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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-κB 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 endotherapia.

Keywords Inflammation · Oxidative and nitrosative stress · Cytokines · Autoimmune · Chronic fatigue syndrome · Myalgic encephalomyelitis

G. Morris
Mumbles Head, Pembrey, Llanelli, UK

M. Berk · M. Maes
Impact Strategic Research Center, School of Medicine, Deakin University, Geelong, Australia

M. Berk
Department of Psychiatry, Orygen Youth Health, Centre for Youth Mental Health and the Florey Institute for Neuroscience and Mental Health, University of Melbourne, Parkville, Melbourne, Australia

P. Galecki
Department of Adult Psychiatry, Medical University of Łódź, Łódź, Poland

P. Galecki
Babinski Memorial Hospital, Łódź, Poland

M. Maes (✉)
Department of Psychiatry, Chulalongkorn University, Por Bor Ror Building 12, Bangkok, Thailand
e-mail: dr.michaelmaes@hotmail.com
URL: http://scholar.google.com/citations?user=1wzMZ7UAAAAJ&hl=en

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)-1β, tumor necrosis factor (TNF)-α, nuclear factor (NF)-κB, 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 ω3-poly-unsaturated 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 neoepitopes [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.

Neoepitopes 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 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-cysteinyl, 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].

Table 1 Signs and drivers of autoimmunity in myalgic encephalomyelitis/chronic fatigue syndrome (MS/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 neoepitopes
↑ 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 neoepitopes
Increased concentrations of homocysteine and DNA hypomethylation
Activated mammalian target of rapamycin, e.g., via increased nitric oxide

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 responsivity 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, andretroviruses, 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 apotopic 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-β1 [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 capase-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 apoptopic 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-κB which we will discuss in the next subsection.

Role of NF-κB in Generating Autoimmunity

NF-κB and Generation of Chronic Inflammation

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

NF-κB and Autoreactive B Cells

Elevated levels of NF-κB additionally increase survival of autoreactive B cells [117]. A further contribution made by elevation of NF-κB 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-κB and Cell Death

There are two NF-κB 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-κB suppresses both PCD types [130, 131] and exerts this prosurvival activity through different anti-apoptotic proteins [123, 132].

NF-κB 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-κB and c-Jun activating kinase (JNK) are activated simultaneously under a variety of stress conditions. Conversely, sustained activation of NF-κB inhibits cytokine-induced JNK activation [133]. Whereas temporary activation of JNK following TNF treatment promotes cell survival, chronic JNK activation encourages cell demise. NF-κB 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-κB suppresses these positive feedback loops by activating caspase inhibitors, BCl-2 compounds, and antioxidants. Hence, elevation of NF-κB leads to a depletion of reactive oxygen species (ROS) and caspase inhibition. NF-κB activation additionally inhibits sustained JNK activity which explains the prosurvival activity of NF-κB [135]. NF-κB, cytokines, and elevated O&NS are conspirational partners in producing a chronic immune-inflammatory 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-β1 and IL-6 support the differentiation of naive T cells towards the Th17 phenotype. These cytokines include IL-1β, 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 RORyt, 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-β1 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-1β 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-1β and TNF-α cause an increase in intestinal endothelial cell permeability via the activation of NF-κB [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-adenoylhomocysteine 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-β1 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-κB 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-κB 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-κB [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 neoepitopes 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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References

1. Morris G, Maes M (2012) A neuro-immune model of myalgic encephalomyelitis/chronic fatigue syndrome. Metab Brain Dis. 0885-7490
2. World Health Organisation (1992) ICD-10 classifications of mental and behavioural disorder: clinical descriptions and diagnostic guidelines. World Health Organisation, Geneva
3. Carruthers BM, van de Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, Staines D, Powles AC, Speight N, Vallings R, Bateman L, Baumgarten-Austrheim B, Bell DS, Carlo-Stella N, Chia J, Darragh A, Jo D, Lewis D, Light AR, Marshall-Gradisbik S, Mena I, Mikovits JA, Miwa K, Murovska M, Pall ML, Stevens S (2011) Myalgic encephalomyelitis: international consensus criteria. J Intern Med 270:327–338
4. Carruthers BM, Kumar Jain A, De Meirleir KL, Peterson DL, Klimas NG, Lerner AM, Bested AC, Flor-Henry P, Joshi P, Powles ACP, Sherkey JA, van de Sande MI (2003) Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working case definition, diagnostic and treatment protocols. JCFS 11:7–97
5. Maes M, Kubera M, Uytterhoeven M, Vrydags N, Bosmans E (2011) Increased plasma peroxides as a marker of oxidative stress in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Med Sci Monit 17:SC11–SC15
6. Morris G, Maes M (2012) Increased nuclear factor-κB and loss of p53 are key mechanisms in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Med Hypotheses 79:607–613
7. Seishima M, Mizutani Y, Shibuya Y, Arakawa C (2008) Chronic fatigue syndrome after human parvovirus B19 infection without persistent viremia. Dermatology 216:341–346
8. Chia JK, Chia AY (2008) Chronic fatigue syndrome is associated with chronic enterovirus infection of the stomach. J Clin Pathol 61:43–48
9. Nicolson GL, Nicolson NL, Haier J (2008) Chronic fatigue syndrome patients subsequently diagnosed with Lyme disease *Borrelia burgdorferi*: evidence for mycoplasma species co-infections. JCFS 14:5–17

