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Autoimmunity's enigmatic role: exploring the connection with myalgic encephalomyelitis/chronic fatigue syndrome

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Abstract

Background Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complicated, heterogeneous condition distinguished by post-exertional neuroimmune exhaustion and multisystem symptoms. Its complexity poses challenges for physicians, researchers and those inflicted by its presence. Due to conflicting evidence and limiting consensus, the association and contribution autoimmunity serves in the pathophysiology or aetiology of ME/CFS is yet to be confirmed. This systematic review synthesises the currently available data to clarify the role autoimmunity has in the pathogenesis of ME/CFS and explore the therapeutic limitations.

Methods This systematic review was conducted in accordance with the PRISMA and Cochrane guidelines. Full-text articles containing the primary key terms "Autoimmunity/Autoimmune" and "ME/CFS" were included provided their suitability to the inclusion and exclusion criteria.

Results Ten publications investigating the role of autoimmunity in ME/CFS were examined. One investigated the role of cytokine signalling; Three investigated the genetic nature of autoimmunity in ME/CFS patients; One examined the immune lineage of ME/CFS patients; Six investigated the presence and role of autoantibodies in ME/CFS patients.

Conclusion The findings generated from this systematic review highlight inconsistent and insufficient evidence to classify ME/CFS as an autoimmune disease. Additionally, it further emphasises the complexity of ME/CFS and highlights the challenges in distinguishing autoreactivity from deregulatory processes. Future research is urgently needed to advance the development of diagnostic and treatment strategies.

PROSPERO Registration Code CRD42024533447.

Keywords Myalgic encephalomyelitis, Chronic fatigue syndrome, ME/CFS, Autoimmunity, Autoantibodies, Autoimmune

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Introduction

Exhaustion, confusion, and pain are a daily occurrence for ~24 million individuals worldwide who are affected by Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). ME/CFS as a perplexing and debilitating illness presents a unique challenge for medical professionals and sufferers alike, in part to the complex and overlapping symptomology [1]. Individuals can present with an array of symptomology ranging from endocrinological, neurological as well as immunological, highlighting the ongoing challenge to diagnose, manage and treat those afflicted by its presence. Despite cumulative evidence supporting its distinction, the absence of a definitive diagnostic test has led to the reliance on diagnostic criteria such as the Fukuda Criteria (1994), the Canadian Consensus Criteria (CCC) (2003), Institute of Medicine (IOM) (2015) and the International Consensus Criteria (ICC) (2011) [1–3]. The aetiology of ME/CFS remains elusive, contributing to its complexity. Recent research has highlighted the role the immune system has in ME/CFS pathogenesis, often times suggesting a potential autoimmune mechanism [4]. Autoimmunity involves the immune system losing its ability to distinguish between self and non-self-tissues, resulting in loss of 'self-tolerance' and derangement of inflammatory signalling [5, 6]. Typically, the classification of such reactions have been based on criteria such as that proposed by Witebsky and Rose, which include the presence of autoantibodies (AAbs) and autoreactivity to self-antigens [7]. Importantly, the application of such criteria to ME/CFS is vital in distinguishing autoimmunological processes from those that are typically dysfunctional. As a differentiating factor between these phenomena is that of autoreactivity. ME/CFS exhibits parallels with various autoimmune conditions, suggesting shared immunopathogenic pathways [1]. However, the contribution autoimmunity has in ME/CFS is subject to ongoing investigation and debate as ME/CFS lacks several key features associated with autoimmunity including tissue damage and validated AAbs reported in autoimmune conditions such as Sjogren's syndrome, Systemic Lupus Erythematosus, and Hashimoto's Thyroiditis. These autoimmune conditions show molecular overlaps with ME/CFS, including dysregulation of immune signalling molecules like cytokine and chemokines (Fig. 1) [3]. While investigations into ME/CFS-specific markers are underway, a comprehensive understanding of potential autoimmune mechanisms remains incomplete. As the post-COVID-19 era may result in an increase of ME/CFS cases, there is an urgent need to unravel its underlying mechanisms [8]. This systematic review aims to comprehensively explore the potential autoimmune markers in ME/CFS. By synthesising existing evidence, we seek to contribute to a deeper understanding of ME/CFS pathogenesis, discuss potential autoimmune pathways, and highlight implications for future research directions and therapeutic advancements.

