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Review

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome – Evidence for an autoimmune disease

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ABSTRACT

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a frequent and severe chronic disease drastically impairing life quality. The underlying pathomechanism is incompletely understood yet but there is convincing evidence that in at least a subset of patients ME/CFS has an autoimmune etiology. In this review, we will discuss current autoimmune aspects for ME/CFS. Immune dysregulation in ME/CFS has been frequently described including changes in cytokine profiles and immunoglobulin levels, T- and B-cell phenotype and a decrease of natural killer cell cytotoxicity. Moreover, autoantibodies against various antigens including neurotransmitter receptors have been recently identified in ME/CFS individuals by several groups. Consistently, clinical trials from Norway have shown that B-cell depletion with rituximab results in clinical benefits in about half of ME/CFS patients. Furthermore, recent studies have provided evidence for severe metabolic disturbances presumably mediated by serum autoantibodies in ME/CFS. Therefore, further efforts are required to delineate the role of autoantibodies in the onset and pathomechanisms of ME/CFS in order to better understand and properly treat this disease.

Contents

Abbreviations: AdR, adrenergic receptor; BAFF, B-lymphocyte activating factor; dUTPase, deoxyuridine 5′-triphosphate nucleotidohydrolase; EBV, Epstein-Barr virus; FM, fibromyalgia; 5-HT, 5-hydroxytryptanime; HHV, human herpes virus; IFNγ, interferon gamma; KIR, killer cell immunoglobulin-like receptor; M AChR, muscarinic acetylcholine receptor; ME/CFS, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome; MS, multiple sclerosis; NK, natural killer cells; PBMC, peripheral blood mononuclear cells; POTS, postural orthostatic tachycardia syndrome; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphisms; TCA, tricarboxylic acid; Tfh, T follicular helper cells; Th, T helper cells; TNFα, tumor necrosis factor alpha; Treg, regulatory T cells.

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1. Introduction

With an estimated prevalence of 0.2–0.3%, Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a multisystem disease with unknown etiology. Patients suffer from persistent exhaustion, cognitive impairment, autonomic dysfunction, chronic pain and flu-like symptoms, leading to a substantial reduction of life quality [1].

ME/CFS disease onset is often reported to be triggered by infections and the link between infections and autoimmune diseases is well established [2]. Although the exact pathogenesis is still unknown, the most plausible hypothesis is that dysregulation of immune system, autonomic nervous system and metabolic disturbances contribute to this complex syndrome, in which severe fatigue and cognitive impairment are a central feature (Fig. 1). Stressful life events are frequently associated with disease onset concomitantly with a history of frequent recurrent infections, immune deficiency and autoimmunity [1,3]. There are numerous studies showing immunological, genetic and metabolic alterations consistent with an autoimmune mechanism. Further, the identification of autoantibodies in ME/CFS patients and the clinical benefit associated with B cell depleting therapy provide strong evidence that, at least in a subset of ME/CFS patients, the disease has an autoimmune etiology.

2. Evidence for autoimmunity in ME/CFS

2.1. Role of infection

Infection by various pathogens, including the Epstein-Barr virus (EBV), the human herpes virus (HHV)-6 and the human parvovirus B19, but also intracellular bacteria, are known as triggers of disease [1,4–6]. In a subset of patients, ME/CFS begins with infectious mononucleosis and evidence for a potential role of EBV in ME/CFS comes from many studies [4,7–9]. In 1984, DuBois et al. first described patients with mononucleosis syndrome suffering from long-lasting fatigue and serological evidence of EBV reactivation [4] followed by a number of studies describing ME/CFS patients with serological evidence of chronic active EBV infection [7–9]. Infectious mononucleosis is known as a risk factor for various autoimmune diseases [2,10]. Several studies show homologies of EBV sequences with human autoantigens such as myelin basic protein for multiple sclerosis (MS) [11]. In a study from our

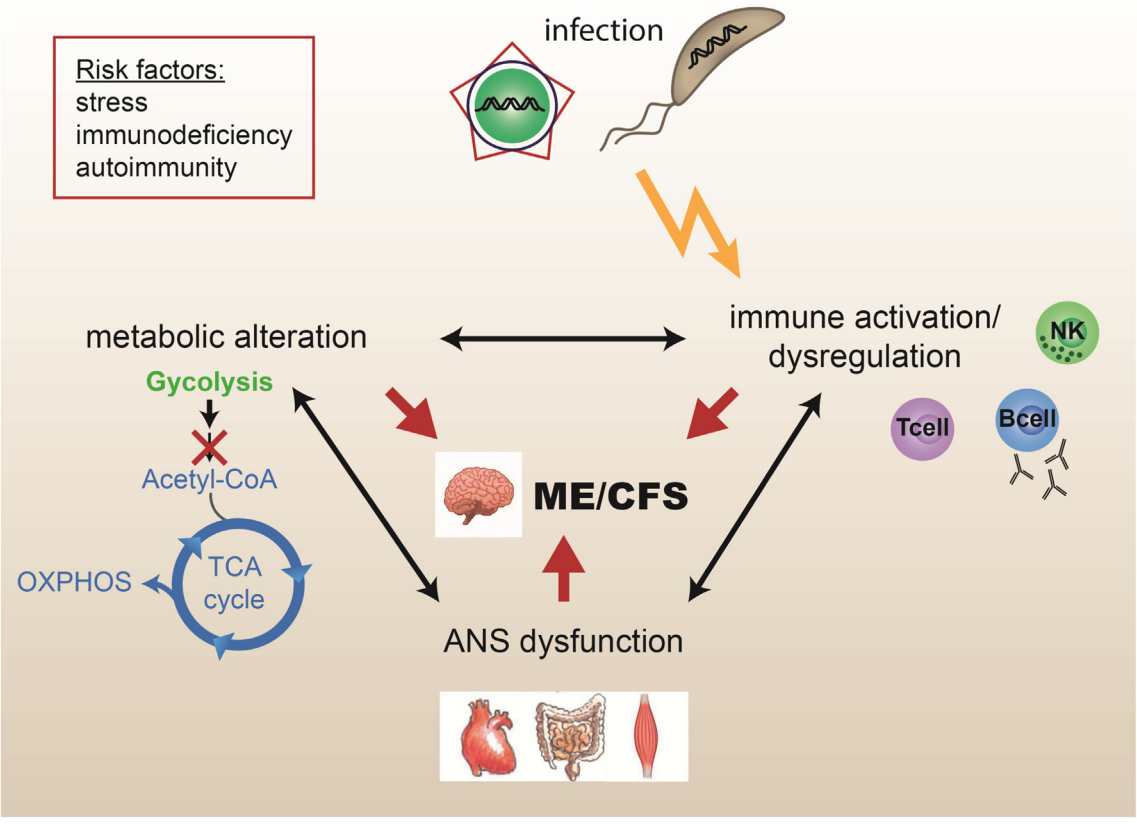

Fig. 1. Potential pathomechanism of ME/CFS. Dysregulation of immune system, autonomic nervous system (ANS) and metabolic disturbances contribute to this complex syndrome, in which severe fatigue and cognitive impairment are core features. In most patients, disease onset is triggered by infection with stress, immune deficiency and autoimmunity as known risk factors. Abbreviations: Acetyl-CoA: acetyl coenzyme A, ANS: autonomic nervous system, ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome, OXPHOS: oxidative phosphorylation, TCA: tricarboxylic acid.

group enhanced IgG reactivity against an EBNA-6 repeat sequence was found in ME/CFS patients [9]. Homologous sequences of various human proteins with an EBNA-6 repeat sequence might be potential targets for antigenic mimicry.