10. Nicolson GL, Gan R, Haier J (2003) Multiple co-infections (*Mycoplasma*, *Chlamydia*, human herpesvirus-6) in blood of chronic fatigue syndrome patients: association with signs and symptoms. APMIS 111:557–566
11. Sleigh KM, Marra FH, Stiver HG (2002) Influenza vaccination: is it appropriate in chronic fatigue syndrome? Am J Respir Med 1:3–9
12. Brenu EW, Staines DR, Baskurt OK, Ashton KJ, Ramos SB, Christy RM, Marshall-Gradisnik SM (2010) Immune and hemorheological changes in chronic fatigue syndrome. J Transl Med 8:1
13. Brenu EW, van Driel ML, Staines DR, Ashton KJ, Ramos SB, Keane J, Klimas NG, Marshall-Gradisnik SM (2011) Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis. J Transl Med 9:81
14. Brenu EW, van Driel ML, Staines DR, Ashton KJ, Hardcastle SL, Keane J, Tajouri L, Peterson D, Ramos SB, Marshall-Gradisnik SM (2012) Longitudinal investigation of natural killer cells and cytokines in chronic fatigue syndrome/myalgic encephalomyelitis. J Transl Med 10:88
15. Klimas N, Salvato F, Morgan R, Fletcher MA (1990) Immunologic abnormalities in chronic fatigue syndrome. J Clin Microbiol 28: 1403–1410
16. Hassan IS, Bannister BA, Akbar A, Weir W, Bofill M (1998) A study of the immunology of the chronic fatigue syndrome: correlation of immunologic markers to health dysfunction. Clin Immunol Immunopathol 87:60–67
17. Maes M, Mihaylova I, Leunis JC (2006) Chronic fatigue syndrome is accompanied by an IgM-related immune response directed against neopitopes formed by oxidative or nitrosative damage to lipids and proteins. Neuro Endocrinol Lett 27:615–621
18. Maes M, Mihaylova I, Leunis JC (2007) Increased serum IgM antibodies directed against phosphatidyl inositol (Pi) in chronic fatigue syndrome (CFS) and major depression: evidence that an IgM-mediated immune response against Pi is one factor underpinning the comorbidity between both CFS and depression. Neuro Endocrinol Lett 28:861–867
19. Shungu DC, Weiduschat N, Murrough JW, Mao X, Pillemer S, Dyke JP, Medow MS, Natelson BH, Stewart JM, Mathew SJ (2012) Increased ventricular lactate in chronic fatigue syndrome. III. Relationships to cortical glutathione and clinical symptoms implicate oxidative stress in disorder pathophysiology. NMR Biomed 25(9): 1073–1087
20. Kennedy G, Spence VA, McLaren M, Hill A, Underwood C, Belch JJ (2005) Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms. Free Radic Biol Med 39:584–589
21. Maes M, Mihaylova I, Kubera M, Uytterhoeven M, Vrydags N, Bosmans E (2009) Coenzyme Q10 deficiency in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is related to fatigue, autonomic and neurocognitive symptoms and is another risk factor explaining the early mortality in ME/CFS due to cardiovascular disorder. Neuro Endocrinol Lett 30:470–476
22. Maes M, Twisk FN (2009) Why myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) may kill you: disorders in the inflammatory and oxidative and nitrosative stress (IO&NS) pathways may explain cardiovascular disorders in ME/CFS. Neuro Endocrinol Lett 30:677–693
23. Maes M, Twisk FNM, Kubera M, Ringel K (2011) Evidence for inflammation and activation of cell-mediated immunity in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS): increased interleukin-1, tumor necrosis factor-α, PMN-elastase, lysozyme and neopterin. J Affect Disord 136:933–939
24. Maes M, Mihaylova I, Kubera M, Bosmans E (2007) Not in the mind but in the cell: increased production of cyclo-oxygenase-2 and inducible NO synthase in chronic fatigue syndrome. Neuro Endocrinol Lett 28:463–469
25. Maes M, Mihaylova I, Bosmans E (2007) Not in the mind of neurasthenic lazybones but in the cell nucleus: patients with chronic fatigue syndrome have increased production of nuclear factor kappa beta. Neuro Endocrinol Lett 28:456–462
