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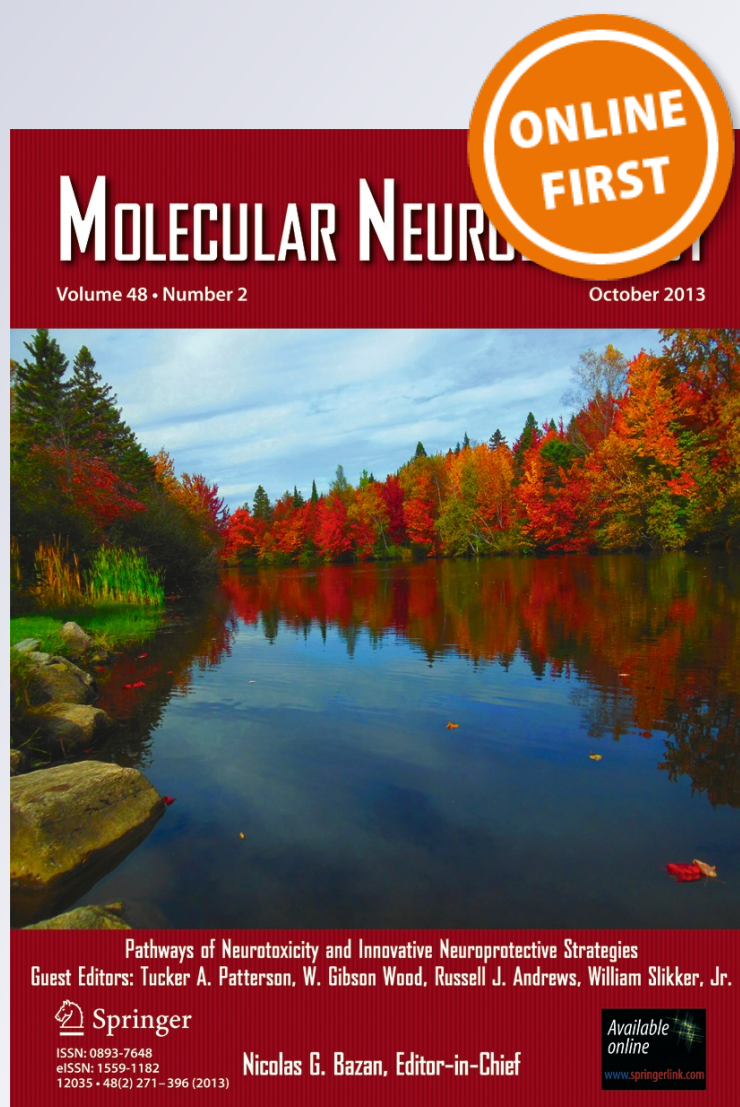
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The Emerging Role of Autoimmunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/cfs)

Gerwyn Morris · Michael Berk · Piotr Galecki · Michael Maes

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Abstract The World Health Organization classifies myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs) as a nervous system disease. Together with other diseases under the G93 heading, ME/cfs shares a triad of abnormalities involving elevated oxidative and nitrosative stress (O&NS), activation of immuno-inflammatory pathways, and mitochondrial dysfunctions with depleted levels of adenosine triphosphate (ATP) synthesis. There is also abundant evidence that many patients with ME/cfs (up to around 60 %) may suffer from autoimmune responses. A wide range of reported abnormalities in ME/cfs are highly pertinent to the generation of autoimmunity. Here we review the potential sources of autoimmunity which are observed in people with ME/cfs. The increased levels of pro-inflammatory cytokines, e.g.,

interleukin-1 and tumor necrosis factor- α , and increased levels of nuclear factor- κ B predispose to an autoimmune environment. Many cytokine abnormalities conspire to produce a predominance of effector B cells and autoreactive T cells. The common observation of reduced natural killer cell function in ME/cfs is a source of disrupted homeostasis and prolonged effector T cell survival. B cells may be pathogenic by playing a role in autoimmunity independent of their ability to produce antibodies. The chronic or recurrent viral infections seen in many patients with ME/cfs can induce autoimmunity by mechanisms involving molecular mimicry and bystander activation. Increased bacterial translocation, as observed in ME/cfs, is known to induce chronic inflammation and autoimmunity. Low ATP production and mitochondrial dysfunction is a source of autoimmunity by inhibiting apoptosis and stimulating necrotic cell death. Self-epitopes may be damaged by exposure to prolonged O&NS, altering their immunogenic profile and become a target for the host's immune system. Nitric oxide may induce many faces of autoimmunity stemming from elevated mitochondrial membrane hyperpolarization and blockade of the methionine cycle with subsequent hypomethylation of DNA. Here we also outline options for treatment involving rituximab and endotherapy.

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Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs) is characterized as a nervous system disease according to the World Health Organization, including the International Classification of Diseases (ICD-10, G93-3) [1, 2]. One defining characteristic of the disease is a global worsening of symptoms

or a prolonged state of relapse following even trivial increases in cognitive or physical activity, i.e., post-exertional malaise/fatigue (PEM) [3–5]. The pathology overlaps with what is observed in other neurological diseases such as multiple sclerosis and Parkinson's disease, which are underpinned by a triad of abnormalities involving activated microglia raised oxidative and nitrosative stress (O&NS) and mitochondrial dysfunction. Morris and Maes [1, 6] detailed the mechanisms by which prolonged pathogen infection leads to a chronic immuno-inflammatory environment in the periphery and ultimately results in activated microglia, raised O&NS, and mitochondrial dysfunctions. The vast majority of ME/cfs patients report multiple recurrent or persistent bacterial and viral infections [7–9]. The multiplicity of infections correlates positively with the number and severity of symptoms in people with ME/cfs. Interestingly, this relationship also extends to neurological symptoms [10]. Concurrent infections appear to globally worsen symptoms [11]. Several authors have reported reduced CD56 bright natural killer cell (NKC) function in ME/cfs [12–14]. Reduced NKC functioning has been a consistent finding with several research teams detecting impaired NKC activity in ME/cfs patients [12, 14, 15]. Strong evidence of T cell exhaustion has been reported in ME/cfs patients by many different teams of workers [15, 16].