Detection of anti-HHV-6 IgM antibodies and HHV-6 antigen in peripheral blood mononuclear cells (PBMC) and mucosa as evidence for HHV-6 reactivation is more frequent in patients with ME/CFS compared to healthy donors, showing that reactivation of persistent HHV-6 infection could be a trigger factor for ME/CFS [12–15]. In studies from our group evidence for an active HHV-6, HHV-7 or B19 infection was found in a subset of patients and was associated with subfebrility and lymphadenopathy [16]. Others, however, showed no difference between severity of symptoms and viral load of HHV-6 and HHV-7 in DNA from saliva and PBMCs among ME/CFS patients and controls [17]. It should be noted that HHV-6 and HHV-7 infect immune cells, preferentially CD4+ T cells, but also CD8+, monocytes/macrophages and natural killer (NK) cells involved in cellular, humoral and innate immune response [18,19]. Infection of immune cells by these viruses lead to changes in cell surface receptor expression, pro-inflammatory and anti-inflammatory cytokine and chemokine expression level modulating local inflammation and immune response. A role for HHV-6 has been proposed in several autoimmune diseases, including MS, autoimmune connective tissue diseases, and Hashimoto's thyroiditis [20]. Molecular mimicry between myelin basic protein and an HHV-6 cell membrane protein is suggested to explain this link in MS [21]. Further, for ME/CFS and Gulf War Illness antibodies against the human dUTPase were reported by Halpin et al. [22]. These autoantibodies mainly occur together with antibodies against at least one of multiple HHV-encoded dUTPases suggesting an antigenic mimicry.

Parvovirus B19 infection has been shown to lead to development of ME/CFS. B19-triggered ME/CFS may be associated with a persistent viremia or may occur without viremia [23] and increased circulating TNF-α and IFN-γ were shown [24]. B19-associated ME/CFS was, in some cases, effectively treated with intravenous IgG [5,25,26]. Documented mechanisms in the pathogenesis of B19-associated autoimmunity include cross reaction of anti-B19 antibodies with human proteins, B19-induced apoptosis which results in presentation of self- antigens to T lymphocytes, and the phospholipase activity to the B19 unique VP1 protein region [23].

2.2. Immune cell alterations

Enhanced levels of immunoglobulins and alterations in B cells are frequently found in autoimmune diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS) [27–30]. Further frequencies of CD21low B cells are frequently increased in these autoimmune diseases [27]. Consistently, alterations of B cell subsets are reported in ME/CFS. Elevated numbers of CD21+ as well as CD19+ and activated CD5+ B cells were described in ME/CFS patients [31,32]. Bradley et al. showed enhanced frequencies of naïve and transitional B cells and diminished plasma blasts [33]. Differently, Brenu et al. did not observe an altered frequency of plasma blasts, but an increase of memory B cells [34]. However, no major alteration of major B cell subpopulations was observed in other studies [3,35]. Mensah et al. reported an increase in CD24+ B cells, a fraction found to be elevated in autoimmune diseases [35]. Further elevated IgG levels in a subset of ME/CFS patients were shown in several studies [3,35,36]. Recently, a whole blood gene expression study discovered a downregulation of genes being involved in B cell differentiation and survival in ME/CFS [37].

T cell activation by infections could play an important role in the onset of autoimmune diseases [38]. In ME/CFS individuals, an increased frequency of activated T cells expressing the activation marker CD26 and HLA-DR has been shown, concomitant to lower levels of CD45RA +CD4+ T cells [31]. Similarly, ME/CFS was associated with higher frequencies of CD38 and HLA-DR co-expressing CD8+ T cells [39]. However, other authors found similar or lower expression of these markers in ME/CFS patients compared to healthy individuals [40,41]. Similarly, there is also evidence for a decreased cytotoxicity of CD 8+ T cells in a subset of ME/CFS patients [42–45]. Of particular interest in autoimmune diseases are T follicular helper cells (Tfh) that induce humoral responses at the germinal centers [46], the anti- inflammatory regulatory T cells (Treg) and the inflammatory T helper 17 (Th17) cells that modulate the activity of autoimmune responses [47]. The frequency of Treg has been addressed by several authors, most of them reporting a paradoxical higher frequency of this cell population in ME/CFS [40,42,48]. However, no studies on the potential role of Tfh and Th17 cells are available in ME/CFS yet.

In contrast to inconsistent B and T cell alterations reported in ME/CFS, diminished numbers of circulating NK cells and reduction of their cytotoxic activity were uniformly shown [31,40,49]. However, enhanced secretion of IFNγ and TNFα by the immunoregulatory CD56 bright NK cell subset was described in ME/CFS [49,50].

In summary, immune dysfunction in ME/CFS, as for other autoimmune disease, is a multifaceted hallmark that requires further studies using new technologies, standardized assays and well defined cohorts to clearly define common patterns.

2.3. Autoantibodies in ME/CFS

Several studies described autoantibodies in ME/CFS mostly against nuclear and membrane structures and neurotransmitter receptors (Table 1).

Table 1
Autoantibodies in ME/CFS.

Autoantigen	Cohorts of patients/control (n)	Autoantibody positive patients/control (%)	Refs.
Nuclear structures			
ANA	60/51	68/15	[51]
	225	23	[53]
	60	57	[54]
	60	68	[55]
	139/149	7/5 (BioPlex ANA screen) 4/6 (IIF)	[57]
Nuclear envelope	60/51	52/2	[51]
	60/30	52/3	[55]
Reticulated speckles	60/30	25/0	[55]
68/48 kDa protein	114/37	13/0	[52]
dsDNA	81	12	[56]
Membrane structures			
Phospholipids	42	38	[58]
Cardiolipin	26	92 (IgM)	[59]
	40	95 (IgM)	[60]
	81	4	[56]
Phosphatidylserine	81	5	[56]
Gangliosides	42/100 (FM)	43	[58]
Neurotransmitter receptors and neurotransmitter			
M AChR	5/11	PET: binding to brain M AChR in ME/CFS	[61]
M1 AChR	60/30	53/0	[54]
M3/4 AChR and ®2-AdR	268/108	significantly elevated compared to healthy controls	[53]
5-HT	42	62	[58]
	81	9	[56]
Other autoantibodies			
Cytoplasmic intermediate filaments	60/30	35/13	[55]
dUTPase	55/151	15/5	[22]
Neopitopes formed by oxidative or nitrosative damage	14/11 16/17	Significantly elevated compared to healthy controls (IgM titers)	[66] [67]

Abbreviations: ANA: antinuclear antibodies; 5-HT: 5-hydroxytryptamine; IIF: indirect immunofluorescence; dUTPase: deoxyuridine 5′-triphosphate nucleotidohydrolase; FM: fibromyalgia.

2.3.1. Autoantibodies against nuclear and membrane structures

Antinuclear antibodies (ANA) were found in one study in 68 % of ME/CFS patients with the majority directed against the nuclear envelope [51]. Further studies showed ANA in 68%, 57%, 23% and 13% of ME/CFS patients [52–55]. Ortega-Hernandez et al. found dsDNA antibodies in 12% of patients [56], but another study failed to show such antibodies in ME/CFS (0.7%) [57].

Klein and Berg described anti-ganglioside antibodies in ME/CFS patients, but not in healthy controls [58]. In addition, they and others found phospholipid autoantibodies in ME/CFS patients [56,58,59] and antibodies against cardiolipin were described in 92–95% of ME/CFS patients in two studies [59,60] but only in 4% in another study [56]. Further autoantibodies against endothelial and neuronal cells were described in 30% and 16% of patients, respectively [56].