Materials and methods

Literature search

This systematic review adheres to the PRISMA (Fig. 2) and Cochrane guidelines for methodological rigor. We undertook a comprehensive database search using the key MeSH terms "Autoimmunity/Autoimmune" and "ME/CFS" (Supplementary 1). Our searches spanned across four major databases. PubMed (9), Embase (68), Scopus (221) and Web of Science (30), yielding a total of 328 publications. After applying our inclusion and exclusion criteria, ten full-text publications were deemed suitable for this review. These publications were categorized based on their primary findings: one publication explored cytokine signalling pathways; three investigated the genetic nature of autoimmunity; one examined immune cell phenotypes; and six focused on the presence and role of AAbs in ME/CFS. To ensure relevance and contemporary understanding, we considered papers published in the period from 1994 to 2024 April.

Inclusion criteria

Generated studies were included in this review if they had two or more of the key search terms in the abstract or title, as well as adhering to the following inclusion criteria: (i) Published in 1994 or later; (ii) Human Participants; (iii) Participants included in the study must be 18 years or older; (iv) Full-text articles; (v) Written in English; (vi)

Observational study design, or observational case-control; and (vii) Diagnosis of ME/CFS was defined using the criteria of either: Fukuda (1994), CCC (2003), ICC (2011), or the IOM (2015).

Exclusion criteria

Generated studies were excluded in this review if they failed to include, at minimum, two key search terms in the abstract or title or had the following exclusion criteria: (i) published before 1994; (ii) did not include the Fukuda criteria; (iii) non-human participants; (iv) the study included children as their primary metric of investigation; (v) the article was inaccessible due to a paywall, non-full-text, interventional report, case report, or review; (vi) the study was not in English; (vii) alternative diagnostic criteria used and not under inclusion criteria: Sect. (7); and (viii) the study design did not include healthy controls.

Selection of studies

Collated studies were managed, organised, and stored using Endnote20™. The titles and abstracts of each