Many studies using peripheral blood measures have shown redox dysregulation, indexed by decreased levels of antioxidants, e.g., zinc, coenzyme Q10, and glutathione [17–20] in ME/cfs and increased O&NS by-products, including higher levels of peroxides and thiobutyric acid, increased isoprostane levels, and elevated protein carbonyl levels [5, 17, 20–22]. A number of studies have demonstrated that oxidative stress measures correlate significantly and positively with symptom severity [5, 17, 19–22].

Other findings of increased O&NS in ME/cfs involve reports demonstrating active expression of immuno-inflammatory pathways known to promote free radical generation and oxidative damage, such as increases in pro-inflammatory cytokines, e.g., interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , nuclear factor (NF)- κ B, cyclo-oxygenase-2 (COX-2), and inducible nitric-oxide synthase (iNOS) [18, 23–27]. Dysregulation of both pro-inflammatory and anti-inflammatory cytokines may occur in ME/cfs [18, 23–26]. In many ME/cfs patients, however, the role of pro-inflammatory cytokines is dominant [13, 28–30]. Abnormalities in IL-6 and transforming growth factor (TGF) β -1 are also commonly reported [1]. Lowered levels of ω 3-polyunsaturated fatty acids in ME/cfs may further increase the inflammatory potential [31].

Prolonged elevated levels of O&NS and pro-inflammatory cytokines play a role in the abnormalities in mitochondrial function reported by several teams of workers [20, 32–34]. Patients with ME/cfs reach perceived exhaustion at a much earlier time point than healthy controls. They display increased lactate and diminished adenosine triphosphate (ATP)

production compared to healthy controls which is even more evident upon repeat exercise testing in both brain and striated muscle. Impairments in oxidative metabolism result in a marked acceleration of glycolysis in striated muscle and a prolonged recovery time needed to restore pre-exercise levels of ATP [35] state [32, 36]. Increasing leptin levels in people suffering from ME/cfs [37] using low-dose cortisone appears to be of benefit [38]. Low levels of leptin are found in people with clinical depression [39], although higher leptin levels have been shown to predict the development of de novo depression [40]. Recently, we have provided evidence that increased production of pro-inflammatory cytokines, immune activation, activated O&NS pathways, and increased lactate and diminished ATP production in ME/cfs may conspire to cause PEM [1, 5, 6, 28].

All the abovementioned abnormalities can conspire to create multiple sources of autoimmune responses and pathology in ME/cfs. The aim of this paper is to review the different types of autoimmune responses in ME/cfs, the etiological factors that play a role in the onset of those autoimmune reactions in ME/cfs and putative treatments of the autoimmune reactions.

Autoimmunity in ME/cfs

Not all patients with ME/cfs suffer from autoimmunity or emerging autoimmune responses. The percentage of positive ME/cfs patients showing autoimmune responses is difficult to establish; however, estimates vary from 30 to 60 % and around 40–50 % show the development of autoimmune reactions directed against a multitude of different neopeptides [27].

B Cells in ME/cfs

Increases in the number of mature CD19 B cells have been reported in ME/cfs patients [41–43]. Klimas et al. [15] reported elevated number of CD20 and CD21 B cells in their trial population. In a recent study, Bradley et al. [44] reported an extremely large and significant increase in the number of naive B cells, which express CD19 and CD20, as a percentage of total lymphocytes and B cells in ME/cfs. The authors also detected significantly greater numbers of transitional B cells and a markedly depleted plasma blast population which they opined pointed to a state of autoimmunity in the patients examined.

Autoimmunity in ME/cfs

Autoantibodies against neurotransmitter receptors, including 5-hydroxytryptamine receptor 1A (HTR1A), dopamine receptor D2 (DRD2) and muscarinic cholinergic receptor 1 (CHRM1), and mu-opioid receptor (OPRM1), have been reported in patients with ME/cfs. Patients with ME/cfs show a higher anti-CHRM1 antibody index and presence of antinuclear antibodies

[45]. Antineuronal antibody levels are particularly elevated in ME/cfs patients with neurologic abnormalities [46]. The presence of autoimmune antibodies in the systemic circulation may result in muscular and or mental fatigue by binding with calcium channels and acetylcholine receptors or antigens in the CNS [47]. Large subgroups of ME/cfs patients display indicators of autoimmune responses directed against antilamine, microtubule-associated proteone, ssDNA, phospholipids, gangliosides, 5-HT, and 68/48kd proteone [48–51]. Table 1 reviews the different autoimmune findings in ME/cfs.

Neopeptides in ME/cfs

IgM-mediated immune responses directed against endogenous molecules are present in ME/cfs patients. These molecules have been damaged undergoing changes in conformation due to high levels of O&NS damage and have become immunogenic as a result. Consequently, autoimmune responses are generated against by-products of lipid peroxidation, such as azelaic acid

Table 1 Signs and drivers of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/cfs)

Signs of autoimmune responses in ME/cfs
↑ Numbers of CD19, CD20, and CD21 B cells
↑ Anti-serotonin (5-HT) antibodies
↑ Anti-5-HTA receptor antibodies
↑ Anti-D2 receptor antibodies
↑ Anti-muscarinic cholinergic receptor antibodies
↑ Antinuclear antibodies
↑ Antineuronal antibodies
↑ Antilamine antibodies
↑ Microtubule-associated proteone antibodies
↑ ssDNA
↑ Anti-phospholipid antibodies
↑ Ganglioside antibodies
↑ 68/48kd proteone
↑ IgM against oxidatively modified neopeptides
↑ IgM against nitric oxide adducts
Positive clinical response to treatment with rituximab
Drivers of autoimmune responses in ME/cfs
Reduced numbers and functions of natural killer cells
Viral infections through molecular mimicry and bystander effects
Mitochondrial apoptosis and necrosis pathways and shortfalls in ATP
Elevated levels of nuclear factor-κB
Cytokines inducing T helper 17 phenotype differentiation
Bacterial translocation with lipopolysaccharide-associated molecular mimicry
Formation of immunogenic oxidatively and nitrosatively modified neopeptides
Increased concentrations of homocysteine and DNA hypomethylation
Activated mammalian target of rapamycin, e.g., via increased nitric oxide

and malondialdehyde (MDA), corrupted anchorage molecules, including oleic, palmitic, and myristic acid and S-farnesyl-L-cysteine, and NO-modified amino acids and proteins, including NO-phenylalanine, NO-arginine, NO-tyrosine, NO-tryptophan, NO-cysteine, and NO-albumin [17]. The concentrations of these damaged molecules correlate significantly and positively with symptom severity. Raised serum IgM levels directed against membrane constituents and anchorage molecules additionally correlate with increases in muscular fatigue and a flu-like malaise [17].