2.3.2. Antibodies against neurotransmitter receptors and neurotransmitter

Antibodies against the muscarinic M1 acetylcholine receptor (AChR) were reported in ME/CFS patients and were associated with muscle weakness [54]. Evidence for a functional role of these antibodies comes from a PET study showing reduced binding of a M AChR ligand in brain in antibody positive ME/CFS patients [61]. Antibodies against ß1 and ß2 adrenergic receptors (AdR) and M2/3 AChR were described in postural tachycardia syndrome, characterized by an increased heart rate in the absence of significant hypotension, as well as in orthostatic hypotension. This finding is of relevance for ME/CFS as 11–40% of ME/CFS patients concurrently suffer from postural orthostatic tachycardia syndrome (POTS) [62–65]. In a study from our group, elevated autoantibodies against both ß2-AdR and M3/4 AChR were found in a subset of ME/CFS patients compared to healthy controls [53]. A high correlation was found between levels of ß2 AdR autoantibodies and elevated IgG1–3 subclasses, activated HLA-DR+ T cells and thyroid peroxidase autoantibodies and ANA. The association of ß2 AdR autoantibodies with immune markers suggests an activation of B and T cells expressing ß2 AdRs. Further, disturbance of the AdR and M AChR function may explain symptoms of autonomic dysregulation in ME/CFS.

No differences between ME/CFS patients and controls were found in levels of autoantibodies directed against receptors for angiotensin, endothelin, *mu*-opioid, serotonin and dopamine [53,54]. However, autoantibodies against serotonin have been associated with ME/CFS [56,58].

2.3.3. Other autoantibodies

The IgM response against autoantigens formed by oxidative or nitrosative damage was studied by Maes et al. [66]. Autoantibodies directed against these neo-antigens, comprising oleic, palmitic and myristic acid, *S*-farnesyl-L-cysteine, by-products of lipid peroxidation, e.g. malondialdehyde, and N-oxide modified amino acids, e.g. nitro-tyrosine and nitro-tryptophan, were significantly higher in ME/CFS patients than in controls. In addition, they observed that the level of these autoantibodies correlates with severity of illness and symptoms. Although increased IgM antibodies against these oxidatively damaged antigens were shown in major depression, too, a higher immune response was found in ME/CFS [67].

2.4. Soluble markers of autoimmunity

Autoimmunity is associated with enhanced levels of circulating inflammatory cytokines playing an important role in the pathogenesis of autoimmune diseases [68]. Elevated levels of cytokines related to Th1- as well as Th2-driven responses were reported for ME/CFS in several studies [42,69–74]. Further cytokine levels in ME/CFS were associated with severity and duration of illness [72–74]. However, alterations in cytokine profiles in ME/CFS were not found in all studies [75,76].

Elevated levels of B lymphocyte activating factor (BAFF) were described in a variety of autoimmune diseases including RA, SLE and pSS [77]. BAFF regulates the survival and maturation of B cells and mediates the IL-10 production of regulatory B cells [78,79]. Elevated levels of BAFF were shown in a subset of patients with ME/CFS in comparison to healthy controls [80]. As the gene expression of the BAFF receptor (TNFRSF13C) is reduced in ME/CFS, increased serum BAFF levels may represent a compensatory mechanism [37]. Interestingly, elevated serum BAFF levels correlated with the autoantibody production in RA, SLE and pSS [81]. In ME/CFS an association between BAFF and autoantibodies was not described so far.

Activin A and B, members of the Transforming Growth Factor β family, are involved in the control of inflammation and muscle mass [82]. Elevated levels of activin B as well as an elevated ratio of activin A or B to the binding protein follostatin in ME/CFS patients were demonstrated in a recent study [83]. An association of increased activin A with inflammatory bowel disease, RA, and asthma was already shown [82].

CD26 is a peptide-cleaving enzyme associated with immune regulation. In various autoimmune diseases, such as MS, Grave's disease, and RA increased numbers of CD26 T cells were found in inflamed tissues and peripheral blood [84]. Fletcher et al. reported a higher frequency of CD26 expressing CD2+ lymphocytes in ME/CFS, but a decreased expression level on T and NK cells [85]. Further, they observed a reduction of the soluble CD26. Reduced serum CD26 levels were also reported for SLE and RA showing an inverse correlation with disease activity [84]. Low CD26 expression on PBMCs in ME/CFS was shown to correlate with reduced post-exercise muscle action potential, increased exercise-mediated lipid peroxidation, reduced quality of life and enhanced pain [86].

Other serum factors, frequently elevated in autoimmune disease like sCD30, sCD23, soluble cytotoxic T lymphocyte-associated antigen-4 (sCTLA-4) or the soluble IL-2 receptor (sIl-2R) are not described in ME/CFS so far [87–92].

2.5. Genetic variants associated with autoimmunity

It is well established that certain HLA alleles are associated with autoimmune diseases. Smith et al. showed an increased prevalence of the class II major histocompatibility complex HLA-DQB1 * 01 allele in ME/CFS patients [93]. Two others variants of HLA-DQB1 in combination with two RAGE-374A variants were associated with ME/CFS [94]. In another study the interaction of killer cell immunoglobulin-like receptors (KIRs) and their HLA class I epitopes were studied. An excess of KIR3DL1 and KIR3DS1 missing their HLA-Bw4Ile80 binding motif was shown in ME/CFS, leading potentially to an ongoing activation [95].

In the last years, genome-wide association studies revealed variants of various genes with either gain- or loss-of-function that are associated with the risk to develop autoimmune diseases. These single nucleotide polymorphisms (SNP) in receptors, enzymes or transcription factors play a role in B cell activation, T cell development, activation and proliferation, and cytokine signaling which are crucial in autoimmune diseases [96–100]. Further, it is becoming increasingly clear that elements of the non-coding genome regulate a variety of normal immune functions and that dysregulation of enhancer elements or long non- coding RNA may play a key role in autoimmunity [101]. So far only polymorphisms in cytokine as well as toll-like receptor signaling pathways and complement cascade were studied showing an association with ME/CFS [102,103]. Due to its regulation of the inflammatory response the glucocorticoid receptor gene NR3C1 has gained interest. Several variants (SNPs) within NR3C1 gene were shown to be significantly associated with ME/CFS [104,105].

2.6. Energy metabolism and autoimmunity

Immunometabolism represents the interface between immunology and metabolism and is an exciting emerging field of research in autoimmunity [106–108]. The metabolic requirements of immune cells depend on their state of resting or activation and differentiation. Their activation results in a metabolic switch to aerobic glycolysis in order to provide

enough energy and bio-precursors to meet the requirements for supporting rapid cell proliferation and immune functions. A growing body of evidence suggests that energy metabolism is crucial for the maintenance of chronic inflammation, not only in terms of energy supply but also in the control of the immune response through metabolic signals [106,107]. It has been suggested that disturbances in this intricate metabolic-immune cross-talk may be closely linked with and contribute to autoimmunity, although the precise pathomechanisms involved still remain to be elucidated [107,108]. It is also striking that several glycolytic enzymes act as autoantigens in rheumatic inflammatory disorders [109], although their role in ME/CFS remains unclear.

The profound and debilitating fatigue experienced by ME/CFS individuals led to the hypothesis that energy metabolism may be dysregulated. Defects in mitochondrial function in ME/CFS were shown in various studies from our group and others [110–113]. Metabolic profile had revealed disturbances related to energy, amino acids, nucleotides, nitrogen metabolism and oxidative stress in ME/CFS [114–119]. A metabolic shift toward aerobic glycolysis resulting in insufficient tricarboxylic acid (TCA) cycle and inadequate ATP production was reported recently, although the underlying basis has yet to be established [116,119]. Interestingly, the 2016 Fluge et al. study points to a secondary metabolic change driven by a serum factor in ME/CFS patients [116].

As dysfunctional metabolic pathways can directly influence and exacerbate defective immune responses, establishing the bioenergetic metabolism status of the different subsets of immune cells in ME/CFS has become a topic of increasing interest.