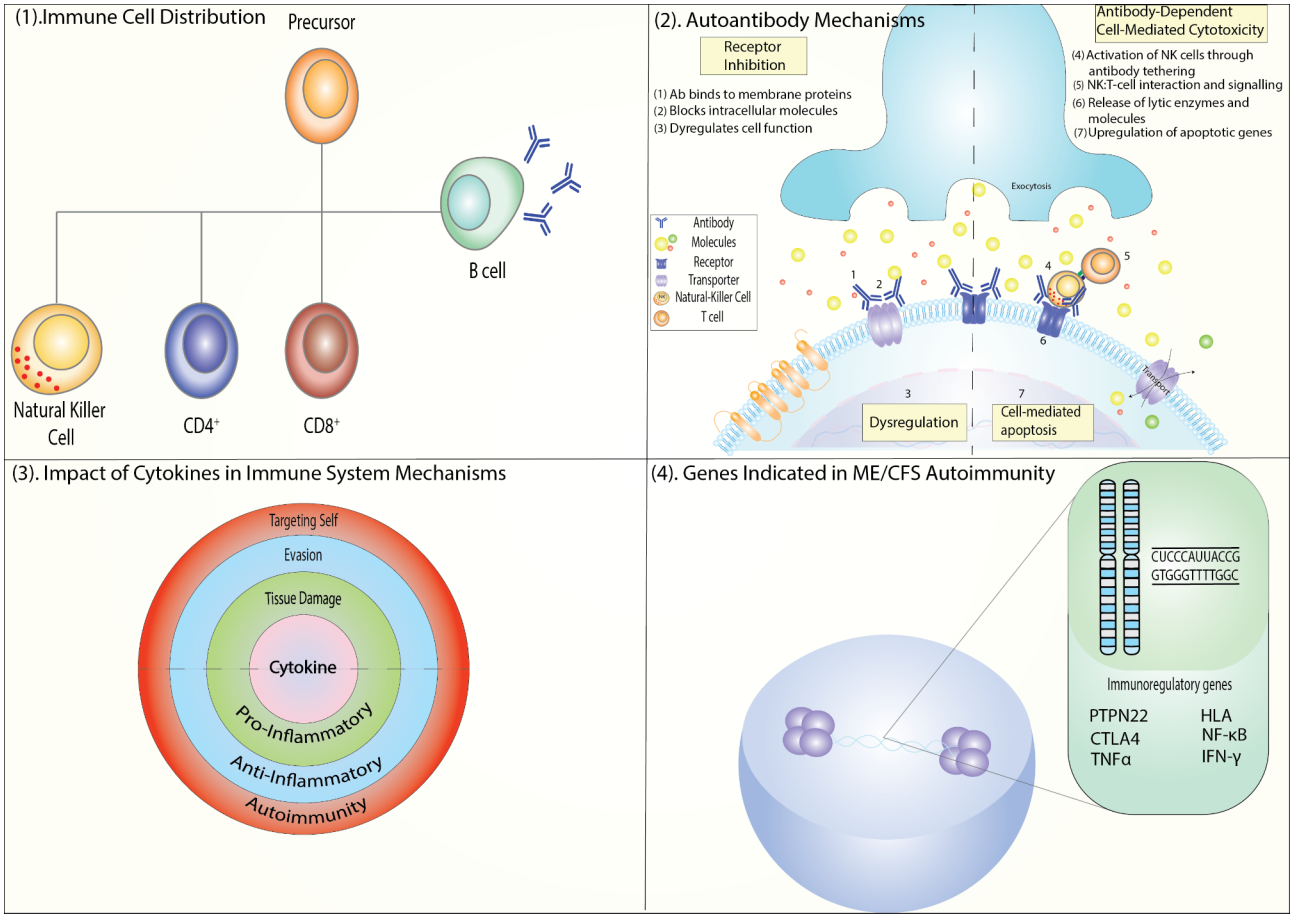

Fig. 1 The impact of autoimmunity in Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS): (1): Distribution of immune phenotypes. (2) The effect autoantibodies have in cell-mediated dysregulation and dynamics. (3) Cytokine involvement in molecular processes and pathology. (4) Genetics of ME/CFS autoimmunity. Abbreviations: HLA, human leukocyte antigen; NF-kB, nuclear factor kappa B; PTPN22, protein tyrosine phosphatase non-receptor type 22; CTLA4, cytotoxic T-lymphocyte associated protein 4; TNFα, tumour necrosis factor alpha; IFNγ, interferon gamma; ME/CFS, myalgic encephalomyelitis/chronic fatigue syndrome; NK, natural killer. Illustrated by JB using Adobe Illustrator 2024

study were screened based on the inclusion and exclusion criteria. All studies selected were independently assessed and reviewed by two authors at different dates. Duplicate studies were screened and removed using the Endnote20™ "delete duplication" feature.

Data extraction

All eligible studies were reviewed and assessed for the following: (i) adherence to defined diagnostic criteria; (ii) year published; (iii) study design; (iv) sample used and or collected; (v) participant number; (vi) number of controls to ME/CFS; (vii) gender; (viii) age; and (ix) experimental methodology.

Statistical analysis

The effect size was calculated using Cohen's d (= μ1 - μ2 / σ). Mean, standard deviation, confidence interval and odds ratios were utilised to generate the effect size. Where applicable, pooled standard deviation and d were analysed using Python 3.12. Odds ratio of generated values were converted to their natural log ((Ln=value)) and then subsequently converted into Cohen's d, d=ln(value) x √ (3/π). This value was then used to demonstrate the effect size. Where applicable, the standard error of the log odds ratio was converted into Cohen's using the approximation method.

Quality analysis

The evaluation of the studies in this review were examined by JB and JD using the Joanna Briggs Institute (JBI) checklist [9]. The checklist assesses the following criteria: (1) group matching; (2) source population; (3) criteria; (4) method of exposure; (5) assessment of exposure; (6) identification of confounding variables; (7) management of outcomes; (9) exposure period selection; (10) statistical analysis. Points 4, 5 and 9 were excluded as outlined (supplementary 1) due to absence of exposure(s). No publications were omitted based on their quality.