26. Meeus M, Mistiaen W, Lambrecht L, Nijs J (2011) Immunological similarities between cancer and chronic fatigue syndrome: the common link to fatigue? Anticancer Res 29:4717–4726
27. Maes M, Mihaylova I, Kubera M, Leunis JC, Twisk FN, Geffard M (2012) IgM-mediated autoimmune responses directed against anchorage epitopes are greater in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) than in major depression. Metab Brain Dis 27(4):415–423
28. Maes M, Twisk FN, Johnson C (2012) Myalgic encephalomyelitis (ME), chronic fatigue syndrome (CFS), and chronic fatigue (CF) are distinguished accurately: results of supervised learning techniques applied on clinical and inflammatory data. Psychiatry Res 200:754–760
29. Lombardi V, Hagen KS, Hunter KW, Diamond JW, Smith-Gagen J, Yang W, Mikovits JA (2011) Xenotropic murine leukemia virus-related virus-associated chronic fatigue syndrome reveals a distinct inflammatory signature. In Vivo 25:307–314
30. Maes M, Twisk FN, Kubera M, Ringel K, Leunis JC, Geffard M (2012) Increased IgA responses to the LPS of commensal bacteria is associated with inflammation and activation of cell-mediated immunity in chronic fatigue syndrome. J Affect Disord 136:909–917
31. Maes M, Mihaylova I, Leunis JC (2005) In chronic fatigue syndrome, the decreased levels of omega-3 poly-unsaturated fatty acids are related to lowered serum zinc and defects in T cell activation. Neuro Endocrinol Lett 26:745–751
32. Vermuelen RCW, Kurt RM, Visser FC, Sluiter W, Scholte HR (2010) Patients with chronic fatigue syndrome performed worse than controls in a controlled repeated exercise study despite a normal oxidative phosphorylation capacity. J Transl Med 8:93
33. Myhill S, Booth NE, McLaren-Howard J (2009) Chronic fatigue syndrome and mitochondrial dysfunction. Int J Clin Exp Med 2:1–16
34. Booth NE, Myhill S, McLaren-Howard J (2012) Mitochondrial dysfunction and the pathophysiology of myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). Int J Clin Exp Med 5:208–220
35. Wong R, Lopaschuk G, Zhu G, Walker D, Catellier D, Burton D, Teo K, Collins-Nakai R, Montague T (1992) Skeletal muscle metabolism in the chronic fatigue syndrome. In vivo assessment by 31P nuclear magnetic resonance spectroscopy. Chest 102:1716–1722
36. Plioplys AV, Plioplys S (1995) Electron-microscopic Investigation of muscle mitochondria in chronic fatigue syndrome. Neuropsychobiol 32:175–181
37. Sharpe MC, Archard LC, Banatvala JE, Borysiewicz LK, Clare AW, David A, Edwards RH, Hawton KE, Lambert HP, Lane RJ, McDonald EM, Mowbray JF, Pearson DJ, Peto TEA, Preedy VR, Smith AP, Smith DG, Taylor DJ, Tyrrell AJ, Wessely S, White PD (1991) A report—chronic fatigue syndrome: guidelines for research. JR Soc Med 84:118–121
38. Cleare AJ, O'Keane V, Miell J (2001) Plasma leptin in chronic fatigue syndrome and a placebo-controlled study of the effects of low-dose hydrocortisone on leptin secretion. Clin Endocrinol (Oxf) 55:113–119
39. Lu XY (2007) The leptin hypothesis of depression: a potential link between mood disorders and obesity? Curr Opin Pharmacol 7:648–652
40. Pasco JA, Jacka FN, Williams LJ, Henry MJ, Nicholson GC, Kotowicz MA, Berk M (2008) Leptin in depressed women: cross-sectional and longitudinal data from an epidemiologic study. J Affect Disord 107(1–3):221–225
41. Nijs J, McGregor NR, De Becker P, Verhas M, Englebienne P, De Meirleir K (2003) Monitoring a hypothetical channelopathy in chronic fatigue syndrome: preliminary observations. JCFS 11: 117–133
42. Nijs J, Coomans D, Nicolson GL, De Becker P, Christian D, De Meirleir K (2003) Immunophenotyping predictive of Mycoplasma infection in patients with chronic fatigue syndrome. JCFS 11:51–69