2.7. Comorbidity with autoimmune diseases

Comorbidity of ME/CFS with various autoimmune or immune-mediated diseases including fibromyalgia (FM), Hashimoto's thyroiditis and POTS is observed (Fig. 2). Especially for FM there is considerable overlap with up to 77% of patients fulfilling disease criteria for both ME/CFS and FM [120]. FM is characterized by chronic widespread pain and is common in autoimmune diseases with around 50% of prevalence in patients with RA and SLE [121,122]. According to the modified ACR 2010 criteria FM has an overall estimated prevalence of 5.4%. In a recent study from our group analyzing clinical subgroups in a large Spanish ME/CFS cohort was reported FM comorbidity ranging from 26% to 91% [123]. In another study including patient cohorts from Norway, UK and USA, a comorbidity for ME/CFS and FM of 30% was observed [124]. In a similar manner Hashimoto's thyroiditis characterized by elevated antibodies against thyroid peroxidase is frequent in autoimmune disease, whereas the overall prevalence is around 0.8% in the general population [125]. Hashimoto's thyroiditis is found in 17–20% in ME/CFS patients [53,123]. Moreover, 11–40% of ME/CFS patients suffer from POTS [62–65]. Interestingly, for both disorders elevated frequencies of autoantibodies directed against AdRs and M AChRs were shown [53,126–128]. Furthermore, a substantial number of ME/CFS patients have a family history of autoimmune diseases [129,130].

3. Therapies targeting autoimmunity in ME/CFS

First clear evidence for a pathogenic role of autoantibodies in ME/CFS comes from two clinical trials with the monoclonal anti-CD20 antibody rituximab [129,131]. Upon depletion of CD20+ B cells with rituximab, a monoclonal antibody directed against the B cell surface protein CD20, approximately 60% of patients experienced a partial or complete, and in some patients sustained clinical remission (Table 2). The delayed onset of response with a median of approximately 4 months in both trials suggests that clinical effects are not directly mediated by depletion of CD20+ B cells, but by diminishing short-lived antibody-producing

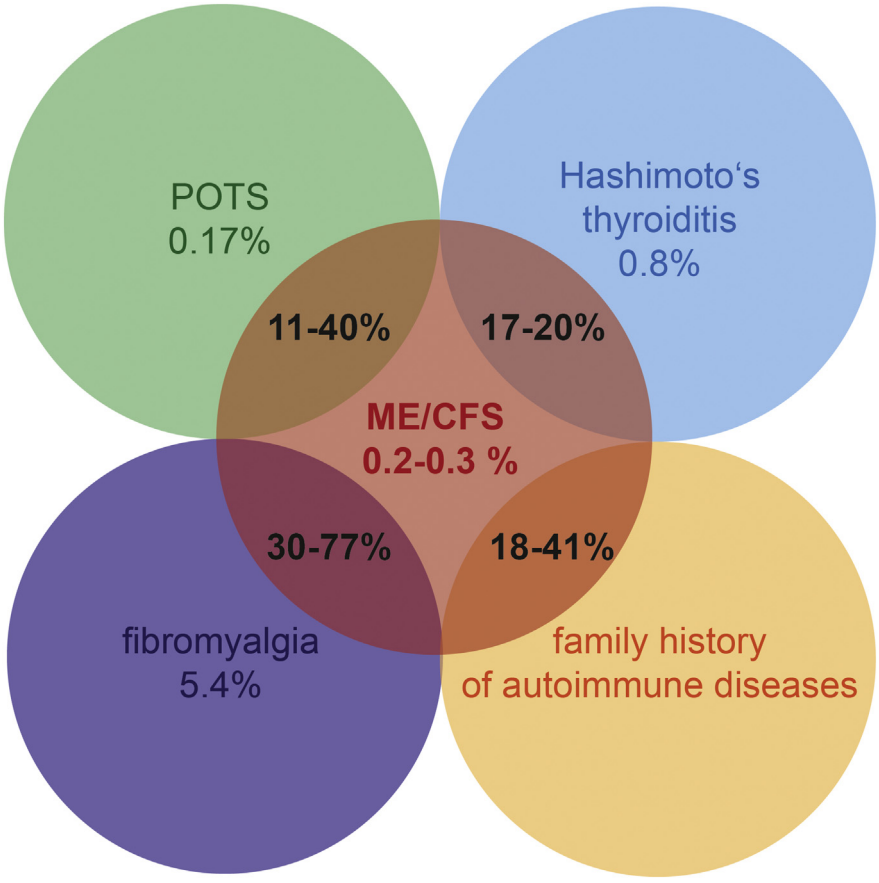

Fig. 2. Autoimmune-associated comorbid conditions in ME/CFS. Overall prevalence of diseases and prevalence for comorbidity with ME/CFS are indicated. Abbreviations: ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome, POTS: postural orthostatic tachycardia syndrome.

Table 2
Clinical trials targeting autoimmunity in ME/CFS.

Dosage	Study design	Patients (n)	Evaluation	Outcome	Refs.
Intravenous IgG					
1 g/kg/m2	RCT	28	FI & SR	No difference	[133]
6×					
2 g/kg/m2	RCT	49	FI & SR	Follow-up m3: 43% vs. 12%	[134]
3×					
0.5 g/1 g/2 g/kg/m2	RCT	99	FI & SR	No difference	[135]
3×					
1 g/kg/m2	RCT	70 (adolescents)	FI	Follow-up m6: 72% vs. 44%	[136]
3×					
Rituximab					
500 mg/m2	RCT	30	FI & SR	Improvement 67% vs. 13%	[131]
2×					
500 mg/m2 6×	Single arm	29	FI & SR	Improvement 62%	[129]
Ongoing Trials					
Cyclophosphamide (Endoxan®)					Fluge et al., unpub.
Immunoadsorption					[137]

Abbreviations: RCT = Randomized controlled trial; FI=Functional Improvement; SR = Symptom reduction, assessed by questionnaires; unpub.: unpublished data.

plasma cells arising from CD20+ memory B cells, followed by subsequent wash-out of autoantibodies. Results from a multicenter controlled trial with rituximab are awaited in spring 2018.

Few other treatment modalities targeting autoimmunity were evaluated in clinical trials in ME/CFS (Table 2). High dose intravenous IgG therapy is efficacious in autoantibody-mediated diseases. Several intravenous IgG studies were performed in ME/CFS during the 80's with two randomized controlled trials with positive and two with a negative outcome [132]. Preliminary data from an ongoing trial in Norway with cyclophosphamide suggests therapeutic efficacy of this broadly immunosuppressive drug (Fluge et al., unpublished data). Immunoadsorption is an apheresis in which IgG is specifically removed from plasma resulting in clinical improvement in various types of autoimmune disease. We performed a pilot trial in 10 patients with ME/CFS and observed first evidence for efficacy [137].

4. Conclusion

There is compelling evidence that autoimmune mechanisms play a role in ME/CFS. However clinical heterogeneity in disease onset (infection versus non-infection triggered), presence of immune-associated symptoms, and divergent immunological alterations point to the existence of subgroups of ME/CFS patients with possibly different pathomechanisms. Therefore, it is important to identify clinically useful diagnostic markers to select patients with autoimmune-mediated disease for clinical trials. The search for autoantibodies is of great importance enabling to develop potential biomarkers for diagnosis and providing a rationale for therapeutic interventions. Encouraging results from first clinical trials warrant larger studies with rituximab and other strategies targeting autoantibodies.

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Author contributions

FS and CS were responsible for the first draft of the protocol, which was critically reviewed, further developed and approved by all authors.

Declaration of competing interests

JB reports personal fees from ALBAJUNA THERAPEUTICS, S.L., outside the submitted work; CS has received grant support for clinical trials and research from Fresenius, Shire, Lost Voices, SolveME, MERUK, IBB, and speaking honoraria from Octapharma and Shire. FS, EC, JCM, SS and MM have no conflict of interest to declare.