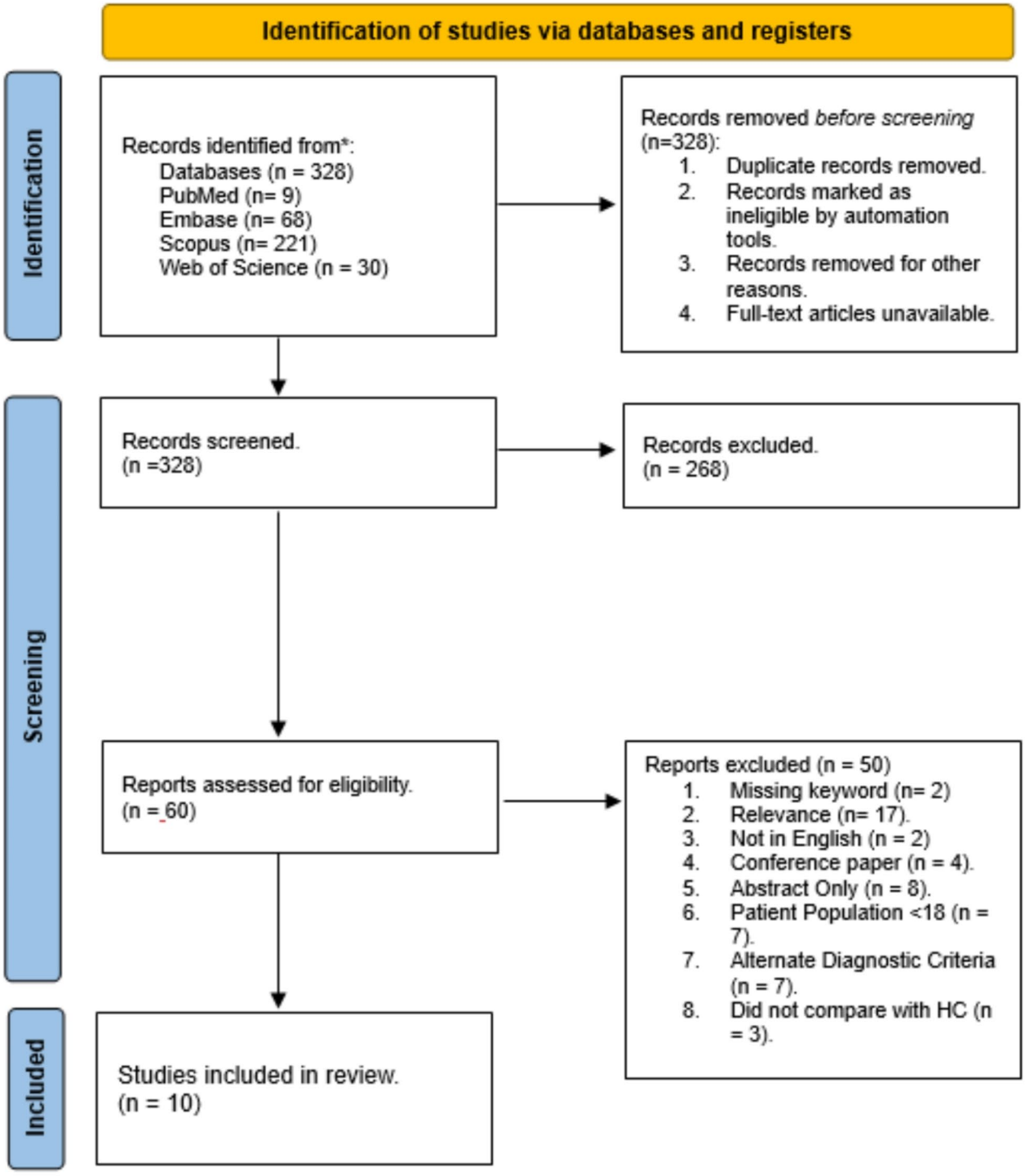

Fig. 2 PRISMA flow diagram of systematic search for autoimmunity. * Records identified by each database; Collated studies were excluded by a human and Endnote20™

Results

Ten papers were identified in PubMed, Embase, Scopus and Web of Science using the provided search terms. All papers were examined and screened using the specified inclusion and exclusion criteria (Table 1).

Literature reporting on proinflammatory cytokines

In investigating the immune signalling pathways, this systematic retrieved one paper exploring the role of inflammatory cytokines in ME/CFS. Maes et al. reported significantly increased pro-inflammatory cytokines to the serotonin receptor, 5-hydroxytryptamine (5-HT) in ME/CFS patients ($p<0.001$). Additionally, 5-HT-ME/CFS-positive patients showed significantly associated symptomatology [10].

Literature reporting on genetics of autoimmunity

Two studies investigated the role of risk alleles in the contribution of autoimmunity in ME/CFS. A report by Steiner et al. demonstrated ME/CFS patients with infection triggered onset (ITO) to have increased frequency of the immunoregulatory alleles *PTPN22* ($p=0.016$) and *CTLA4* ($p=0.001$). Intriguingly, ME/CFS patients w/ITO and HC had no change in their relative frequency [11]. A study by Lande et al. investigated the association of bona fide autoimmune alleles, human leukocyte antigen (HLA), in ME/CFS patients in a large cohort. Their results demonstrated HLA risk alleles HLA-C and HLA-DBQ1 frequencies to be significantly increased compared to HC ($p<0.05$) [12]. However, upon stratification and linkage disequilibrium analysis, HLA risk frequency was significantly correlated with ME/CFS comorbidity with other autoimmune diseases (ADs). AD frequency was highest for Hashimoto's, psoriasis, and rheumatoid arthritis. Despite HLA findings, when data was stratified, ME/CFS patients with comorbidity had increased frequency of HLA alleles. Therefore, it is difficult to ascertain the specificity of ME/CFS being directly correlated to autoimmunity. Contrasting this, one study Wang et al. reported ME/CFS patients to have significantly enriched immunomodulatory genes: *IKZF2*, *IKZF3*, *ABCE1*, *BACH2*, *CD3D*, and *HSPA8* ($p<0.05$). Intriguingly, females had significant enrichment of *IFIT1*, *ISG15* and *IF16* ($p<0.05$). Similar stratification of their patient cohort found bedridden patients with previously known AD (30%) to have further upregulation of these genes [13].