Factors that Contribute to Autoimmune Responses in ME/cfs

Role of NK Cells in Autoimmunity

NKCs, via their rapid response and their ability to destroy infected cells, are the primary defense against pathogen invasion and are active before the adaptive immune system comes into play [52, 53]. They also interact with other immune cells, however, and also perform a major role in immune regulation particularly in controlling excessive T cell proliferation and regulating the activation and differentiation of myeloid dendritic cells. NKCs facilitate dendritic cell (DC) development and T cell differentiation, but NKCs can also inhibit (auto)immune responses by destroying autologous myeloid and lymphoid cells.

NKCs resident in peripheral blood may be subdivided into two subsets, i.e., those that express CD56 or CD16 receptors. CD56dimCD16+ NKCs make up approximately 90 % of NKCs found in blood. These NKCs attack and kill target cells with great efficiency but secrete only very low levels of cytokines. CD56brightCD16– NKCs, however, constitute less than 10 % of the entire NKCs population and are found in much greater concentrations in secondary lymphoid tissues [54]. This NKC subset, when activated produces a range cytokines, including interferon (IFN)-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF), but need to be activated before they are able to exert a cytotoxic function [55].

There is also a growing understanding of the role of NKCs in the genesis of autoimmunity [56, 57]. Some studies report that NKCs impede or impair autoimmune responses [58, 59]. Others, however, suggest that NKCs have an enabling role in autoimmunity, possibly due to their innate ability to supply an early source of cytokines with subsequent activation of antigen presentation cells (APCs) and hence the development of inflammatory and ultimately pathogenic Th1 (and Th17) responses [60, 61]. The weight of evidence suggests that NKCs can regulate inflammation and induce loss of self-tolerance at a number of different levels during immune activation and, thus, suggest that NKCs play quite different roles in different kinds of autoimmunity. It appears that their roles also vary

during different stages of the processes which ultimately cumulate in the development of autoimmune diseases.

NKC functions (and sometimes numbers) decline in majority of autoimmune diseases as the diseases progress [62]. There has been a great deal of debate regarding a primary role for NKCs in engendering autoimmunity [57, 62]. The weight of evidence indicates that NKCs promote the creation of autoreactive Th1 cells and subsequent disease at a relatively early stage in the genesis of autoimmune disease [60, 61]. Once autologous T cells come into play, NKCs are controlled by cytokines (in particular IL-21) secreted by these cells which lead to a functional deficiency and even a partial deletion of the NKCs in question [63]. IL-21 can stimulate the activation and differentiation of T and NKC populations, but can also induce the death of those cells [64, 65]. The effect of IL-21 on NKCs seems dependent on the functionality of the cells [64, 65]. T cells appear to have two different contrasting kinds of influence on NKCs. Firstly, T cells act to support NKC activity under conditions of chronic infections, via the provision of IL-2. Secondly, T cells attenuate NKC activity via the release of IL-21 [63]. The bidirectional interaction or cross talk between NKCs and T cells is facilitated by colocalization [66, 67] and dendritic cells act as communicative bridges between the two cell types [63, 68]. Numerous studies have reported that people with autoimmune diseases like systemic lupus erythematosus (SLE) and multiple sclerosis (MS) have depleted numbers of NKCs compared to controls and that those cells display grossly impaired functions [69, 70].

The results of four magnetic resonance imaging (MRI) studies involving intravenous daclizumab in MS provide very strong evidence supporting the role of NKCs in disease pathology [71]. Daclizumab (Zenapax®) is a humanized monoclonal anti-CD25 antibody that was first approved in renal allograft rejection and later examined in clinical trials in MS [71]. The numbers of CD16-NKCs increased in the peripheral blood of patients treated with daclizumab. Moreover, the reduction in disease activity, measured by MRI, correlated significantly and positively with the expansion of this NKC subset [72]. NKCs obtained from the blood of patients during therapy destroyed autologous-activated T cells, while NKCs taken from the same patients before therapy did not [72]. This supports the notion that reduced numbers and function of CD56 bright CD16-NKCs contribute to the development or maintenance of the disease [54]. By inference, the frequently reported finding that NKCs and NKC activity are significantly lowered in ME/cfs may play a role in the onset of autoimmunity in that disorder.

Role of Viral Infections in Autoimmunity

Viral infections lead to the synthesis of IFN- α and IFN- β . If the levels of IFN- α and IFN- β are high enough, subsequent activation of IL-12 and NKCs is blocked [73, 74]. With some

viruses, however, this IFN response is not sufficient to block high levels of IFN- γ and IL-12 [74] and this situation may lead to autoimmunity. Bystander activation, molecular mimicry, and chronic viral infection possibly involving epitope spreading are potential processes that may generate immunoreactivity eventually producing autoimmunity.