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References

[1] Carruthers BM, van de Sande MI, De Meirleir KL, Klimas NG, Broderick G, Mitchell T, et al. Myalgic encephalomyelitis: international consensus criteria. J Intern Med 2011;270:327–38.
[2] Ascherio A, Munger KL. EBV and autoimmunity. Curr Top Microbiol Immunol 2015; 390:365–85.
[3] Guenther S, Loebel M, Mooslechner AA, Knops M, Hanitsch LG, Grabowski P, Wittke K, Meisel C, Unterwalder N, Volk HD, Scheibenbogen C. Frequent IgG subclass and mannose binding lectin deficiency in patients with chronic fatigue syndrome. Hum Immunol 2015;76(10):729–35. https://doi.org/10.1016/j.humimm.2015.09.028.
[4] DuBois RE, Seeley JK, Brus I, Sakamoto K, Ballow M, Harada S, et al. Chronic mononucleosis syndrome. South Med J 1984;77:1376–82.
[5] Jacobson SK, Daly JS, Thorne GM, McIntosh K. Chronic parvovirus B19 infection resulting in chronic fatigue syndrome: case history and review. Clin Infect Dis 1997;24:1048–51.
[6] Ortega-Hernandez OD, Shoenfeld Y. Infection, vaccination, and autoantibodies in chronic fatigue syndrome, cause or coincidence? Ann N Y Acad Sci 2009;1173: 600–9.
[7] Manian FA. Simultaneous measurement of antibodies to Epstein-Barr virus, human herpesvirus 6, herpes simplex virus types 1 and 2, and 14 enteroviruses in chronic fatigue syndrome: is there evidence of activation of a nonspecific polyclonal immune response? Clin Infect Dis 1994;19:448–53.
[8] Loebel M, Strohschein K, Giannini C, Koelsch U, Bauer S, Doebis C, et al. Deficient EBV-specific B- and T-cell response in patients with chronic fatigue syndrome. PLoS One 2014;9:e85387.
[9] Loebel M, Eckey M, Sotzny F, Hahn E, Bauer S, Grabowski P, et al. Serological profiling of the EBV immune response in chronic fatigue syndrome using a peptide microarray. PLoS One 2017;12:e0179124.

