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

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
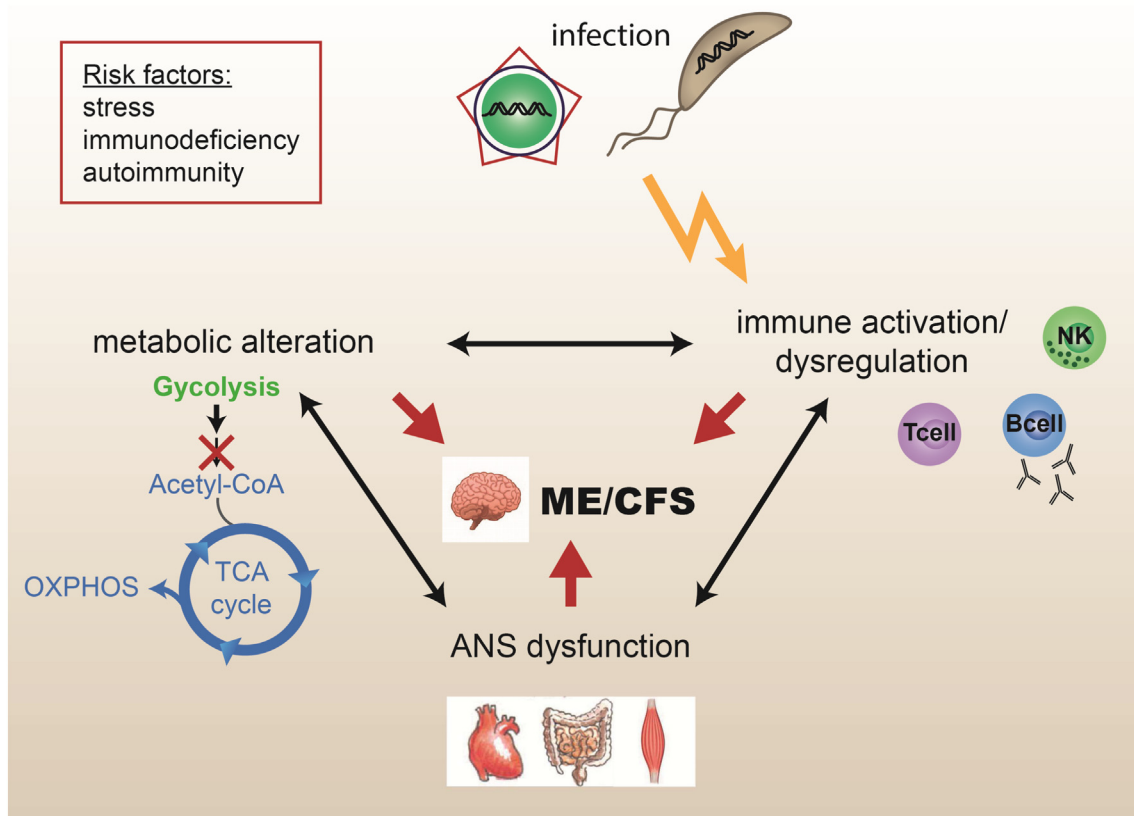
Fig. 1. Potential pathomechanism of ME/CFS. Dysregulation of immune system, autonomic nervous system (ANS) and metabolic disturbances contribute to this complex syndrome, in which severe fatigue and cognitive impairment are core features. In most patients, disease onset is triggered by infection with 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 CD8+ 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
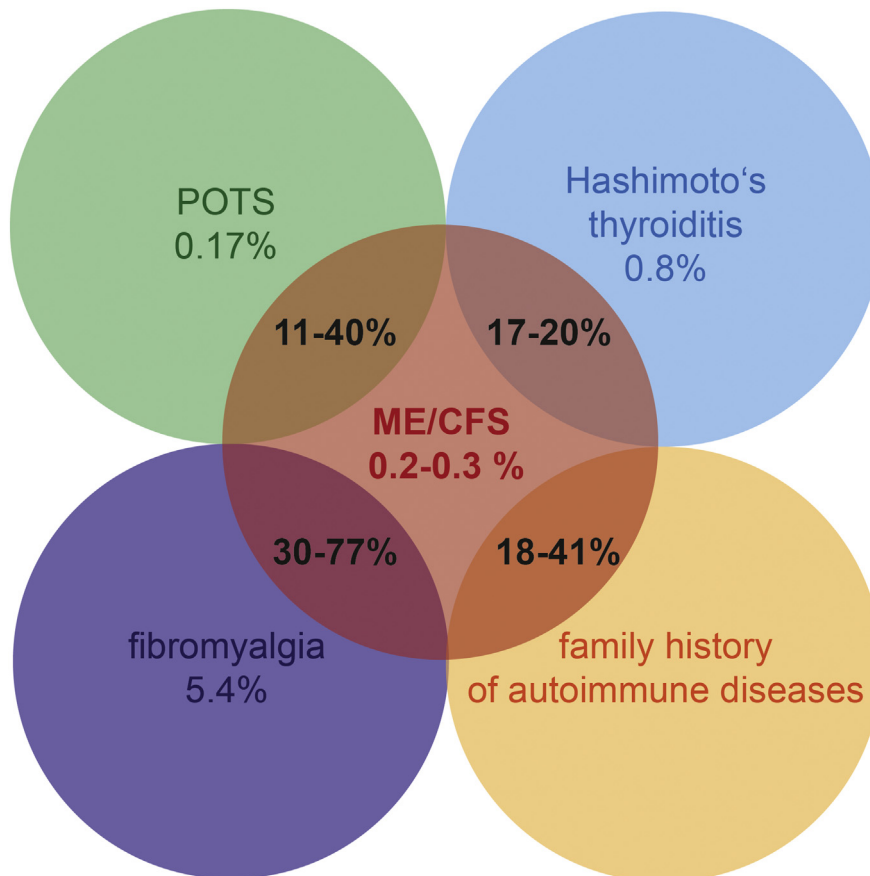

Fig. 2. Autoimmune-associated comorbid conditions in ME/CFS. Overall prevalence of diseases and prevalence for comorbidity with ME/CFS are indicated. Abbreviations: ME/CFS: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome, POTS: postural orthostatic tachycardia syndrome.

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

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