Virus Infections and Molecular Mimicry

Molecular mimicry involves the presence of a shared epitope between a virus or other pathogen and a host [75]. Cross-reactivity between viruses and hosts are commonplace. In order for overt autoimmune disease to emerge, the cross-reactivity must involve an epitope which has a crucial part to play in biochemical cascades, and damage to its structure can lead to pathology. If no such epitope is involved, a state of autoimmune responsiveness may exist but no disease emerges [75]. Chronic viral infections may cause immune- and O&NS-mediated damage because of the chronic presence of viral antigens activating the immune system. For example, numerous viruses are associated with the onset and maintenance of MS. Approximately two dozen different viruses are isolated from the CNS of MS patients, including herpesviruses, paramyxoviruses, and retroviruses, etc. [76, 77]. Numerous studies have reported exacerbation of disease activity following virus infections [78, 79]. Despite the isolation of those viral entities, none have been definitively established as being the causative pathogen [80]. Viral antigens may display very similar if not identical three-dimensional structure and/or sequence homology to host epitopes [75]. Such viral infections can ultimately lead to primed or activated preexisting self-reacting T cells leading to autoimmunity. Such preexisting autologous T cells would in the case of MS include a T cell population which is reactive to myelin. Myelin-specific T cells have been detected in patients with MS and healthy controls [81]. This is essentially the mechanism of autoimmunity induced by molecular mimicry. Infection by viruses lacking homologous structures may cause autoimmunity via the bystander activation mechanism. This involves a localized intense cytokine response resulting in the proliferation and migration of self-reactive T cells into the CNS [82]. This will be dealt with in more detail in a subsequent section.

Viral Infections and Bystander Effects

Bystander T cell activation produces functional and phenotypic changes in T cells without the need for engagement of the T cell receptor (TCR) [83]. While other receptors on cell membranes may be involved [84], cytokines alone are capable of provoking this mode of activation [85]. T cells are normally activated via the engagement of highly specific TCRs and MHC molecules [86, 87]. These cells may also be activated unconventionally by TCR independent or bystander signaling

[83]. Bystander T cell occurs in herpes simplex virus, LCMV, and HIV infections leading to polyclonal expansion of memory T cells and subsequent production of protective or pathological cytokines. The underlying mechanism of bystander IFN- γ activation is dependent on pro-inflammatory cytokines, mainly IL-12 and IL-18 [88].

For example, a role for bystander activation may account for the cell death resulting from HIV infection which cannot be explained by direct infection of T cells [89]. Activation of immuno-inflammatory pathways has been proposed as a major cause of cell death [90], a proposal supported by a considerable body of evidence [90]. Virus infections also provoke activation of dendritic cells and macrophages. These activated antigen-presenting cells can also activate preprimed autoreactive T cells, which in turn may drive autoimmune responses. Additionally, T cells which are virus-specific might also instigate bystander activation. Briefly, CD8⁺ T cells recognize virally infected cells at sites of infection and secrete toxic granules causing the death of these cells. The apoptotic fragments, the CD8⁺ T cells and macrophages then generate cytokines, such as TNF- α , and NO, which drive bystander destruction of cells in the immediate vicinity even when not infected. This produces exaggerated immunopathology at the sites of viral infection [77].

Mitochondrial Apoptosis Pathways and Autoimmunity

Apoptosis and Necrosis

Identification of apoptotic cells by phagocytes drives the production of anti-inflammatory cytokines, e.g., IL-10, prostaglandins, and TGF- β 1 [90, 91] and actively restrains the activity of pro-inflammatory cytokines generated by engagement of Toll-like receptors (TLRs) [92]. Apoptotic cells may block TLR-dependent release of pro-inflammatory cytokines and chemokines, processes which are regulated at the transcriptional level [93]. Thus, the stimulation of phagocyte receptors not only results in the immune silent removal of dying cells but produces signals leading to the production of anti-inflammatory agents which generate an immunosuppressive milieu during the elimination of apoptotic cells. During necrotic cell death, on the other hand, the uncoordinated and massive release of cell contents may cause profound immuno-inflammatory responses. The other homeostatic process involved in apoptotic elimination is the generation of anti-inflammatory compounds, which inhibit immuno-inflammatory pathways and enable the removal of apoptosed cells. Failure to efficiently remove apoptotic cells can generate autoimmunity [94]. Impaired apoptotic clearance means that apoptotic cells can undergo necrotic death which involves the lysis of cells releasing their contents thus provoking activation of immuno-inflammatory pathways directed against the voided intracellular antigens and DNA [95, 96]. This provides an immunogenic impetus involved in the

etiopathology of several human autoimmune disorders notably systemic lupus erythematosus and rheumatoid arthritis [97].

In the extrinsic pathway, released ligands (signaling molecules) bind to death receptors such as Fas on the target cell resulting in the induction of apoptosis [98]. Apoptotic signals triggered by Fas (Apo1/CD95) can be transmitted via two pathways depending on cell type [99]. Fas excitation activates caspase-8 in type 1 cells by express formation of the DISC3 (death activating complex) [100, 101], which subsequently activates caspase-3, once again resulting in apoptosis. In type 2 cells, DISC formation is less marked and alterations in mitochondrial membrane potential (DCm) occur before the activation of caspase-8 and caspase-3. Active caspase-8 can also regulate the escape of cytochrome c in the intrinsic pathway [101, 102].

The intrinsic pathway is activated by cellular stress, especially elevated free radicals and elevated O&NS. Once the stress signal is received, proapoptotic proteins such as BAX and BID induce the release of cytochrome c together with other contents from the mitochondria [103] into the cytoplasm. Once released, cytochrome c interacts with ATP to form a complex that ultimately activates caspase-9, one of the apoptosis initiator proteins [104]. Consequently, an apoptosome is formed, which ultimately activates a final effector protein that initiates caspase-3 degradation [104]. Low ATP concentrations, as are frequently observed in ME/cfs, inhibit this apoptotic pathway.

Mitochondrial ATP, Apoptosis, Necrosis, and Autoimmunity

A number of studies have reported that depletion of intracellular ATP completely inhibits Fas-mediated apoptosis and that ATP-dependent steps occur down- and upstream of caspase-3-like protease activation [105]. Hence, a shortfall of ATP can inhibit the intrinsic as well as the extrinsic pathway. It is not surprising, therefore, to learn that the ATP concentration is a major determinant of cell demise by apoptosis and necrosis [105]. Moreover, apoptotic signals are communicated to the cell nucleus via an ATP-dependent mechanism [106]. Indeed, this active transport mechanism is vital for generating apoptotic changes of the nucleus [107]. Necrosis is regarded as a form of highly regulated programmed death enabled by communication between an array of ATP-dependent kinase enzymes [108, 109]. Death receptor signaling is regarded as a major driver of ATP-dependent necrosis and may occur without any perceptible depletion in cellular ATP. This pathway is regulated by a series of protein kinases with one particular threonine kinase namely RIP-1 being the key regulator. Energy availability is a key driver of different types of necrosis [110]. Below a certain ATP threshold, an instigated apoptotic response fails but necrosis takes over. Hence, the level of ATP depletion is involved in the type of cell death [111]. We conclude that shortfalls in ATP may either drive programmed

cell death (PCD) towards the necrotic pathway or indeed inhibit cellular death altogether or dramatically increase the survival time of effector T cells in the immune system. In either case, a shortfall of ATP may be a driver of autoimmunity and chronic inflammation. Another cause (or indeed consequence) of chronic inflammation is activated NF- κ B which we will discuss in the next subsection.