43. Tirelli U, Marotta G, Improta S, Pinto A (1994) Immunological abnormalities in patients with chronic fatigue syndrome. Scand J Immunol 40:601–608
44. Bradley AS, Ford B, Bansal AS (2013) Altered functional B-cell subset populations in patients with chronic fatigue syndrome compared to healthy controls. 172(1):73–80. doi:10.1111/cei.12043
45. Tanaka S, Kuratsune H, Hidaka Y, Hakariya Y, Tatsumi KI, Takano T, Kanakura Y, Amino N (2003) Autoantibodies against muscarinic cholinergic receptor in chronic fatigue syndrome. Int J Mol Med 12: 225–230
46. Buchwald MD, Wener MH, Komaroff AL (1991) Antineuronal antibody levels in chronic fatigue syndrome patients with neurologic abnormalities. Arthritis Rheum 34:1485–1486
47. Plioplys AV (1997) Antimuscle and anti-CNS circulating antibodies in chronic fatigue syndrome. Neurology 48:1717–1719
48. Bassi N, Amital D, Amital H, Doria A, Shoenfeld Y (2008) Chronic fatigue syndrome: characteristics and possible causes for its pathogenesis. Isr Med Assoc J 10:79–82
49. Nishikai M (2007) Antinuclear antibodies in patients with chronic fatigue syndrome. Nippon Rinsho 65:1067–1070
50. Maes M (2010) An intriguing and hitherto unexplained co-occurrence: depression and chronic fatigue syndrome are manifestations of shared inflammatory, oxidative and nitrosative (IO&NS) pathways. Prog Neuropsychopharmacol. Biol Psychiatry 35:784–794
51. Klein R, Berg PA (1995) High incidence of antibodies to 5-hydroxytryptamine, gangliosides and phospholipids in patients with chronic fatigue and fibromyalgia syndrome and their relatives: evidence for a clinical entity of both disorders. Eur J Med Res 1:21–26
52. Ljunggren HG, Karre K (1990) In search of the 'missing self': MHC molecules and NK cell recognition. Immunol Today 11: 237–244
53. Yokoyama WM, Kim S, French AR (2004) The dynamic life of natural killer cells. Annu Rev Immunol 22:405–429
54. Lunemann A, Lunemann JD, Munz C (2009) Regulatory NK-cell functions in inflammation and autoimmunity. Mol Med 15:352–358
55. Strowig T, Brilot F, Munz C (2008) Noncytotoxic functions of NK cells: direct pathogen restriction and assistance to adaptive immunity. J Immunol 180:7785–7791
56. Zimmer J, Bausinger H, de la Salle H (2001) Autoimmunity mediated by innate immune effector cells. Trends Immunol 22:300–301
57. Shi F, Ljunggren HG, Sarvetnick N (2001) Innate immunity and autoimmunity: from self-protection to self-destruction. Trends Immunol 22:97–101
58. Smeltz RB, Wolf NA, Swanborg RH (1999) Inhibition of autoimmune T cell responses in the DA rat by bone marrow-derived NK cells in vitro: implications for autoimmunity. J Immunol 163:1390–1397
59. Matsumoto Y, Kohyama K, Aikawa Y, Shin T, Kawazoe Y, Suzuki Y, Tanuma N (1998) Role of natural killer cells and TCRγδ T cells in acute autoimmune encephalomyelitis. Eur J Immunol 28:1681–1688
60. Shi FD, Takeda K, Akira S, Sarvetnick N, Ljunggren HG (2000) IL-18 directs autoreactive T cells and promotes autodestruction in the central nervous system via induction of IFN-γ by NK cells. J Immunol 165:3099–3104
61. Shi FD, Wang HB, Li H, Hong S, Taniguchi M, Link H, Van Kaer L, Ljunggren HG (2000) Natural killer cells determine the outcome of B cell-mediated autoimmunity. Nat Immunol 1:245–251
62. Trinchieri G (1989) Biology of natural killer cells. Adv Immunol 47:187–376
63. Liu R, Van Kaer L, La Cava A, Price M, Campagnolo DI, Collins M, Young DA, Vollmer TL, Shi FD (2006) Autoreactive T cells mediate NK cell degeneration in autoimmune disease. J Immunol 176:5247–5254
64. Kasaian MT, Whitters MJ, Carter LL, Lowe LD, Jussif JM, Deng B, Johnson KA, Witek JS, Senices M, Konz RF, Wurster AL, Donaldson DD, Collins M, Young DA, Grusby MJ (2002) IL-21 limits NK cell responses and promotes antigen-specific T cell activation: a mediator of the transition from innate to adaptive immunity. Immunity 16:559–569
65. Vollmer TL, Liu R, Price M, Rhodes S, La Cava A, Shi FD (2005) Differential effects of IL-21 during initiation and progression of autoimmunity against neuroantigen. J Immunol 174:2696–2701
66. Fehniger TA, Cooper MA, Nuovo GJ, Cella M, Facchetti F, Colonna M, Caligiuri MA (2003) $CD56^{bright}$ natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. Blood 101:3052–3057
67. Ferlazzo G, Pack M, Thomas D, Paludan C, Schmid D, Strowig T, Bougras G, Muller WA, Moretta L, Munz C (2004) Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. Proc Natl Acad Sci U S A 101:16606–16611
68. Zingoni A, Sornasse T, Cocks BG, Tanaka Y, Santoni A, Lanier LL (2004) Cross-talk between activated human NK cells and CD4+ T cells via OX40–OX40 ligand interactions. J Immunol 173:3716–3724
69. Vranes Z, Poljakovic Z, Marusic M (1989) Natural killer cell number and activity in multiple sclerosis. J Neurol Sci 94:115–123
70. Infante-Duarte C, Weber A, Kratzschmar J, Prozorovski T, Pikol S, Hamann I, Bellmann-Strobl J, Aktas O, Dorr J, Wuerfel J, Sturzebecher CS, Zipp F (2005) Frequency of blood CX3CR1-positive natural killer cells correlates with disease activity in multiple sclerosis patients. FASEB J 19:1902–1904
71. Schippling DS, Martin R (2008) Spotlight on anti-CD25: daclizumab in MS. Int MS J 15:94–98
72. Bielekova B, Catalfamo M, Reichert-Scrivner S, Packer A, Cerna M, Waldmann TA, McFarland H, Henkart PA, Martin R (2006) Regulatory CD56z(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. Proc Natl Acad Sci U S A 103:5941–5946
73. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 17:189–220
74. Su HC, Nguyen KB, Salazar-Mather TP, Ruzek MC, Dalod MY, Biron CA (2001) NK cell functions restrain T cell responses during viral infections. Eur J Immunol 31:3048–3055
75. Fujinami RS, Oldstone MB, Wroblewska Z, Frankel ME, Koprowski H (1983) Molecular mimicry in virus infection: crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. Proc Natl Acad Sci U S A 80:2346–2350
76. Li Y, Peng T, Yang Y, Niu C, Archard LC, Zhang H (2000) High prevalence of enteroviral genomic sequences in myocardium from cases of endemic cardiomyopathy (Keshan disease) in China. Heart 83:696–701
77. Fujinami RS, von Herrath MG, Christen U, Whitton JL (2006) Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev 19:80–94
78. Andersen O, Lygner PE, Bergstrom T, Andersson M, Vahlne A (1993) Viral infections trigger multiple sclerosis relapses: a prospective seroepidemiological study. J Neurol 240:417–422
79. Edwards S, Zvartau M, Clarke H, Irving W, Blumhardt LD (1998) Clinical relapses and disease activity on magnetic resonance imaging associated with viral upper respiratory tract infections in multiple sclerosis. J Neurol Neurosurg Psychiatry 64:736–741
80. Johnson RT (1998) Chronic inflammatory and demyelinating diseases. In: Viral infections of the nervous system. 2nd Ed. Lippincott-Raven, Philadelphia, pp. 227–263
81. Wucherpfennig KW, Ota K, Endo N, Seidman JG, Rosenzweig A, Weiner HL, Hafler DA (1990) Shared human T cell receptor Vb usage to immunodominant regions of myelin basic protein. Science 248: 1016–1019
82. McCoy L, Tsunoda I, Fujinami RS (2006) Multiple sclerosis and virus induced immune responses: autoimmunity can be primed by