Literature reporting immune phenotype in ME/CFS

One paper examined the immune cell parameters in ME/CFS patients compared to HC. Steiner et al. reported that ME/CFS patients compared to HCs have no change in CD4+or CD8+T-cells [11]. Their results further stratified their patient cohort into two distinct groups, ME/CFS patients who reported initiation of disease as a result of infection triggered onset and those without. Following stratification, ME/CFS patients w/ITO had significantly decreased CD19+B cell counts ($p<0.05$). CD19+B cell depletion has been shown in SLE and RA, providing further evidence for an autoimmune mechanism in ITO patients.

Literature reporting on autoimmune-mediated autoantibodies in ME/CFS

Five studies examined the role of autoimmune-mediated AAbs in ME/CFS. Three of the five studies found increased AAbs in ME/CFS patients. Firstly, a report by Danilenko et al. showed significantly increased AAbs against β2-glycoprotein-I, neural antigens, and voltage-gated Ca2+channels in ME/CFS patients compared to HC ($p<0.01$) [14]. Secondly, De Bellis et al. investigated the role of anti-pituitary (APA) and anti-hypothalamic antibodies (AHA) in ME/CFS patients. Compared to HC, ME/CFS patients exhibited higher prevalence of APA and AHA titres ($p<0.01$). Additionally, their results demonstrated APA and/or AHA titre to be associated with HPA dysfunction [15]. Thirdly, Ryabkova et al. reported the effect of AAbs in ME/CFS patients compared to HC's and those with comorbid fibromyalgia (FM). Their results demonstrated ME/CFS patients with fibromyalgia (ME/CFS+FM) to have significantly increased AAbs to anti-GABA receptors [16]. However, ME/CFS patients without fibromyalgia (ME/CFS-FM) showed no significant difference compared to HC. Fourthly, Maes et al. 2012 showed there to be elevated serum IgM to three specific anchorage molecules in ME/CFS patients and HC [17]. They demonstrated ME patients to have higher autoimmune responses to oxidative and acetylcholine self-epitopes, as well as being associated with perceived symptomology. However, there was no changes found in nitrosative self-epitopes. Fifthly, an investigation by Fonseca et al. (2024) examined a large IgG EBV peptide cohort in ME/CFS patients compared to HC to highlight the immune responses in ITO. The study developed a 26-antibody classifier that was able to discriminate ME/CFS patients from HC. However, this classifier was not able to distinguish ME/CFS patients with ITO from those w/ITO, as well as those who had an unknown disease origin [18]. Finally, one study by Skowera et al. (2002) reported that ME/CFS patients had no association with ANA autoimmunity, specifically ANAs targeting the nuclear envelope [19].

Discussion

With increased attention to ME/CFS research, investigations have attempted to determine the contribution autoimmunological mechanisms serve in the pathogenesis of ME/CFS. The aim of this systematic review was to assess