Role of NF- κ B in Generating Autoimmunity

NF- κ B and Generation of Chronic Inflammation

The increased levels of NF- κ B, which are frequently observed in ME/cfs [24], may also play a role in the generation of autoimmunity. The activation of NF- κ B initiates a number of transcriptional events leading to autoregulation of inflammatory cascades via modulation of NF- κ B activation [112]. NF- κ B can be activated by numerous stimuli. Such stimuli include bacterial endotoxins [113], TNF- α , IL-1 β [1, 114], elevated O&NS [1, 115], mitogens, and viral proteins [114]. NF- κ B activation stimulates the transcription of TNF- α and IL-1 β , both of which in turn are known activators of NF- κ B [1]. An inflammatory stimulus such a bacterial endotoxin leads to the activation of NF- κ B, which enhances TNF- α and IL-1 β production resulting in the amplification of the initial inflammatory signal [112]. NF- κ B during an inflammatory response activates the synthesis of iNOS that produces NO [116].

NF- κ B and Autoreactive B Cells

Elevated levels of NF- κ B additionally increase survival of autoreactive B cells [117]. A further contribution made by elevation of NF- κ B to autoimmunity is the consequent upregulation of B cell activating factor (BAFF), a crucial cytokine in the survival of B cells and resting mature B cells [118, 119]. BAFF is not only a crucial survival factor during B cell development but is also an essential factor in the development of B cell tolerance. Breakdown of the mechanisms regulating BAFF expression results in exaggerated BAFF synthesis that diminishes B cell tolerance and drives B cell hyperplasia and leads to autoimmune phenomena [120].

NF- κ B and Cell Death

There are two NF- κ B pathways involved in the regulation of cell survival or cell death [121]. The classical pathway is activated by bacterial or viral infections or elevated levels of pro-inflammatory cytokines [122]. This is the pathway which is normally involved in the inhibition of PCD [123, 124]. The second or alternative pathway is stimulated by a range of TNF molecules [125] and is particularly important in premature B cell survival [126]. Two well-illuminated pathways lead to PCD [127], i.e., the mitochondrial or intrinsic pathway and, secondly,

the death receptor (DR) or extrinsic pathway [128]. Both pathways are caspase-dependent [128, 129]. NF- κ B suppresses both PCD types [130, 131] and exerts this prosurvival activity through different anti-apoptotic proteins [123, 132].

NF- κ B promotes the expression of different compounds of the Bcl-2 family which impede apoptosis through mitochondrial mechanisms involving caspase-8-governed Bid cleavage and cytochrome c release [123, 132]. NF- κ B and c-Jun activating kinase (JNK) are activated simultaneously under a variety of stress conditions. Conversely, sustained activation of NF- κ B inhibits cytokine-induced JNK activation [133]. Whereas temporary activation of JNK following TNF treatment promotes cell survival, chronic JNK activation encourages cell demise. NF- κ B activation restrains the chronic phase of JNK activation and therefore protects cells against cytotoxicity induced by TNF- α [134].

Multiple positive feedback loops exist between the instigators of cellular death, O&NS, caspases, and JNK. Crucially, NF- κ B suppresses these positive feedback loops by activating caspase inhibitors, BCL-2 compounds, and antioxidants. Hence, elevation of NF- κ B leads to a depletion of reactive oxygen species (ROS) and caspase inhibition. NF- κ B activation additionally inhibits sustained JNK activity which explains the prosurvival activity of NF- κ B [135]. NF- κ B, cytokines, and elevated O&NS are conspiratorial partners in producing a chronic immunoinflammatory state and consequent immune sequelae involving a number of different mechanisms [1]. We now turn to causes of autoimmunity produced by abnormal levels of cytokines and O&NS, two other characteristics of ME/cfs.

Cytokines and T Cell-Mediated Autoimmunity

IL-6 in combination with TGF- β induces the production of Th17 cells [136]. Several cytokines apart from TGF- β 1 and IL-6 support the differentiation of naive T cells towards the Th17 phenotype. These cytokines include IL-1 β , IL-21, and IL-23 [136, 137]. IL-6 actually orchestrates the differentiation of Th17 cells by activating STAT-3 leading to the subsequent production of IL-21 and IL-23 [138]. These latter cytokines are activators of ROR γ t, the transcription factor governing the shift of naive T towards Th17 cells. IL-21 is essential to this process and may drive Th17 differentiation in the absence of IL-6 [136, 139]. IL-23 is not essential for the differentiation of Th17 T cells but is vital for their survival and their function. Hence, Th17 T cells are not pathogenic in the absence of IL-23 [136, 140]. IL-1 amplifies the generation of Th17 cells induced by TGF- β 1 and IL-6 and may cause a Th17 shift in the absence of IL-6 [136, 139, 140]. IL-1 overproduction results in the conversion of FOXP3 T regulatory (Treg) into Th17 cells [141, 142]. It is noteworthy that while elevated levels of IL-2 normally suppress the generation of Th17 cells, elevations of IL-2 in the presence of elevated IL-1 have a stimulatory effect [143]. IL-6 has another pivotal role in the

production of Th17 cytokines by inhibiting the differentiation and function of FOXP3 regulatory T cells. FOXP3 inhibits the transcription of ROR gamma and hence suppresses Th17 differentiation [144].