molecular mimicry and augmented by bystander activation. Autoimmunity 39:9–19
83. Bangs SC, McMichael AJ, Xu XN (2006) Bystander T cell activation—implications for HIV infection and other diseases. Trends Immunol 27:518–524
84. Bierer BE, Hahn WC (1993) T cell adhesion, avidity regulation and signaling: a molecular analysis of CD2. Semin Immunol 5:249–261
85. Unutmaz D, Pilri P, Abrignani S (1994) Antigen-independent activation of naive and memory resting T cells by a cytokine combination. J Exp Med 180:1159–1164
86. Mathew A, Kurane I, Green S, Vaughn DW, Kalayanarooj S, Suntayakorn S, Ennis FA, Rothman AL (1999) Impaired T cell proliferation in acute dengue infection. J Immunol 162:5609–5615
87. Zivna I, Green S, Vaughn DW, Kalayanarooj S, Stephens HA, Chandanayingyong D, Nisalak A, Ennis FA, Rothman AL (2002) T cell responses to an HLA-B*07-restricted epitope on the dengue NS3 protein correlate with disease severity. J Immunol 168:5959–5965
88. Rowland CA, Lertmemongkolchai G, Bancroft A, Haque A, Lever MS, Griffin KF, Jackson MC, Nelson M, O'Garra A, Grencis R, Bancroft GJ, Lukaszewski RA (2006) Critical role of type 1 cytokines in controlling initial infection with *Burkholderia mallei*. Infect Immun 74:5333–5340
89. Douek DC, Picker LJ, Koup RA (2003) T cell dynamics in HIV-1 infection. Annu Rev Immunol 21:265–304
90. Silvestri G, Feinberg MB (2003) Turnover of lymphocytes and conceptual paradigms in HIV infection. J Clin Invest 112:821–824
91. Fadok VA, Henson PM (1998) Apoptosis: getting rid of the bodies. Curr Biol 8:R693–R695
92. Chung EY, Liu J, Homma Y, Zhang Y, Brendolan A, Saggese M, Han J, Silverstein R, Selleri L, Ma X (2007) Interleukin-10 expression in macrophages during phagocytosis of apoptotic cells is mediated by homeodomain proteins Pbx1 and Prep-1. Immunity 27:952–964
93. Cvetanovic M, Ucker DS (2004) Innate immune discrimination of apoptotic cells: repression of proinflammatory macrophage transcription is coupled directly to specific recognition. J Immunol 172:880–889
94. Hanayama R, Tanaka M, Miyasaka K, Aozasa K, Koike M, Uchiyama Y, Nagata S (2004) Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. Science 304:1147–1150
95. Erwig LP, Henson PM (2007) Immunological consequences of apoptotic cell phagocytosis. Am J Pathol 171:2–8
96. Nagata S, Hanayama R, Kawane K (2010) Autoimmunity and the clearance of dead cells. Cell 140:619–630
97. Gaipl US, Franz S, Voll RE, Sheriff A, Kalden JR, Herrmann M (2004) Defects in the disposal of dying cells lead to autoimmunity. Curr Rheumatol Rep 6:401–407
98. Arends MJ, Wyllie AH (1991) Apoptosis: mechanisms and roles in pathology. Int. Rev. Exp Pathol 32:223–254
99. Scaffidi C, Fulda S, Srinivasan A, Friesen C, Li F, Tomaselli KJ, Debatin KM, Krammer PH, Peter ME (1998) Two CD95 (APO-1/Fas) signaling pathways. EMBO J 17:1675–1687
100. Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, Peter ME (1997) FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 16:2794–2804
101. Salvesen GS, Dixit VM (1997) Caspases: intracellular signaling by proteolysis. Cell 91:443–446
102. Goonetilleke UR, Ward SA, Gordon SB (2009) Could proteomic research deliver the next generation of treatments for pneumococcal meningitis? Interdiscip Perspect Infect Dis 2009:214–216
103. Thornberry NA, Lazebnik Y (1998) Caspases: enemies within. Science 281:1312–1316
104. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X (1997) Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91:479–489
105. Eguchi Y, Shimizu S, Tsujimoto Y (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 57:1835–1840
106. Melchior F, Gerace L (1995) Mechanisms of nuclear protein import. Curr Opin Cell Biol 7:310–318
107. Yasuhara N, Eguchi Y, Tachibana T, Imamoto N, Yoneda Y, Tsujimoto Y (1997) Essential role of active nuclear transport in apoptosis. Genes Cells 2:55–64
108. Zong WX, Thompson CB (2006) Necrotic death as a cell fate. Genes Dev 20:1–15
109. Proskuryakov SY, Konoplyannikov AG, Gabai VL (2003) Necrosis: a specific form of programmed cell death? Exp Cell Res 283:1–16
110. Xu Y, Huang S, Liu ZG, Han J (2006) Poly(ADP-ribose) polymerase-1 signalling to mitochondria in necrotic cell death requires RIP1/TRAF2-mediated JNK1 activation. J Biol Chem 281:8788–8795
111. Chen Y (2009) Necrosis: an energy-dependent programmed cell death? UTMJ 86:110–112
112. Blackwell TS, Christman JW (1997) The role of nuclear factor-kappa B in cytokine gene regulation. Am J Respir Cell Mol Biol 17:3–9
113. Zhang G, Ghosh S (2000) Molecular mechanisms of NF-kappaB activation induced by bacterial lipopolysaccharide through Toll-like receptors. J Endotoxin Res 6:453–457
114. Siebenlist U, Franzoso G, Brown K (1994) Structure, regulation and function of NF-kappa B. Annu Rev Cell Biol 10:405–455
115. Gloire G, Legrand-Poels S, Piette J (2006) NF-kappaB activation by reactive oxygen species: fifteen years later. Biochem Pharmacol 72:1493–1505
116. Jones E, Adcock IM, Ahmed BY, Punchard NA (2007) Modulation of LPS stimulated NF-kappaB mediated nitric oxide production by PKCepsilon and JAK2 in RAW macrophages. J Inflamm (Lond) 4:23
117. Enzler T, Bonizzi G, Silverman GJ, Otero DC, Widhopf GF, Anzelon-Mills A, Rickert RC, Karin M (2006) Alternative and classical NF-kappa B signaling retain autoreactive B cells in the splenic marginal zone and result in lupus-like disease. Immunity 25:403–415
118. Wu Y, Bressette D, Carrell JA, Kaufman T, Feng P, Taylor K, Gan Y, Cho YH, Garcia AD, Gollatz E, Dimke D, LaFleur D, Migone TS, Nardelli B, Wei P, Ruben SM, Ullrich SJ, Olsen HS, Kanakaraj P, Moore PA, Baker KP (2000) Tumor necrosis factor (TNF) receptor superfamily member TACI is a high affinity receptor for TNF family members APRIL and BLyS. J Biol Chem 275:35478–35485
119. Rahman ZS, Rao SP, Kalled SL, Manser T (2003) Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. J Exp Med 198:1157–1169
120. Liu Z, Davidson A (2011) BAFF and selection of autoreactive B cells. Trends Immunol 32:388–394
121. Senftleben U, Karin M (2002) The IKK/NF-kappa B pathway. Crit Care Med 30:S18–S26
122. Li ZW, Chu W, Hu Y, Delhase M, Deerinck T, Ellisman M, Johnson R, Karin M (1999) The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. J Exp Med 189:1839–1845
123. Karin M, Lin A (2002) NF-kappaB at the crossroads of life and death. Nat Immunol 3:221–227
124. Karin M, Delhase M (2000) The I kappa B kinase (IKK) and NF-kappa B: key elements of proinflammatory signalling. Semin Immunol 12:85–98
125. Senftleben U, Cao Y, Xiao G, Greten FR, Krahn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M (2001) Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. Science 293:1495–1499
126. Bonizzi G, Karin M (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25:280–288