[10] Niller HH, Wolf H, Ay E, Minarovits J. Epigenetic dysregulation of epstein-barr virus latency and development of autoimmune disease. Adv Exp Med Biol 2011;711: 82–102.
[11] Gabibov AG, Belogurov Jr AA, Lomakin YA, Zakharova MY, Avakyan ME, Dubrovskaya VV, et al. Combinatorial antibody library from multiple sclerosis patients reveals antibodies that cross-react with myelin basic protein and EBV antigen. FASEB J 2011;25:4211–21.
[12] Ablashi DV, Eastman HB, Owen CB, Roman MM, Friedman J, Zabriskie JB, et al. Frequent HHV-6 reactivation in multiple sclerosis (MS) and chronic fatigue syndrome (CFS) patients. J Clin Virol 2000;16:179–91.
[13] Chapenko S, Krumina A, Kozireva S, Nora Z, Sultanova A, Viksna L, et al. Activation of human herpesviruses 6 and 7 in patients with chronic fatigue syndrome. J Clin Virol 2006;37(Suppl. 1):S47–51.
[14] Fremont M, Metzger K, Rady H, Hulstaert J, De Meirleir K. Detection of herpesviruses and parvovirus B19 in gastric and intestinal mucosa of chronic fatigue syndrome patients. In Vivo 2009;23:209–13.
[15] Di Luca D, Zorzenon M, Mirandola P, Colle R, Botta GA, Cassai E. Human herpesvirus 6 and human herpesvirus 7 in chronic fatigue syndrome. J Clin Microbiol 1995;33: 1660–1.
[16] Chapenko S, Krumina A, Logina I, Rasa S, Chistjakovs M, Sultanova A, et al. Association of active human herpesvirus-6, -7 and parvovirus b19 infection with clinical outcomes in patients with myalgic encephalomyelitis/chronic fatigue syndrome. Adv Virol 2012;2012:205085.
[17] Oakes B, Hoagland-Henefield M, Komaroff AL, Erickson JL, Huber BT. Human endogenous retrovirus-K18 superantigen expression and human herpesvirus-6 and human herpesvirus-7 viral loads in chronic fatigue patients. Clin Infect Dis 2013; 56:1394–400.
[18] Miyake F, Yoshikawa T, Sun H, Kakimi A, Ohashi M, Akimoto S, et al. Latent infection of human herpesvirus 7 in CD4(+) T lymphocytes. J Med Virol 2006;78:112–6.
[19] De Bolle L, Van Loon J, De Clercq E, Naesens L. Quantitative analysis of human herpesvirus 6 cell tropism. J Med Virol 2005;75:76–85.
[20] Broccolo F, Fusetti L, Ceccherini-Nelli L. Possible role of human herpesvirus 6 as a trigger of autoimmune disease. ScientificWorldJournal 2013;2013:867389.
[21] Tejada-Simon MV, Zang YC, Hong J, Rivera VM, Zhang JZ. Cross-reactivity with myelin basic protein and human herpesvirus-6 in multiple sclerosis. Ann Neurol 2003; 53:189–97.
[22] Halpin P, Williams MV, Klimas NG, Fletcher MA, Barnes Z, Ariza ME. Myalgic encephalomyelitis/chronic fatigue syndrome and gulf war illness patients exhibit increased humoral responses to the herpesviruses-encoded dUTPase: implications in disease pathophysiology. J Med Virol 2017;89:1636–45.
[23] Kerr JR. The role of parvovirus B19 in the pathogenesis of autoimmunity and autoimmune disease. J Clin Pathol 2016;69:279–91.
[24] Kerr JR, Barah F, Mattey DL, Laing I, Hopkins SJ, Hutchinson IV, et al. Circulating tumour necrosis factor-alpha and interferon-gamma are detectable during acute and convalescent parvovirus B19 infection and are associated with prolonged and chronic fatigue. J Gen Virol 2001;82:3011–9.
[25] Kerr JR, Cunniffe VS, Kelleher P, Bernstein RM, Bruce IN. Successful intravenous immunoglobulin therapy in 3 cases of parvovirus B19-associated chronic fatigue syndrome. Clin Infect Dis 2003;36:e100-.
[26] McGhee SA, Kaska B, Liebhaber M, Stiehm ER. Persistent parvovirus-associated chronic fatigue treated with high dose intravenous immunoglobulin. Pediatr Infect Dis J 2005;24:272–4.
[27] Thorarinsdottir K, Camponeschi A, Cavallini N, Grimsholm O, Jacobsson L, Gjertsson I, Martensson IL. CD21(-/low) B cells in human blood are memory cells. Clin Exp Immunol 2016;185(2):252–62. https://doi.org/10.1111/cei.12795.
[28] Isnardi I, Ng YS, Menard L, Meyers G, Saadoun D, Srdanovic I, et al. Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. Blood 2010;115:5026–36.
[29] Saadoun D, Terrier B, Bannock J, Vazquez T, Massad C, Kang I, et al. Expansion of autoreactive unresponsive CD21-/low B cells in Sjogren's syndrome-associated lymphoproliferation. Arthritis Rheum 2013;65:1085–96.
[30] Wehr C, Eibel H, Masilamani M, Illges H, Schlesier M, Peter HH, et al. A new CD21low B cell population in the peripheral blood of patients with SLE. Clin Immunol 2004;113:161–71.
[31] Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. J Clin Microbiol 1990;28:1403–10.
[32] Tirelli U, Marotta G, Improta S, Pinto A. Immunological abnormalities in patients with chronic fatigue syndrome. Scand J Immunol 1994;40:601–8.
[33] Bradley AS, Ford B, Bansal AS. Altered functional B cell subset populations in patients with chronic fatigue syndrome compared to healthy controls. Clin Exp Immunol 2013;172:73–80.
[34] Brenu EW, Huth TK, Hardcastle SL, Fuller K, Kaur M, Johnston S, et al. Role of adaptive and innate immune cells in chronic fatigue syndrome/myalgic encephalomyelitis. Int Immunol 2014;26:233–42.
[35] Mensah F, Bansal A, Berkovitz S, Sharma A, Reddy V, Leandro MJ, et al. Extended B cell phenotype in patients with myalgic encephalomyelitis/chronic fatigue syndrome: a cross-sectional study. Clin Exp Immunol 2016;184:237–47.
[36] Lobel M, Mooslechner AA, Bauer S, Gunther S, Letsch A, Hanitsch LG, et al. Polymorphism in COMT is associated with IgG3 subclass level and susceptibility to infection in patients with chronic fatigue syndrome. J Transl Med 2015;13:264.
[37] Nguyen CB, Alsoe L, Lindvall JM, Sulheim D, Fagermoen E, Winger A, et al. Whole blood gene expression in adolescent chronic fatigue syndrome: an exploratory cross-sectional study suggesting altered B cell differentiation and survival. J Transl Med 2017;15:102.
[38] Mills KH. TLR-dependent T cell activation in autoimmunity. Nat Rev Immunol 2011;11:807–22.
[39] Landay AL, Jessop C, Lennette ET, Levy JA. Chronic fatigue syndrome: clinical condition associated with immune activation. Lancet 1991;338:707–12.
[40] Curriu M, Carrillo J, Massanella M, Rigau J, Alegre J, Puig J, et al. Screening NK-, B- and T-cell phenotype and function in patients suffering from chronic fatigue syndrome. J Transl Med 2013;11:68.
[41] Swanink CM, Vercoulen JH, Galama JM, Roos MT, Meyaard L, van der Ven-Jongekrijg J, et al. Lymphocyte subsets, apoptosis, and cytokines in patients with chronic fatigue syndrome. J Infect Dis 1996;173:460–3.
[42] Brenu EW, van Driel ML, Staines DR, Ashton KJ, Ramos SB, Keane J, et al. Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/Myalgic encephalomyelitis. J Transl Med 2011;9:81.
[43] Hardcastle SL, Brenu EW, Johnston S, Nguyen T, Huth T, Wong N, et al. Characterisation of cell functions and receptors in chronic fatigue syndrome/Myalgic encephalomyelitis (CFS/ME). BMC Immunol 2015;16:35.
[44] Brenu EW, Broadley S, Nguyen T, Johnston S, Ramos S, Staines D, et al. A preliminary comparative assessment of the role of CD8+ T cells in chronic fatigue syndrome/myalgic encephalomyelitis and multiple sclerosis. J Immunol Res 2016; 2016:9064529.
[45] Theorell J, Bileviciute-Ljungar I, Tesi B, Schlums H, Johnsgaard MS, Asadi-Azarbaijani B, et al. Unperturbed cytotoxic lymphocyte phenotype and function in myalgic encephalomyelitis/chronic fatigue syndrome patients. Front Immunol 2017;8:723.
[46] Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly - TFH cells in human health and disease. Nat Rev Immunol 2013;13:412–26.
[47] Afzali B, Lombardi G, Lechler RI, Lord GM. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 2007;148:32–46.
[48] Ramos S, Brenu E, Broadley S, Kwiatek R, Ng J, Nguyen T, et al. Regulatory T, natural killer T and gammadelta T cells in multiple sclerosis and chronic fatigue syndrome/myalgic encephalomyelitis: a comparison. Asian Pac J Allergy Immunol 2016;34: 300–5.
[49] Brenu EW, van Driel ML, Staines DR, Ashton KJ, Hardcastle SL, Keane J, et al. Longitudinal investigation of natural killer cells and cytokines in chronic fatigue syndrome/myalgic encephalomyelitis. J Transl Med 2012;10:88.
[50] Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, et al. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood 2001;97:3146–51.
[51] Konstantinov K, von Mikecz A, Buchwald D, Jones J, Gerace L, Tan EM. Autoantibodies to nuclear envelope antigens in chronic fatigue syndrome. J Clin Invest 1996;98:1888–96.
[52] Nishikai M, Tomomatsu S, Hankins RW, Takagi S, Miyachi K, Kosaka S, et al. Autoantibodies to a 68/48 kDa protein in chronic fatigue syndrome and primary fibromyalgia: a possible marker for hypersomnia and cognitive disorders. Rheumatology 2001;40:806–10.
[53] Loebel M, Grabowski P, Heidecke H, Bauer S, Hanitsch LG, Wittke K, et al. Antibodies to beta adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome. Brain Behav Immun 2016;52:32–9.
[54] Tanaka S, Kuratsune H, Hidaka Y, Hakariya Y, Tatsumi KI, Takano T, et al. Autoantibodies against muscarinic cholinergic receptor in chronic fatigue syndrome. Int J Mol Med 2003;12:225–30.
[55] von Mikecz A, Konstantinov K, Buchwald DS, Gerace L, Tan EM. High frequency of autoantibodies to insoluble cellular antigens in patients with chronic fatigue syndrome. Arthritis Rheum 1997;40:295–305.
[56] Ortega-Hernandez OD, Cuccia M, Bozzini S, Bassi N, Moscavitch S, Diaz-Gallo LM, et al. Autoantibodies, polymorphisms in the serotonin pathway, and human leukocyte antigen class II alleles in chronic fatigue syndrome: are they associated with age at onset and specific symptoms? Ann N Y Acad Sci 2009;1173:589–99.
[57] Op De Beeck K, Vermeersch P, Verschueren P, Westhovens R, Marien G, Blockmans D, et al. Antinuclear antibody detection by automated multiplex immunoassay in untreated patients at the time of diagnosis. Autoimmun Rev 2012;12:137–43.
[58] Klein R, Berg PA. 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 1995;1:21–6.
[59] Hokama Y, Empey-Campora C, Hara C, Higa N, Siu N, Lau R, et al. Acute phase phospholipids related to the cardiolipin of mitochondria in the sera of patients with chronic fatigue syndrome (CFS), chronic Ciguatera fish poisoning (CCFP), and other diseases attributed to chemicals, Gulf War, and marine toxins. J Clin Lab Anal 2008;22:99–105.
[60] Hokama Y, Campora CE, Hara C, Kuribayashi T, Le Huynh D, Yabusaki K. Anticardiolipin antibodies in the sera of patients with diagnosed chronic fatigue syndrome. J Clin Lab Anal 2009;23:210–2.
[61] Yamamoto S, Ouchi Y, Nakatsuka D, Tahara T, Mizuno K, Tajima S, et al. Reduction of [11C](+)3-MPB binding in brain of chronic fatigue syndrome with serum autoantibody against muscarinic cholinergic receptor. PLoS One 2012;7:e51515.
[62] Reynolds GK, Lewis DP, Richardson AM, Lidbury BA. Comorbidity of postural orthostatic tachycardia syndrome and chronic fatigue syndrome in an Australian cohort. J Intern Med 2014;275:409–17.
[63] Hoad A, Spickett G, Elliott J, Newton J. Postural orthostatic tachycardia syndrome is an under- recognized condition in chronic fatigue syndrome. QJM 2008;101:961–5.
[64] Jones JF, Nicholson A, Nisenbaum R, Papanicolaou DA, Solomon L, Boneva R, et al. Orthostatic instability in a population-based study of chronic fatigue syndrome. Am J Med 2005;118:1415.
[65] Dahan S, Tomljenovic L, Shoenfeld Y. Postural orthostatic tachycardia syndrome (POTS)—a novel member of the autoimmune family. Lupus 2016;25:339–42.