Th17 cells are encephalitogenic in the presence of IL-23 and readily cross the blood–brain barrier (particularly if inflamed) and induce neuroinflammation [140] via a number of mechanisms. These mechanisms include the secretion of granzyme B causing neuron damage and stimulating the recruitment of CD4 + T cells, neutrophils, and dendritic cells into the CNS [145]. Th17 most readily cross the blood barrier if it is already inflamed. Elevated levels of IL-1 β and TNF- α lead to the disruption of endothelial tight junctions in the blood–brain barrier [146, 147] and the gut [148, 149]. These pro-inflammatory cytokines may not only facilitate neuroinflammation by causing disruption of the blood–brain barrier (BBB), but also do so by increasing the permeability of tight junctions in the gut and allowing translocation of bacteria and endotoxins into the bloodstream.

Bacterial Translocation, Neuroinflammation, and Autoimmunity

Increased bacterial translocation, a new pathway in ME/cfs [17, 30], is another factor that may cause autoimmunity in ME/cfs. IL-1 β and TNF- α cause an increase in intestinal endothelial cell permeability via the activation of NF- κ B [149] and disrupt endothelial cell tight junctions allowing the translocation of gram-negative bacteria and LPS or endotoxins into the mesenteric lymph nodes or the circulation leading to activation of immuno-inflammatory responses [18, 30, 150]. The microvascular endothelial tight junctions in the BBB are very similar to those in the intestine, and hence, molecular entities such as Th17 T cells which can damage the tight junctions in the intestine may also damage the tight junctions in the brain. Circulating bacterial LPS disrupt the integrity of the BBB [151] and lead to the activation of microglia [152]. Microglia are responsible for activating and propagating the immune response in the CNS following pathogen invasion [153, 154]. Microglia (and astrocytes) are able to “perceive” pathogens via their pattern recognition receptors. Binding of pathogens promotes recruitment and antigen-specific activation of infiltrating leucocytes [154]. Activation of microglia and subsequent activation of astrocytes result in neuropathological changes, eventually leading to the development of neuroinflammation of neurodegenerative disease [1, 6].

Bacterial LPS can also generate neurological diseases which are autoimmune in origin. For example, the central oligosaccharides of the LPS of *Campylobacter jejuni* demonstrate a high degree of mimicry with gangliosides [155]. Cross-reactivity between anti-LPS and anti-ganglioside antibodies plays a pivotal role in the etiology of Guillain–Barré syndrome [18, 156]. LPS appears to stimulate the release of

glutamate, ultimately removing the voltage-sensitive Mg²⁺ block of NMDA receptors, promoting excitotoxicity [157]. LPS molecular mimicry resulting from gram-negative bacteria associated with Alzheimer’s disease (beta-amyloid), AIDS dementia (gp120 and gp41), or multiple sclerosis (myelin basic protein) may well explain the transient neurological and neuropsychiatric symptoms often associated with these diseases [157]. Some authors suggest that LPS-associated molecular mimicry may in fact cause MS and Parkinson’s disease [158, 159] and that increased intestinal permeability plays a role in both disorders [160, 161].

We have discussed a number of mechanisms whereby autoimmunity in the periphery could induce a state of autoimmunity in the CNS. Indeed the existence of autoantibodies to neurotransmitters and anchorage molecules has been repeatedly demonstrated in ME/cfs. The next section concentrates on autoimmunity induced by elevated O&NS. The basic principle underpinning this data is that epitopes may be damaged by exposure to prolonged O&NS and thus lose their immunogenic tolerance and become a target for the hosts’ immune system [17].

Autoantibodies Resulting from O&NS-Modified Epitopes

IgM-related immune responses have been reported in ME/cfs patients directed towards corrupted components of lipid membranes and anchorage molecules, such as oleic, palmitic, and myristic acid, and S-farnesyl-L-cysteine [17, 29]. IgM autoantibodies are also produced against derivatives of lipid peroxidation, e.g., azelaic acid and malondialdehyde (MDA), and nitrosatively modified amino acids or proteins, such as NO-tyrosine, NO-phenylalanine, NO-arginine, NO-tryptophan, NO-cysteinyl, and NO-albumin. Interestingly, IgM-mediated immune responses directed against the same neoepitopes (e.g., lipid membrane fatty acids, anchorage molecules and NO-adducts) and comparable immuno-inflammatory and O&NS processes have been observed in major depression [18, 27, 50]. Recently, we have reviewed that these shared immuno-inflammatory, O&NS and autoimmune pathways may underpin the comorbidity between ME/cfs and major depression [50]. These secondary autoimmune responses directed against neoepitopes are significantly greater in ME/cfs than in major depression [27].

Lipid peroxidation induced by elevated ROS leads to the genesis of highly reactive aldehydes, including 4-hydroxynonenal (4-HNE) and MDA. These entities can engage in covalent bonding with proteins, modifying their structure, which in turn affects their biological functions [162] potentially making them highly immunogenic [163, 164]. The importance of NO in disease etiology lies with generation of superoxide, ultimately leading to the production of peroxynitrite (ONOO[−]). This entity is a powerful nitrating and oxidizing agent which for example reacts with tyrosine

residues to produce nitrotyrosine (NT) [165]. Elevated NT levels have been found in many pathologies [166, 167]. Additionally, modifications of DNA and proteins by peroxynitrite may amplify their immunogenicity, causing a break in immune tolerance [163, 167, 168].

The role of elevated O&NS in the generation of antibodies is therefore one mechanism by which these signaling molecules underpin the development of autoimmunity. The next section deals with autoimmune pathology initiated by elevated NO, which are frequently observed in ME/cfs [17, 29]. The section will be a brief and simple summary of the molecular pathways involved.