127. Luo JL, Kamata H, Karin M (2005) IKK/NF-kappaB signaling: balancing life and death—a new approach to cancer therapy. J Clin Invest 115:2625–2632
128. Danial NN, Korsmeyer SJ (2004) Cell death: critical control points. Cell 116:205–219
129. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407: 770–776
130. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D (1995) Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. Nature 376:167–170
131. Doi TS, Marino MW, Takahashi T, Yoshida T, Sakakura T, Old LJ, Obata Y (1999) Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. Proc Natl Acad Sci U S A 96:2994–2999
132. Kucharczak J, Simmons MJ, Fan Y, Gelinas C (2003) To be, or not to be: NF-kappaB is the answer—role of Rel/NF-kappaB in the regulation of apoptosis. Oncogene 22:8961–8982
133. Zhang Y, Chen F (2004) Reactive oxygen species (ROS), trouble-makers between nuclear factor-kappaB (NF-kappaB) and c-Jun NH(2)-terminal kinase (JNK). Cancer Res 64:1902–1905
134. Wullaert A, Heyninck K, Beyaert R (2006) Mechanisms of crosstalk between TNF-induced NF-kappaB and JNK activation in hepatocytes. Biochem Pharmacol 72:1090–1101
135. Sakon S, Xue X, Takekawa M, Sasazuki T, Okazaki T, Kojima Y, Piao JH, Yagita H, Okumura K, Doi T, Nakano H (2003) NF-kappaB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. EMBO J 22:3898–3909
136. Gonzales-Garcia C, Martin-Saavedra FM, Ballestar A, Ballestar S (2009) The Th17 lineage: answers to some immunological questions. Inmunologia 28:32–45
137. Waite JC, Skokos D (2012) Th17 response and inflammatory autoimmune diseases. Int J Inflamm 2012:819467
138. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR (2007) IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 8:967–974
139. Finckh A, Gabay C (2008) At the horizon of innovative therapy in rheumatology: new biologic agents. Curr Opin Rheumatol 20:269–275
140. Jadidi-Niaragh F, Mirshafiey A (2011) Th17 cell, the new player of neuroinflammatory process in multiple sclerosis. Scand J Immunol 74:1–13
141. Kryczek I, Wei S, Vatan L, Escara-Wilke J, Szeliga W, Keller ET, Zou W (2007) Cutting edge: opposite effects of IL-1 and IL-2 on the regulation of IL-17+ T cell pool IL-1 subverts IL-2-mediated suppression. J Immunol 179:1423–1426
142. Joosten LAB (2010) Excessive interleukin-1 signaling determines the development of Th1 and Th17 responses in chronic inflammation. Arthr Rheumatism 62:320–322
143. Deknuydt F, Bioley G, Valmon D, Ayyoub M (2009) IL-1β and IL-2 convert human Treg into T_H17 cells. Clin Immunol 131:298–307
144. Ziegler SF, Buckner JH (2009) FOXP3 and the regulation of Treg/Th17 differentiation. Microbes Infect 11:594–598
145. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A (2007) Human TH17 lymphocytes promote blood–brain barrier disruption and central nervous system inflammation. Nat Med 13:1173–1175
146. McColl BW, Rothwell NJ, Allan SM (2008) Systemic inflammation alters the kinetics of cerebrovascular tight junction disruption after experimental stroke in mice. J Neurosci 28:9451–9462
147. Sharief MK, Ciardi M, Thompson EJ, Sorice F, Rossi F, Vullo V, Cirelli A (1992) Tumour necrosis factor-alpha mediates blood–brain barrier damage in HIV-1 infection of the central nervous system. Mediators Inflamm 1:191–196
148. Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA, Said HM (2004) TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. Am J Physiol Gastrointest Liver Physiol 286:G367–G376
149. Al-Sadi RM, Ma TY (2007) IL-1beta causes an increase in intestinal epithelial tight junction permeability. J Immunol 178:4641–4649
150. Lakhan SE, Kirchgessner A (2010) Gut inflammation in chronic fatigue syndrome. Nutr Metab (Lond) 7:79
151. Wang H, Sun J, Goldstein H (2008) Human immunodeficiency virus type 1 infection increases the in vivo capacity of peripheral monocytes to cross the blood–brain barrier into the brain and the in vivo sensitivity of the blood–brain barrier to disruption by lipopolysaccharide. J Virol 82:7591–7600
152. Xing B, Bachstetter AD, Eldik LJ (2011) Microglial p38α MAPK is critical for LPS-induced neuron degeneration, through a mechanism involving TNFα. Mol Neurodegener 6:84
153. Chauhan VS, Marriott I (2007) Bacterial infections of the central nervous system: a critical role for resident glial cells in the initiation and progression of inflammation. Curr Immunol 3:133–143
154. Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lokensgard JR, Peterson PK (2004) Role of microglia in central nervous system infections. Clin Microbiol Rev 17:942–964
155. Yu RK, Usuki S, Ariga T (2006) Ganglioside molecular mimicry and its pathological roles in Guillain–Barré syndrome and related diseases. Infect Immun 74:6517–6527
156. Ang CW, Noordzij PG, de Klerk MA, Endtz HP, van Doorn PA, Laman JD (2002) Ganglioside mimicry of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity in rabbits. Infect Immun 70:5081–5085
157. Wang YS, White TD (1999) The bacterial endotoxin lipopolysaccharide causes rapid inappropriate excitation in rat cortex. J Neurochem 72:652–660
158. Sherbet G (2009) Bacterial infections and the pathogenesis of autoimmune conditions. BJMP 2:6–13
159. Tufekci KU, Genc S, Genc K (2011) The endotoxin-induced neuroinflammation model of Parkinson's disease. Parkinsons Dis 2011:487450
160. Yacyshyn B, Meddings J, Sadowski D, Bowen-Yacyshyn MB (1996) Multiple sclerosis patients have peripheral blood CD45RO + B cells and increased intestinal permeability. Dig Dis Sci 41: 2493–2498
161. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, Estes JD, Dodiya HB, Keshavarzian A (2011) Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PLoS One 6:e28032
162. Grune T, Michel P, Sitte N, Eggert W, Albrecht-Nebe H, Esterbauer H, Siems WG (1997) Increased levels of 4-hydroxynonenal modified proteins in plasma of children with autoimmune diseases. Free Radic Biol Med 23:357–360
163. Kurien BT, Scofield RH (2008) Autoimmunity and oxidatively modified autoantigens. Autoimmun Rev 7:567–573
164. Khan MF, Wu X, Kaphalia BS, Boor PJ, Ansari GA (1997) Acute hematopoietic toxicity of aniline in rats. Toxicol Lett 92:31–37
165. Weinberg JB, Granger DL, Pisetsky DS, Seldin MF, Misukonis MA, Mason SN, Pippen AM, Ruiz P, Wood ER, Gilkeson GS (1994) The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: increased nitric oxide production and nitric oxide synthase expression in MRL-lpr/lpr mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered NG-monomethyl-L-arginine. J Exp Med 179:651–660
166. Khan F, Siddiqui AA, Ali R (2006) Measurement and significance of 3-nitrotyrosine in systemic lupus erythematosus. Scand J Immunol 64:507–514
167. Khan F, Ali R (2006) Antibodies against nitric oxide damaged poly L-tyrosine and 3-nitrotyrosine levels in systemic lupus erythematosus. J Biochem Mol Biol 39:189–196