Table 1 Summary of selected studies

Author	Year	Diagnostic Criteria	Investigation	Findings	Study Size	Cohen's Effect Size
Danilenko et al.	2022	Fukuda + CCC	Autoantibodies	Increased AAbs to neural antigens β2-glycoprotein-I ($p < 0.01$), NF-200 ($p < 0.01$), S100 ($p < 0.01$),MBP ($p < 0.05$),Hol-R ($p < 0.0001$),Gly-R ($p < 0.01$),GABA-R ($p < 0.01$),DA-R ($p < 0.01$),Ser-R ($p < 0.001$),ds-DNA ($p < 0.001$) IFN-γ ($p < 0.01$) and voltage-gated Ca2 + channels ($p < 0.01$).	33 ME/CFS and 20 HC	Not provided.
De Bellis et al.	2021	Fukuda	Autoantibodies	Higher APA and AHA titre; associated with HPA dysfunction ($p < 0.01$). Hormone levels GH ($p < 0.001$), Basal cortisol ($p < 0.001$), Cortisol peak ($p < 0.002$) and ACTH ($p < 0.001$)	30 ME/CFS and 25 HC	GH = -2.13 IGF-1 = -1.79 Basal cortisol = -5.37 Cortisol peak = -2.18 ACTH = -2.74
Ryabkova et al.	2022	Fukuda CCC	Autoantibodies	Increased AAbs to GABA-receptors.	11 ME/CFS + FM, 11 ME/CFS-FM and 11 HC	Not provided
Skowera et al.	2002	Fukuda	Autoantibodies	ANAs are absent in ME/CFS patients.	100 ME/CFS and 111 HC	ANA = 1.576
Maes et al.	2012	Fukuda	Autoantibodies	Elevated serum IgM to anchorage molecules palmitic acid ($p = 0.0001$), myristic acid ($p = 0.00003$) and S-farnesyl-L-cysteine ($p = 0.00001$); and NO-phenylalanine ($p < 0.005$)	16 ME/CFS and 17 HC	Palmitic Acid = 1.91 Myristic Acid = None S-farnesyl-L-cysteine = 1.99 NO-phenylalanine = -0225
Fonseca et al.	2024	CCC	Autoantibodies	ME/CFS patients with infectious triggered onset can be distinguished from healthy controls. Peptide sequences associated with specific antibodies are unlikely to be inducers of autoimmune B-cell responses.	92 ME/CFS and 50 HC	Not Provided
Maes et al.	2013	Fukuda	Cytokines	Increased proinflammatory cytokines specific to 5-HT-receptor ($p < 0.001$). 5-HT-positive ME/CFS patients have exacerbated symptoms.	117 ME/CFS and 35 HC	TNFa = 1.69 IL-1 = 1.17 Neopterin = 1.20
Lande et al.	2020	CCC	Genetics	HLA class I and class II alleles are associated with ME/CFS compared to HC. HLA-C ($p < 0.0001$), HLA-B*57:01($p < 0.004$), HLA-DBQ1 ($p < 0.005$), HLA-B*44:02 ($p < 0.03$), HLA-B*08:01 ($p < 0.01$) and HLA-DPB1 ($p < 0.02$).	426 ME/CFS and 4511 HC	HLA-C = 0.409 HLA-DBQ1 = 0.223 Proportionality of carrying either one or both alleles = 0.409
Wang et al.	2022	IOM	Genetics	Enriched immunomodulatory genes: *IKZF2*, *IKZF3*, *ABCE1*, *BACH2*, *CD3D*, *and HSPA8* ($p < 0.05$). Females have significant enrichment of *IFIT1*, *ISG15* and *IF16 ($p < 0.05$).*	166 ME/CFS and 201 HC	Not provided
Steiner et al.	2020	CCC	Immune Phenotype + Genetics	ME/CFS patients with w/ITO have decreased CD19 + B-cells ($p < 0.05$). However, no changes in CD4 + or CD8+. ME/CFS + ITO have increased frequency of *PTPN22* ($p = 0.016$) and *CTLA-4* ($p = 0.001$).	305 ME/CFS and 201 HC	PTN22all = 0.223 PTN22ito = 0.269 PTN22w/ito = 0.047 CTLA4all = 0.161 CTLA4ito = 0.234 CTLAw/ito = -0.077 IRF5all = -0.0341 IRF5ito = -0.0341 IRF5w/ito = -0.212 TNFall = -0.0459 TNFito = -0.0642 TNFw/ito = 0.005 TNF1all = -0.144 TNF1ito = -0.0961 TNF1w/ito = -0.329

[1] ME/CFS Myalgic encephalomyelitis/chronic fatigue syndrome, HC healthy control, ITO infection triggered onset, w/ITO without infection triggered onset, NK natural killer cells, PBMC peripheral blood mononuclear cell, ICC international consensus criteria, CCC Canadian consensus criteria, IOM Institute of medicine. HLA-A-B-C are MHC class I and HLA-DBQ1, DRB1, DQB1 and DQA1 are MHC class II. TNF and TNF1 are SNPs rs1800629 G > A and rs1799724 C > T respectively. Cohen's term d effect sizes as small (d = 0.2), medium (d = 0.5), and large (d > 0.8).

the available literature by critically examining studies that explored the role of autoimmunity in ME/CFS, particularly in terms of its significance, and to assess whether it may be classified as an autoimmune disease. Subsequent findings from the reports assessed in this systematic review provide valuable insights to our current understanding of the immune landscape within ME/CFS and question its relevancy at the cellular and molecular level. Overall, eligible literature reported on AAbs to neural antigen glycoprotein-I, GABA-receptors, as along with elevated serum IgM, increased proinflammatory cytokines, and the association of HLA alleles with ME/CFS (Table 1).