Nitric Oxide, the Methionine Cycle, and Autoimmunity

Nitrous oxide irreversibly inhibits methionine synthase, the enzyme responsible for the remethylation of homocysteine to methionine, via the oxidation of the essential cofactor cobalamin [169]. This inhibition leads to elevated levels of homocysteine and depleted concentrations of methionine. Increased concentrations of homocysteine and S-adenosylhomocysteine in the intracellular space result in hypomethylation of DNA [170]. This state can lead to the overexpression of many genes involved in autoimmunity [171, 172]. Such hypomethylation of DNA is observed in a number of autoimmune diseases [173], e.g., rheumatoid arthritis [174], SLE [172], and inflammatory arthritis [175]. Hypomethylation of lymphocyte DNA produces an increase in self reactivity in antigen-specific T cells [176] and stimulates the production and proliferation of autologous B cells [177]. The increase in autoreactive B cells numbers and function is considered to be a major contributing element in autoimmunity induced by abnormalities in this lymphocyte population which will be considered in the final section of the paper. Before considering this area in detail, however, the next section focuses on the role of NO as a regulator of mitochondrial membrane potential and the mammalian target of rapamycin (mTOR).

NO acts as a signaling molecule which regulates mitochondrial membrane potential [178]. Prolonged elevated NO concentrations lead to mitochondrial membrane hyperpolarization [179] and subsequent stimulation of mTOR [180]. mTOR is a serine/threonine protein kinase and a monitor of the mitochondrial transmembrane potential which controls protein synthesis, cell growth, cell proliferation, and survival [181]. mTOR is additionally a major regulator of T cell homeostasis [182] and chronic activation disrupts the regulation of the immune response producing effects predisposing to autoimmunity. These effects include increased proliferation of autoreactive T cells coupled with an increase in B cell autoantibody production [183]. Activated mTOR drives the differentiation of naive T lymphocytes towards the effector phenotype [184] and inhibits Treg differentiation and FOXP3 expression thus not only reducing Treg numbers but also Treg

cell function [182, 185, 186]. Natural CD4 + FOXP3 Treg cells are not terminally differentiated and display developmental plasticity. Activated mTOR “reprograms” natural Tregs into Th17 or Th1 T lymphocytes [185]. mTOR also controls the activation and maturation of plasmacytoid dendritic cells [184, 187]. The net effect of activated mTOR is the increase in IFN- α produced by plasmacytoid dendritic cells and an increase in the costimulatory capacity of myeloid dendritic cells [187].

Elevated levels of leptin, a cytokine heavily involved in energy regulation, also activates mTOR [188]. It is perhaps not surprising therefore that elevated levels of leptin are found in many autoimmune conditions [189]. The following section examines the effects of elevated levels of this cytokine on different players involved in the immune response.

Leptin and Autoimmunity

Leptin levels are elevated by prolonged activation of pro-inflammatory cytokines [190] and subsequently have profound effects on the immune system further predisposing to an autoimmune environment. Leptin exerts a stimulatory effect on NO production [191] and further upregulates the activity of pro-inflammatory cytokines [183, 192]. Elevated levels of leptin potentiate the production of IL-12, IFN- γ , and IL-2 and suppress the synthesis of IL-10 and IL-4 [193, 194]. Activated T cell survival and proliferation are both enhanced by leptin [195, 196], while the proliferation of FOXP3 CD4 + CD25+ Tregs is inhibited by this cytokine [183]. Evidence suggests that elevated levels of leptin leads to the activation and improved survival of resting T and B lymphocytes with subsequent secretion of TNF- α and IL-6 by both cell types [197]. Leptin also modulates the secretion of pro-inflammatory cytokines and chemokines by macrophages [193], monocytes [198], and eosinophils [199]. Dendritic cell survival and Th1 priming are both increased by elevated leptin levels as is their production of pro-inflammatory and Th1 cytokines [200].

All in all, elevated levels of leptin may induce a de novo autoimmune environment by inducing the activation of T and B cells while driving T cell differentiation towards the Th1 or Th17 phenotype or by potentiating the deleterious effects of an already chronically activated immune system. In the forthcoming final section, we consider the management role of B cells and their contribution to autoimmune pathology which exists quite independently of their ability to produce antibodies.

Antibody-Independent Generation of Autoimmunity by B Lymphocytes

B cells synthesize cytokines in response to a wide range of extracellular signals. These signals include microbial endotoxins, antigens, and T cells [201, 202]. In individuals with autoimmune disorders, cytokine-producing B cells are found

in the blood and primary lymphoid tissues [201, 203]. Cytokines produced by B cells are able to modulate the development and function of many pivotal immune cells, including T, NK, and dendritic cells [204]. This management role of B cells probably explains the immunoregulatory antibody-independent activities of B cells [205].

Interestingly, rituximab therapy induces prolonged remission in several autoimmune diseases without any significant depletion of serum autoantibody titers [206]. Rituximab (Rituxan® or MabThera®) is a monoclonal antibody which targets CD20 (a B cell surface marker) and consequently causes a profound B cell depletion [206]. The CD20 monoclonal antibody modulates the titer and functions of Treg and effector T cells in many autoimmune diseases [204, 207]. This observation adds to the accumulating body of evidence that B cells may be pathogenic independently of their ability to produce antibodies [201, 205]. The mechanisms that regulate B cell cytokine synthesis are therefore a focus of interest.

B cells are able to differentiate into Th-1-like and Th-2-like effector subsets that produce distinct cytokines, such as IFN- γ and IL-4, respectively [204, 205, 208–210]. B cells may produce chemokines and cytokines independently or in response to antigen, TLR ligands, T cells, or combinations of these stimuli [201, 202]. B cell cytokine production is heavily context-dependent and is the result of the balance of signals received by the B cell receptor and the CD40 antigen. CD40 and BCR signaling results in the proliferation of B cells secreting TNF- α and IL-6. These cytokines act in an autocrine manner to further accelerate B cell differentiation and amplifies the immune response via the generation and maintenance of a positive feedback loop. CD40 stimulation alone activates B cells which generate IL-10 only [211]. As stated above, the cytokines made by B cells have a management role as they are involved in modulating the activation or function of CD4⁺ T cells [201]. Regulatory B cells or Bregs for example act to inhibit pro-inflammatory cytokines and effector T cell differentiation and promote the differentiation of Tregs via the production of IL-10 or TGF- β 1 and hence dampen down a hyperaggressive immune response [203]. Reduced numbers or functions of these regulatory subsets are evident in a number of different autoimmune diseases [212, 213]. Similarly, TGF- β -expressing B cells stimulate the conversion of CD25⁺ CD4⁺ T cells into FOXP3⁺ Treg cells [214]. Thus, cytokines synthesized by B cells can suppress T cell immune responses directly or can promote a predominance of Treg cells which attenuate effector T cell proliferation and functions.