[66] Maes M, Mihaylova I, Leunis JC. 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 2006;27:615–21.
[67] Maes M, Mihaylova I, Kubera M, Leunis JC, Twisk FN, Geffard M. 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 2012;27:415–23.
[68] Moudgil KD, Choubey D. Cytokines in autoimmunity: role in induction, regulation, and treatment. J Interf Cytokine Res 2011;31:695–703.
[69] Khaiboullina SF, DeMeirleir KL, Rawat S, Berk GS, Gaynor-Berk RS, Mijatovic T, et al. Cytokine expression provides clues to the pathophysiology of Gulf War illness and myalgic encephalomyelitis. Cytokine 2015;72:1–8.
[70] Skowera A, Cleare A, Blair D, Bevis L, Wessely SC, Peakman M. High levels of type 2 cytokine- producing cells in chronic fatigue syndrome. Clin Exp Immunol 2004;135:294–302.
[71] Fletcher MA, Zeng XR, Barnes Z, Levis S, Klimas NG. Plasma cytokines in women with chronic fatigue syndrome. J Transl Med 2009;7:96.
[72] Milrad SF, Hall DL, Jutagir DR, Lattie EG, Ironson GH, Wohlgemuth W, et al. Poor sleep quality is associated with greater circulating pro-inflammatory cytokines and severity and frequency of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) symptoms in women. J Neuroimmunol 2017;303:43–50.
[73] Montoya JG, Holmes TH, Anderson JN, Maecker HT, Rosenberg-Hasson Y, Valencia IJ, et al. Cytokine signature associated with disease severity in chronic fatigue syndrome patients. Proc Natl Acad Sci U S A 2017;114:E7150-E8.
[74] Hornig M, Montoya JG, Klimas NG, Levine S, Felsenstein D, Bateman L, et al. Distinct plasma immune signatures in ME/CFS are present early in the course of illness. Sci Adv 2015;1.
[75] Blundell S, Ray KK, Buckland M, White PD. Chronic fatigue syndrome and circulating cytokines: a systematic review. Brain Behav Immun 2015;50:186–95.
[76] Clark LV, Buckland M, Murphy G, Taylor N, Vleck V, Mein C, et al. Cytokine responses to exercise and activity in patients with chronic fatigue syndrome: case-control study. Clin Exp Immunol 2017;190:360–71.
[77] Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. Clin Exp Immunol 2009;158:155–63.
[78] Saulep-Easton D, Vincent FB, Quah PS, Wei A, Ting SB, Croce CM, et al. The BAFF receptor TACI controls IL-10 production by regulatory B cells and CLL B cells. Leukemia 2016;30:163–72.
[79] Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol 2009;9:491–502.
[80] Lunde S, Kristoffersen EK, Sapkota D, Risa K, Dahl O, Bruland O, et al. Serum BAFF and APRIL levels, T-lymphocyte subsets, and Immunoglobulins after B-cell depletion using the monoclonal anti-CD20 antibody rituximab in Myalgic Encephalopathy/Chronic Fatigue Syndrome. PLoS One 2016;11:e0161226.
[81] Pers JO, Daridon C, Devauchelle V, Jousse S, Saraux A, Jamin C, et al. BAFF overexpression is associated with autoantibody production in autoimmune diseases. Ann N Y Acad Sci 2005;1050:34–9.
[82] Hedger MP, de Kretser DM. The activins and their binding protein, follistatin-diagnostic and therapeutic targets in inflammatory disease and fibrosis. Cytokine Growth Factor Rev 2013;24:285–95.
[83] Lidbury BA, Kita B, Lewis DP, Hayward S, Ludlow H, Hedger MP, et al. Activin B is a novel biomarker for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) diagnosis: a cross sectional study. J Transl Med 2017;15:60.
[84] Hosono O, Ohnuma K, Dang NH, Morimoto C. CD26: a key molecule in immune regulation and autoimmune diseases. Mod Rheumatol 2003;13:199–204.
[85] Fletcher MA, Zeng XR, Maher K, Levis S, Hurwitz B, Antoni M, et al. Biomarkers in chronic fatigue syndrome: evaluation of natural killer cell function and dipeptidyl peptidase IV/CD26. PLoS One 2010;5:e10817.
[86] Fenouillet E, Vigouroux A, Steinberg JG, Chagvardieff A, Retornaz F, Guieu R, et al. Association of biomarkers with health-related quality of life and history of stressors in myalgic encephalomyelitis/chronic fatigue syndrome patients. J Transl Med 2016;14:251.
[87] Okumura M, Hidaka Y, Kuroda S, Takeoka K, Tada H, Amino N. Increased serum concentration of soluble CD30 in patients with Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab 1997;82:1757–60.
[88] Ichikawa Y, Yoshida M, Yamada C, Horiki T, Hoshina Y, Uchiyama M. Circulating soluble CD30 levels in primary Sjogren's syndrome, SLE and rheumatoid arthritis. Clin Exp Rheumatol 1998;16:759–60.
[89] Oflazoglu E, Simpson EL, Takiguchi R, Grewal IS, Hanifin JM, Gerber HP. CD30 expression on CD1a+ and CD8+ cells in atopic dermatitis and correlation with disease severity. Eur J Dermatol 2008;18:41–9.
[90] Yoshikawa T, Nanba T, Kato H, Hori K, Inamoto T, Kumagai S, et al. Soluble FcεRII/CD23 in patients with autoimmune diseases and Epstein-Barr virus-related disorders: analysis by ELISA for soluble FcεRII/CD23. ImmunoMethods 1994;4:65–71.
[91] Pawlak E, Kochanowska IE, Frydecka I, Kielbinski M, Potoczek S, Bilinska M. The soluble CTLA-4 receptor: a new marker in autoimmune diseases. Arch Immunol Ther Exp 2005;53:336–41.
[92] Dejica D. Serum soluble IL-2 receptor as a marker of lymphocyte activation in some autoimmune diseases. Effect of immunosuppressive therapy. Roum Arch Microbiol Immunol 2001;60:183–201.
[93] Smith J, Fritz EL, Kerr JR, Cleare AJ, Wessely S, Mattey DL. Association of chronic fatigue syndrome with human leucocyte antigen class II alleles. J Clin Pathol 2005;58:860–3.
[94] Carlo-Stella N, Bozzini S, De Silvestri A, Sbarsi I, Pizzochero C, Lorusso L, et al. Molecular study of receptor for advanced glycation endproduct gene promoter and identification of specific HLA haplotypes possibly involved in chronic fatigue syndrome. Int J Immunopathol Pharmacol 2009;22:745–54.
[95] Pasi A, Bozzini S, Carlo-Stella N, Martinetti M, Bombardieri S, De Silvestri A, et al. Excess of activating killer cell immunoglobulinlike receptors and lack of HLA-Bw4 ligands: a twoedged weapon in chronic fatigue syndrome. Mol Med Rep 2011;4:535–40.
[96] Kochi Y. Genetics of autoimmune diseases: perspectives from genome-wide association studies. Int Immunol 2016;28:155–61.
[97] Frederiksen B, Liu E, Romanos J, Steck AK, Yin X, Kroehl M, et al. Investigation of the vitamin D receptor gene (VDR) and its interaction with protein tyrosine phosphatase, non-receptor type 2 gene (PTPN2) on risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). J Steroid Biochem Mol Biol 2013;133:51–7.
[98] Sigurdsson S, Nordmark G, Garnier S, Grundberg E, Kwan T, Nilsson O, et al. A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5. Hum Mol Genet 2008;17:2868–76.
[99] Hirschfield GM, Liu X, Xu C, Lu Y, Xie G, Lu Y, et al. Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. N Engl J Med 2009;360:2544–55.
[100] Ting WH, Chien MN, Lo FS, Wang CH, Huang CY, Lin CL, et al. Association of Cytotoxic T- lymphocyte-associated protein 4 (CTLA4) gene polymorphisms with autoimmune thyroid disease in children and adults: case-control study. PLoS One 2016;11:e0154394.
[101] Wu GC, Pan HF, Leng RX, Wang DG, Li XP, Li XM, et al. Emerging role of long noncoding RNAs in autoimmune diseases. Autoimmun Rev 2015;14:798–805.
[102] Carlo-Stella N, Badulli C, De Silvestri A, Bazzichi L, Martinetti M, Lorusso L, et al. A first study of cytokine genomic polymorphisms in CFS: positive association of TNF-857 and IFNgamma 874 rare alleles. Clin Exp Rheumatol 2006;24:179–82.
[103] Rajeevan MS, Dimulescu I, Murray J, Falkenberg VR, Unger ER. Pathway-focused genetic evaluation of immune and inflammation related genes with chronic fatigue syndrome. Hum Immunol 2015;76:553–60.
[104] Lee E, Cho S, Kim K, Park T. An integrated approach to infer causal associations among gene expression, genotype variation, and disease. Genomics 2009;94:269–77.
[105] Rajeevan MS, Smith AK, Dimulescu I, Unger ER, Vernon SD, Heim C, et al. Glucocorticoid receptor polymorphisms and haplotypes associated with chronic fatigue syndrome. Genes Brain Behav 2007;6:167–76.
[106] Procaccini C, Galgani M, De Rosa V, Matarese G. Intracellular metabolic pathways control immune tolerance. Trends Immunol 2012;33:1–7.
[107] Spies CM, Straub RH, Buttgereit F. Energy metabolism and rheumatic diseases: from cell to organism. Arthritis Res Ther 2012;14:216.
[108] Freitag J, Berod L, Kamradt T, Sparwasser T. Immunometabolism and autoimmunity. Immunol Cell Biol 2016;94:925–34.
[109] Chang X, Wei C. Glycolysis and rheumatoid arthritis. Int J Rheum Dis 2011;14:217–22.
[110] Booth NE, Myhill S, McLaren-Howard J. Mitochondrial dysfunction and the pathophysiology of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Int J Clin Exp Med 2012;5:208–20.
[111] Maes M, Twisk FN, Ringel K. Inflammatory and cell-mediated immune biomarkers in myalgic encephalomyelitis/chronic fatigue syndrome and depression: inflammatory markers are higher in myalgic encephalomyelitis/chronic fatigue syndrome than in depression. Psychother Psychosom 2012;81:286–95.
[112] Castro-Marrero J, Cordero MD, Saez-Francas N, Jimenez-Gutierrez C, Aguilar-Montilla FJ, Aliste L, et al. Could mitochondrial dysfunction be a differentiating marker between chronic fatigue syndrome and fibromyalgia? Antioxid Redox Signal 2013;19:1855–60.
[113] Myhill S, Booth NE, McLaren-Howard J. Targeting mitochondrial dysfunction in the treatment of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) - a clinical audit. Int J Clin Exp Med 2013;6:1–15.
[114] Armstrong CW, McGregor NR, Sheedy JR, Buttfield I, Butt HL, Gooley PR. NMR metabolic profiling of serum identifies amino acid disturbances in chronic fatigue syndrome. Clin Chim Acta 2012;413:1525–31.
[115] Armstrong CW, McGregor NR, Lewis DP, Butt HL, Gooley PR. Metabolic profiling reveals anomalous energy metabolism and oxidative stress pathways in chronic fatigue syndrome patients. Metabolomics 2015;11:1626–39.
[116] Fluge O, Mella O, Bruland O, Risa K, Dyrstad SE, Alme K, et al. Metabolic profiling indicates impaired pyruvate dehydrogenase function in myalgic encephalopathy/chronic fatigue syndrome. JCI Insight 2016;1:e89376.
[117] Germain A, Ruppert D, Levine SM, Hanson MR. Metabolic profiling of a myalgic encephalomyelitis/chronic fatigue syndrome discovery cohort reveals disturbances in fatty acid and lipid metabolism. Mol BioSyst 2017;13:371–9.
[118] Naviaux RK, Naviaux JC, Li K, Bright AT, Alaynick WA, Wang L, et al. Metabolic features of chronic fatigue syndrome. Proc Natl Acad Sci U S A 2016;113:E5472-0.
[119] Yamano E, Sugimoto M, Hirayama A, Kume S, Yamato M, Jin G, et al. Index markers of chronic fatigue syndrome with dysfunction of TCA and urea cycles. Sci Rep 2016;6:34990.
[120] Aaron LA, Herrell R, Ashton S, Belcourt M, Schmaling K, Goldberg J, et al. Comorbid clinical conditions in chronic fatigue: a co-twin control study. J Gen Intern Med 2001;16:24–31.
[121] Buskila D, Sarzi-Puttini P. Fibromyalgia and autoimmune diseases: the pain behind autoimmunity. Isr Med Assoc J 2008;10:77–8.
[122] Wolfe F, Ross K, Anderson J, Russell IJ, Hebert L. The prevalence and characteristics of fibromyalgia in the general population. Arthritis Rheum 1995;38:19–28.
[123] Castro-Marrero J, Faro M, Aliste L, Saez-Francas N, Calvo N, Martinez-Martinez A, et al. Comorbidity in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: a nationwide population-based cohort study. Psychosomatics 2017;58:533–43.
[124] Jason LA, McManimen S, Sunnquist M, Newton JL, Strand EB. Examining those meeting IOM criteria versus IOM plus fibromyalgia. Neurology 2017;5:19–28.