Immunological signalling mechanisms are governed by cellular and humoral interactions that ultimately impact various physiological and pathophysiological responses. This review highlighted one seminal study that outlined the pivotal role cytokines serve in the pathogenesis of ME/CFS (Sect. 3.1). Maes and colleagues outlined the specificity cytokines may have to significant receptors, such as that of 5-HT in ME/CFS patients [8]. This concerted cytokine phenomenon was shown to augment the activity of the 5-HT receptor and resulted in those positive for this response to have exacerbated symptomology. Additionally, Maes and colleagues also previously highlighted the impact cytokines have in ME/CFS pathogenesis. Several noteworthy cytokines, such as TNFα, IL-6, neopterin, lysozyme, PMN-elastase and IL-1β were shown to be dysregulated in ME/CFS patients compared to HCs [20]. Understandably, these cytokines play a crucial role in various immunological pathways and responses, which can have both protective and detrimental outcomes. In autoimmune conditions such as RA, TNFα has been shown to be detrimental to the disease severity. Management of RA with anti- TNFα agents such as infliximab and etanercept have shown to be efficacious in treating the disease [21]. There is limited literature reporting on the contribution cytokine signalling plays in ME/CFS, with emphasis to autoimmunity. However, the role of cytokines in autoimmunity is non-specific and a deeper understanding of the intricate cytokine network is fundamental to signifying their impact in this condition's pathogenesis as well as highlighting them as potential therapeutic targets for ME/CFS.

Like many autoimmune diseases, the stratification of immune phenotypes helps raise the resolution in evaluating the molecular landscape that precedes disease development. In evaluating this, this systematic review assessed one study that investigated the immune cell lineages of ME/CFS patients (Sect. 3.3). Comparative analysis performed by Steiner et al. revealed several noteworthy results in immune cell distribution in ME/CFS patients [9]. Firstly, they showed there to be no significant changes in CD4+, CD8+T-cell or B-cell populations when compared to HC. Secondly, as viral mediated infection is primarily associated with acquisition of ME/CFS disease, the author stratified their data set into two groups for comparative analysis: those with ITO and those w/ITO. Subsequently, this did reveal patients w/ITO to have significantly reduced CD19+B-cell counts, whereas T and B-cell distribution across the cohorts showed no significant differences. Although a commendable finding, these results further highlight the complexity involved in ME/CFS research. Corresponding to recent reports evaluating immune cell distribution in ME/CFS patients, investigations are still yet to generate reproducible consensus as to whether immune cell distribution is a contributing factor to ME/CFS pathogenesis, or whether their activity is implicated in the maintenance of the condition [22–24]. This is likely due to methodological inconsistency and robustness, as well as smaller patient cohorts utilised in studies. Therefore, future studies with more robust methods and larger patient populations are required to determine the attribution immune cell lineages play in either the onset, or maintenance of ME/CFS.

The findings from this systematic review included five papers that examined AAbs in ME/CFS patients compared to HC (Sect. 3.4). Three of the five studies analysed in this systematic review demonstrated increases in AAbs in ME/CFS patients [14, 16, 19]. Firstly, Danilenko et al. reported AAbs to β2-glycoprotein-I, neural antigens, and voltage-gated Ca2+channels [12]. These findings raise intriguing questions about the potential role of AAbs in ME/CFS, as transmembrane signalling molecules, such as that responsible for calcium modulation, has been previously discussed in a review by Shoenfeld et al. highlighting the molecular landscape in autoimmunity [25, 26]. Nevertheless, the results generated by Danilenko et al. fails to establish autoimmunity in ME/CFS as a driver for ME/CFS, but rather highlights a compelling association that warrants further investigation in understanding the role of AAbs in ME/CFS pathogenesis. Secondly, in building on the role of AAbs in ME/CFS, De Bellis and colleagues investigated the presence of APA and AHA AAbs in ME/CFS patients [13]. Their results exhibited a high prevalence for APA and AHA titres in ME/CFS patients when compared to HC. Intriguingly, they also identified significant association between APA and AHA titres with HPA dysfunction. These findings suggest that the presence of AAbs against neuroendocrine systems does indeed result in varying forms of dysregulation, however comprehensive mechanistic studies are required to unravel the precise interplay between the presence of AAbs and symptomatic presentation. Thirdly, Ryabkova et al. explored the role of AAbs in ME/CFS patients with (ME/CFS+FM) and without comorbid FM (ME/CFS-FM), compared to HC [14]. Their study revealed ME/

CFS+FM patients to have significantly increased AAbs targeting anti-GABA receptors. Juxtapose to this finding, ME/CFS-FM patients showed no significant differences in any AAb titre compared to HC. These findings, consequently, suggest that specific AAbs may be associated with the presence of FM as a comorbidity, however further research is required to elucidate the precise role these AAbs serve in the pathogenesis of ME/CFS alone. As previous research has demonstrated GABA to be significantly impacted in FM patients without other comorbidities [27], it is pivotal that further research focuses on delineating ME/CFS from FM. Fourthly, Maes et al. demonstrated ME/CFS patients to have elevated serum levels of IgM specific to anchorage molecules as well as increased autoimmune responses to oxidative and acetylcholine self-epitopes [15]. Intriguingly, these findings were associated with patient perceived symptomology. However, there was no changes found in nitrosative self-epitopes. Fifthly, through the identification of specific antibodies against EBV peptides, Fonseca et al. demonstrated no significant differences in IgG-EBV peptides sequence homology with human proteins, suggesting that ITO may be linked to the broader nature of ME/CFS pathogenesis, rather than autoimmune B-cell responses [18]. Finally, in contrast to the aforementioned studies, Skowera and colleagues reported there is no association between ME/CFS and ANAs, particular those targeting the nuclear envelope [17]. This negative finding accentuates the ongoing complexity of ME/CFS and the need for nuanced understanding in the role AAbs may serve in ME/CFS autoimmune pathogenesis.

