cell stimulus, pain
Urocortin	Inflammation, vasodilation
Vasoactive intestinal peptide	Vasodilation, mast cell activation
<u>Proteoglycans</u>	
Chondroitin sulfate	Cartilage synthesis, anti-inflammatory
Heparin	Angiogenesis, nerve growth factor stabilization
Hyaluronic acid	Connective tissue, nerve growth factor stabilization
Serglycin	Storage of granule proteases
<u>De novo synthesized</u>	
<u>Chemokines</u>	
<u>CCL2, CXCL8, MIP-1α, MCP-1</u>	
<u>Cytokines</u>	
Interleukins (IL)-1,2,3,4,5,6,8,9,10,13,16,18	Inflammation, leukocyte migration, pain
IFN- α , IFN- β , IFN- γ ; MIF; TGF β ; TNF,	Inflammation, leukocyte proliferation/activation
<u>Growth Factors</u>	
SCF, β -FGF, neurotrophin 3, NGF,	Growth of a variety of cells
PDGF, TGF β , VEGF	
<u>Nitric oxide</u>	Vasodilation
<u>Phospholipid metabolites</u>	
Leukotriene B ₄	Leukocyte chemotaxis
Leukotriene C ₄	Vasoconstriction, pain
Platelet activating factor	Platelet activation, vasodilation
Prostaglandin D ₂	Bronchoconstriction, pain

Table 5. Microglia Mediators

Cytokines

- IL-1 β
- IL-6
- TNF

Chemokines

- CCL2
 - CXCL8 (IL-8)
 - CCL5 (MCP-1)
-

Figure 1.