[125] Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol 1977;7:481–93.
[126] Li H, Yu X, Liles C, Khan M, Vanderlinde-Wood M, Galloway A, et al. Autoimmune basis for postural tachycardia syndrome. J Am Heart Assoc 2014;3:e000755.
[127] Li J, Zhang Q, Liao Y, Zhang C, Hao H, Du J. The value of acetylcholine receptor antibody in children with postural tachycardia syndrome. Pediatr Cardiol 2015;36:165–70.
[128] Yu X, Stavrakis S, Hill MA, Huang S, Reim S, Li H, et al. Autoantibody activation of beta- adrenergic and muscarinic receptors contributes to an "autoimmune" orthostatic hypotension. J Am Soc Hypertens 2012;6:40–7.
[129] Fluge O, Risa K, Lunde S, Alme K, Rekeland IG, Sapkota D, et al. B-lymphocyte depletion in Myalgic encephalopathy/ chronic fatigue syndrome. An open-label phase II study with rituximab maintenance treatment. PLoS One 2015;10:e0129898.
[130] Endicott NA. Chronic fatigue syndrome in private practice psychiatry: family history of physical and mental health. J Psychosom Res 1999;47:343–54.
[131] Fluge O, Bruland O, Risa K, Storstein A, Kristoffersen EK, Sapkota D, et al. Benefit from B- lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double- blind and placebo-controlled study. PLoS One 2011;6:e26358.
[132] Whiting P, Bagnall AM, Sowden AJ, Cornell JE, Mulrow CD, Ramirez G. Interventions for the treatment and management of chronic fatigue syndrome: a systematic review. JAMA 2001;286:1360–8.
[133] Peterson PK, Shepard J, Macres M, Schenck C, Crosson J, Rechtman D, et al. A controlled trial of intravenous immunoglobulin G in chronic fatigue syndrome. Am J Med 1990;89:554–60.
[134] Lloyd A, Hickie I, Wakefield D, Boughton C, Dwyer J. A double-blind, placebo-controlled trial of intravenous immunoglobulin therapy in patients with chronic fatigue syndrome. Am J Med 1990;89:561–8.
[135] Vollmer-Conna U, Hickie I, Hadzi-Pavlovic D, Tymms K, Wakefield D, Dwyer J, et al. Intravenous immunoglobulin is ineffective in the treatment of patients with chronic fatigue syndrome. Am J Med 1997;103:38–43.
[136] Rowe KS. Double-blind randomized controlled trial to assess the efficacy of intravenous gammaglobulin for the management of chronic fatigue syndrome in adolescents. J Psychiatr Res 1997;31:133–47.
[137] Scheibenbogen C, Loebel M, Freitag H, Krueger A, Bauer S, Antelmann M, Doehner W, Scherbakov N, Heidecke H, Reinke P, Volk HD, Grabowski P. Immunoadsorption to remove ß2 adrenergic receptor antibodies in Chronic Fatigue Syndrome CFS/ME. PLoS One 2018;13(3):e0193672. https://doi.org/10.1371/journal.pone.0193672.