168. Kurien BT, Hensley K, Bachmann M, Scofield RH (2006) Oxidatively modified autoantigens in autoimmune diseases. Free Radic Biol Med 41:549–556
169. Christensen B, Refsum H, Garras A, Ueland PM (1992) Homocysteine remethylation during nitrous oxide exposure of cells cultured in media containing various concentrations of folates. J Pharmacol Exp Ther 261:1096–1105
170. Castro R, Rivera I, Struys EA, Jansen EE, Ravasco P, Camilo ME, Blom HJ, Jakobs C, Tavares de Almeida I (2003) Increased homocysteine and S-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. Clin Chem 49:1292–1296
171. Li Y, Liu Y, Strickland FM, Richardson B (2010) Age-dependent decreases in DNA methyltransferase levels and low transmethylation micronutrient levels synergize to promote overexpression of genes implicated in autoimmunity and acute coronary syndromes. Exp Gerontol 45:312–322
172. Zhao M, Tang J, Gao F, Wu X, Liang Y, Yin H, Lu Q (2010) Hypomethylation of IL10 and IL13 promoters in CD4+ T cells of patients with systemic lupus erythematosus. J Biomed Biotechnol 2010:931018
173. Xu X, Yang P, Shu Z, Bai Y, Wang CY (2012) DNA methylation in the pathogenesis of autoimmunity. In: Gu W, Wang Y (eds) Gene discovery for disease models. Wiley, Hoboken
174. Karouzakis E, Gay RE, Michel BA, Gay S, Neidhart M (2009) DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 60:3613–3622
175. Kim YI, Logan JW, Mason JB, Roubenoff R (1996) DNA hypomethylation in inflammatory arthritis: reversal with methotrexate. J Lab Clin Med 128:165–172
176. Richardson B, Scheinbart L, Strahler J, Gross L, Hanash S, Johnson M (1990) Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 33:1665–1673
177. Richardson B (1986) Effect of an inhibitor of DNA methylation on T cells. II. 5-Azacytidine induces self-reactivity in antigen-specific T4+ cells. Hum Immunol 17:456–470
178. Beltran B, Quintero M, Garcia-Zaragoza E, O'Connor E, Esplugues JV, Moncada S (2002) Inhibition of mitochondrial respiration by endogenous nitric oxide: a critical step in Fas signaling. Proc Natl Acad Sci U S A 99:8892–8897
179. Perl A, Telarico T, Singh R, Fernandez D (2010) Activation of the nitric oxide–mitochondrial hyperpolarization–mTOR–Rab4 signaling pathway precedes disease development in lupus T cells. J Immunol 184:93.36
180. Nagy G, Koncz A, Telarico T, Fernandez D, Ersek B, Buzas E, Perl A (2010) Central role of nitric oxide in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus. Arthritis Res Ther 12:210
181. Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. Genes Dev 18:1926–1945
182. Pierdominici M, Vacirca D, Delunardo F, Ortona E (2011) mTOR signaling and metabolic regulation of T cells: new potential therapeutic targets in autoimmune diseases. Curr Pharm Des 17:3888–3897
183. Fernandez-Riejos P, Najib S, Santos-Alvarez J, Martin-Romero C, Perez-Perez A, Gonzalez-Yanes C, Sanchez-Margalet V (2010) Role of leptin in the activation of immune cells. Mediators Inflamm 2010:568343
184. Delgoffe GM, Powell JD (2009) mTOR: taking cues from the immune microenvironment. Immunology 127:459–465
185. Yurchenko E, Shio MT, Huang TC, Da Silva Martins M, Szyf M, Levings MK, Oliver M, Piccirillo CA (2012) Inflammation-driven reprogramming of CD4+ Foxp3+ regulatory T cells into pathogenic Th1/Th17 T effectors is abrogated by mTOR inhibition in vivo. PLoS ONE 7:e35572
186. Haxhinasto S, Mathis D, Benoist C (2008) The AKT-mTOR axis regulates de novo differentiation of CD4 + Foxp3+ cells. J Exp Med 205:565–574
187. Fernandez D, Perl A (2010) mTOR signaling: a central pathway to pathogenesis in systemic lupus erythematosus? Discov Med 9:173–178
188. Procaccini C, De Rosa V, Galgani M, Carbone F, Cassano S, Greco D, Qian K, Auvinen P, Calì G, Stallone G, Formisano L, La Cava A, Matarese G (2012) Leptin-induced mTOR activation defines a specific molecular and transcriptional signature controlling CD4+ effector T cell responses. J Immunol 189:2941–2953
189. Matarese G, Leiter EH, La Cava A (2007) Leptin in autoimmunity: many questions, some answers. Tissue Antigens 70:87–95
190. Faggioni R, Feingold KR, Grunfeld C (2001) Leptin regulation of the immune response and the immunodeficiency of malnutrition. FASEB J 15:2565–2571
191. Bettowski J, Wojcicka G, Jamroz A (2003) Stimulatory effect of leptin on nitric oxide production is impaired in dietary-induced obesity. Obes Res 11:1571–1580
192. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD, Diehl AM (1998) Leptin regulates proinflammatory immune responses. FASEB J 12:57–65
193. Napoleone E, DI Santo A, Amore C, Baccante G, di Febbo C, Porreca E, de Gaetano G, Donati MB, Lorenzet R (2007) Leptin induces tissue factor expression in human peripheral blood mononuclear cells: a possible link between obesity and cardiovascular risk? J Thromb Haemost 5:1462–1468
194. Martin-Romero C, Santos-Alvarez J, Goberna R, Sanchez-Margalet V (2000) Human leptin enhances activation and proliferation of human circulating T lymphocytes. Cell Immunol 199:15–24
195. Fernandez-Riejos P, Goberna R, Sanchez-Margalet V (2008) Leptin promotes cell survival and activates Jurkat T lymphocytes by stimulation of mitogen-activated protein kinase. Clin Exp Immunol 151: 505–518
196. Sanchez-Margalet V, Martin-Romero C, Gonzalez-Yanes C, Goberna R, Rodriguez-Bano J, Muniain MA (2002) Leptin receptor (Ob-R) expression is induced in peripheral blood mononuclear cells by in vitro activation and in vivo in HIV-infected patients. Clin Exp Immunol 129:119–124
197. Zarkesh-Esfahani H, Pockley G, Metcalfe RA, Bidlingmaier M, Wu Z, Ajami A, Weetman AP, Strasburger CJ, Ross RJ (2001) High-dose leptin activates human leukocytes via receptor expression on monocytes. J Immunol 167:4593–4599
198. Kiguchi N, Maeda T, Kobayashi Y, Fukazawa Y, Kishioka S (2009) Leptin enhances CC-chemokine ligand expression in cultured murine macrophage. Biochem Biophys Res Commun 384:311–315
199. Wong CK, Cheung PF, Lam CW (2007) Leptin-mediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation. Eur J Immunol 37:2337–2348
200. Mattioli B, Giordani L, Quaranta MG, Viora M (2009) Leptin exerts an anti-apoptotic effect on human dendritic cells via the PI3K–Akt signaling pathway. FEBS Lett 583:1102–1106
201. Lund FE (2008) Cytokine-producing B lymphocytes—key regulators of immunity. Curr Opin Immunol 20:332–338
202. Pistoia V (1997) Production of cytokines by human B cells in health and disease. Immunol Today 18:343–350
203. Mizoguchi A, Bhan AK (2006) A case for regulatory B cells. J Immunol 176:705–710
204. Lund FE, Randall TD (2010) Effector and regulatory B cells: modulators of CD4(+) T cell immunity. Nat Rev Immunol 10:236–247
205. de Goer de Herve MG, Durali D, Dembele B, Giuliani M, Tran TA, Azzarone B, Eid P, Tardieu M, Delfraissy JF, Taoufik Y (2011) Interferon-alpha triggers B cell effector 1 (Be1) commitment. PLoS One 6:e19366
206. Sanz I, Anolik JH, Looney RJ (2007) B cell depletion therapy in autoimmune diseases. Front Biosci 12:2546–2567
207. Stasi R (2010) Rituximab in autoimmune hematologic diseases: not just a matter of B cells. Semin Hematol 47:170–179