B cells may be categorized based on their pattern of cytokine secretion into B effector-1 and effector-2 cells (Be-1 and Be-2 cells). B cells in the presence of Th1-like cytokines and primed by T cells or TLR ligands secrete Th1-like cytokines, including IFN- γ . These Be-1 cells produce minimal levels of IL-4 or IL-13 but do secrete IL-10, TNF- α and IL-6 [208,

210]. In contrast, B cells in the presence of Th2-type cytokines and primed by T cells/antigen secrete IL-2, IL-4, and IL-13, but low levels of IFN- γ and IL-12 [209, 210]. These Be-2 cells may also release IL-10, TNF- α , and IL-6. Finally, regulatory B cells [215] produce IL-10 following stimulation with antigen combinations, CD40L and TLR ligands [204, 216]. Hence, in the predominant cytokine environment present in people with ME/cfs, one would expect an imbalance of Be-1–Be-2 effector cells and Bregs. CD40 and BCR signaling would be expected in an environment containing high levels of pro-inflammatory cytokines and antigens, and hence, effector B cells would be expected to greatly outnumber regulatory B cells and make a major contribution to the breakdown of immune homeostasis evidenced in this disease.

Conclusions and Future Directions

We have endeavored to describe the many potential sources of autoimmunity in people with ME/cfs. Low NKC function is a source of disrupted homeostasis and prolonged effector T cell survival. Low ATP production and mitochondrial dysfunction is a source of autoimmunity by inhibiting apoptotic and stimulating necrotic cell death pathways and hence decreasing immunosuppression at the termination of the immune response and increasing inflammation. Elevated levels of pro-inflammatory and other cytokine species conspire together to impair the normal homeostatic mechanisms which govern T and B cell activation differentiation and survival. This leads to an imbalance of regulatory and effector lymphocytes. Elevated O&NS damage lipids and proteins leading to the formation of neoepitopes which become immunogenic leading to the disruption of many essential cellular processes. Elevated levels of NF- κ B not only contribute to prolonged lymphocyte survival but also increase the generation of autoreactive B cells. Elevated levels of pro-inflammatory cytokines result in elevated levels of NO and leptin. Both entities lead to disruption of homeostatic mechanisms via interaction of mTOR. Elevated levels of NO lead to blockade of the methionine cycle and hypomethylation of DNA. Finally, increased levels of pro-inflammatory cytokines and NF- κ B conspire to disrupt epithelial tight junctions in the intestine allowing the potential translocation of bacterial LPS into the general circulation. This can have dire consequences and is a crucial element underpinning the pathology in a number of neuro-autoimmune disorders.

Rituximab has recently been evaluated as a treatment for ME/cfs and has displayed considerable efficacy [217]. In a study, 30 patients with ME/cfs were randomized to rituximab or placebo, in a placebo-controlled double-blind study and monitored for a year. Significant positive responses were recorded in 67 % of the rituximab-treated patients and 13 % of the placebo arm. The intervention produced a global improvement in symptoms without the production of any serious

side effects. The positive response to rituximab experienced by patients was delayed by between 2 and 7 months, and this phenomenon convinced the authors that ME/cfs was, at least partly, an autoimmune disease.

Rituximab is gathering momentum as a treatment of many autoimmune diseases [218] particularly where the patients are unresponsive to first and second line treatments [219]. When taken together, the trial data demonstrates that the great majority of patients respond well to rituximab. The benefit applies to people with a wide range of autoimmune diseases which are licensed indications such as rheumatoid arthritis [220] and other autoimmune diseases such as SLE [221] and Sjorgens syndrome [222] where the drug is prescribed off license. Concerns have been raised regarding the side effect profile of rituximab probably because of its historical success in successfully treating certain cancers. However, an exhaustive review of all peer-reviewed data undertaken by Gurcan et al. [219] concluded that the incidence of serious or severe unwanted effects was very low.

Rituximab reduces T and B cells [223]. It must be stressed that the effectiveness of rituximab is not dependent on secreted antibody as rituximab does not alter plasma cell numbers in serum or CSF [224]. This observation suggests that rituximab may have effects other than as a monoclonal antibody. Perhaps one of the most surprising effects is the inhibition of Th17 T cell production [225]. Th17 T cells are pivotal players in producing chronic inflammation and neurotoxicity in several other autoimmune diseases such as MS. Rituximab also reduces IL-2 levels directly and thus may help to suppress a chronically activated immune system [226]. Rituximab may also achieve the inhibition of the immune response by inhibiting the activation of NF- κ B [227], which is another driver of autoimmunity. Rituximab also improves the function of Treg cells [228]. Impaired Treg function is also another element in the induction autoimmunity and neurotoxicity. The contribution of B cells to pathology in MS is evidenced by the effectiveness of rituximab [229]. T lymphocytes from relapsing remitting MS patients show an attenuated response to stimulation by antigen following treatment with rituximab [230]. This observation supports the hypothesis that B cell activity is also required to maintain active disease processes in this illness [230]. The drug has also delivered clinically important benefits when used as a treatment in a number of peripheral neurological diseases [231].

Another potentially synergistic approach may well be the use of endotherapia. Endotherapia (GEMSP) has recently been successfully trialed as a treatment of MS [232]. Endotherapia is an immunopathological strategy addressing pathology which seems to underpin chronic incurable diseases whose etiology is multifactorial. It involves the combination of an evaluation of circulating immunoglobulins directed against specific neo-epitopes that created ROS elevation. GEMSP is a preparation of numerous small molecules, including fatty acids, anchorage

molecules, antioxidants, radical scavengers, amino acids, ligated to linear chain of poly-L-lysine (PLL) which are non-immunogenic [232] and inhibit inflammation and O&NS processes. Each individual linkage affords significant advantages. Importantly, it prevents metabolic degradation of the linked molecules and enables a long half-life and confers stability on the various linked chemical entities. Membrane permeability is increased and the induction of various viral and bacterial components. These features combine to induce neuroprotection [232].

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