208. Harris DP, Goodrich S, Gerth AJ, Peng SL, Lund FE (2005) Regulation of IFN-gamma production by B effector 1 cells: essential roles for T-bet and the IFN-gamma receptor. J Immunol 174: 6781–6790
209. Harris DP, Goodrich S, Mohrs K, Mohrs M, Lund FE (2005) Cutting edge: the development of IL-4-producing B cells (B effector 2 cells) is controlled by IL-4, IL-4 receptor alpha, and Th2 cells. J Immunol 175:7103–7107
210. Harris DP, Haynes L, Sayles PC, Duso DK, Eaton SM, Lepak NM, Johnson LL, Swain SL, Lund FE (2000) Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat Immunol 1:475–482
211. Duddy ME, Alter A, Bar-Or A (2004) Distinct profiles of human B cell effector cytokines: a role in immune regulation? J Immunol 172: 3422–3427
212. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, Mauri C (2010) $CD19^{+}CD24^{hi}CD38^{hi}$ B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. Immunity 32:129–140
213. Pers JO, Lemoine S, Morva A, Saraux A, Jamin C, Youinou P (2011) Impaired regulatory capacities of B lymphocytes in systemic lupus erytematosus. Ann Rheum Dis 70:A59
214. Gros MJ, Naquet P, Guinamard RR (2008) Intrinsic TGF-beta 1 regulation of B cells. J Immunol 180:8153–8158
215. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF (2008) A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. Immunity 28:639–650
216. Mauri C, Ehrenstein MR (2008) The 'short' history of regulatory B cells. Trends Immunol 29:34–40
217. Fluge O, Bruland O, Risa K, Storstein A, Kristoffersen EK, Sapkota D, Næss H, Dahl O, Nyland H, Mella O (2011) Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. PLoS ONE 6:e26358
218. Eisenberg R (2005) Update on rituximab. Ann Rheum Dis 64:iv55–iv57
219. Gurcan HM, Keskin DB, Stern JN, Nitzberg MA, Shekhani H, Ahmed AR (2009) A review of the current use of rituximab in autoimmune diseases. Int Immunopharmacol 9:10–25
220. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T (2004) Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 350:2572–2581
221. Tokunaga M, Saito K, Kawabata D, Imura Y, Fujii T, Nakayamada S, Tsujimura S, Nawata M, Iwata S, Azuma T, Mimori T, Tanaka Y (2007) Efficacy of rituximab (anti-CD20) for refractory systemic lupus erythematosus involving the central nervous system. Ann Rheum Dis 66:470–475
222. Devauchelle-Pensec V, Pennec Y, Morvan J, Pers JO, Daridon C, Jousse-Joulin S, Roudaut A, Jamin C, Renaudineau Y, Roue IQ, Cochener B, Youinou P, Saraux A (2007) Improvement of Sjogren's syndrome after two infusions of rituximab (anti-CD20). Arthritis Rheum 57:310–317
223. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA (2006) Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. J Neuroimmunol 180:63–70
224. Petereit HF, Moeller-Hartmann W, Reske D, Rubbert A (2008) Rituximab in a patient with multiple sclerosis—effect on B cells, plasma cells and intrathecal IgG synthesis. Acta Neurol Scand 117: 399–403
225. Ireland S, Monson N (2011) Potential impact of B cells on T cell function in multiple sclerosis. Mult Scler Int 2011:423971
226. Lederer JA, Liou JS, Kim S, Rice N, Lichtman AH (1996) Regulation of NF-kappa B activation in T helper 1 and T helper 2 cells. J Immunol 156:56–63
227. Jazirehi AR, Huerta-Yepez S, Cheng G, Bonavida B (2005) Rituximab (chimeric anti-CD20 monoclonal antibody) inhibits the constitutive nuclear factor-{kappa}B signaling pathway in non-Hodgkin's lymphoma B-cell lines: role in sensitization to chemotherapeutic drug-induced apoptosis. Cancer Res 65:264–276
228. Vigna-Perez M, Hernandez-Castro B, Paredes-Saharopulos O, Portales-Perez D, Baranda L, Abud-Mendoza C, Gonzalez-Amaro R (2006) Clinical and immunological effects of rituximab in patients with lupus nephritis refractory to conventional therapy: a pilot study. Arthritis Res Ther 8:R83
229. McFarland HF (2008) The B cell—old player, new position on the team. N Engl J Med 358:664–665
230. Bar-Or A, Fawaz L, Fan B, Darlington PJ, Rieger A, Ghorayeb C, Calabresi PA, Waubant E, Hauser SL, Zhang J, Smith CH (2010) Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? Ann Neurol 67:452–461
231. Monson NL, Cravens PD, Frohman EM, Hawker K, Racke MK (2005) Effect of rituximab on the peripheral blood and cerebrospinal fluid B cells in patients with primary progressive multiple sclerosis. Arch Neurol 62:258–264
232. Geffard M, Duleu S, Bessede A, Vigier V, Bodet D, Mangas A, Covenas R (2012) GEMSP: a new therapeutic approach to multiple sclerosis. Cent Nerv Syst Agents Med Chem 12:173–181