The evidence as a collective, suggests that immune dysregulation and the presence of AAb in people with ME/CFS may play a role in the contribution to specific symptoms or comorbidities. However, there is a lack of evidence to suggest ME/CFS is an autoimmune condition. It is critical to recognise the broader and more specific limitations of these studies, such as that of small sample sizes and the need for further mechanistic research to establish causal relationships and clinical significance. Notably, the heterogeneity of AAb profiles amongst ME/CFS patients underscores the complexity of this phenomenon and highlights the necessity for personalised approached to diagnosis and treatment. Future studies should endeavour to uncover the precise mechanisms underlying autoimmunity in ME/CFS and its clinical applications, as well as utilise the various diagnostic autoimmune tests, such as ANA, rheumatoid factor, anti-dsDNA and others in distinguishing autoimmune presentations, from those of immune dysregulation. Additionally, studies should aim to stratify cohorts appropriately, as a control measure, to ensure the specific contribution of each variable is accurately identified.

In understanding the intricate relationship between the genetic risk alleles and autoimmunity in ME/CFS, this systematic review critically assessed four seminal studies that explored the genetic predisposition to immunomodulatory genes in ME/CFS (Sect. 3.2). Steiner et al., investigated the role of genetic risk alleles in ME/CFS patients with ITO. Their study unveiled a notable pattern: ME/CFS patients with ITO exhibited an increased frequency in immunoregulatory alleles, particularly *PTPN22* and *CTLA4* [11]. Surprisingly, this heightened frequency was absent in ME/CFS patients without ITO and HC. Both these gene signatures have been well characterised in various other autoimmune diseases, as they primarily effect T-cell regulation [28–30]. Thus, these findings may suggest that distinct genetic signatures may be associated with ITO ME/CFS. Likewise, a comprehensive analysis by Wang and colleagues explored the presence of enriched immunomodulatory genes in ME/CFS patients. Their research displayed significant enrichment of the immunomodulatory genes: *IKZF2*, *IKZF3*, *ABCE1*, *BACH2*, *CD3D*, and *HSPA8*, in ME/CFS patients [13]. Notably, enrichment was particularly pronounced in females and was further heightened in bedridden patients with previously known ADs. These findings in conjunction raise intriguing questions about the potential link between the expression of immunomodulatory genes and disease severity, particularly in the context of comorbid ADs. Additionally, these findings also underline the necessity for further research to better differentiate the molecular significance autoimmune markers may have in comorbid ME/CFS patients. The application of such practices will better facilitate discerning the significance comorbid ADs impact the ME/CFS immune landscape and ensure the appropriate nomenclature be utilised.

In contrast to the focus of immunomodulatory genes, Lande and colleagues focused their efforts on characterising the presence of bona fide autoimmune diagnostic alleles, HLA alleles. HLA testing is a commonly used diagnostic tool used in conjugation with the clinical picture. Specific genetic markers, such as HLA-B7, are associated with autoimmune conditions, namely ankylosing spondylitis, and are used to indicate risk of having certain autoimmune conditions [31]. In their study, intriguing results displayed significantly increased frequencies of HLA risk alleles: HLA-C and HLA-DBQ1 in ME/CFS patients compared to HC [12]. However, upon further stratification and linkage disequilibrium analysis it was shown that ME/CFS HLA risk allele frequency was significantly correlated with ME/CFS patients who had a comorbidity with other AD, such as Hashimoto's thyroiditis, psoriasis, and rheumatoid arthritis. All of which test positive for HLA alleles independently [32–34]. Despite these ADs having differing HLA frequencies, this further highlights the complexities in establishing direct and

specific correlation between ME/CFS and autoimmunity, particularly when comorbid ADs are present. Additionally, the presence of an autoantibody alone is often not enough to establish a diagnosis, but rather be used as an indicator for further clinical assessment.

Collectively, these studies provide valuable insights into the delicate, yet complex genetic nature of ME/CFS. Genetic predisposition to aberrative immunoregulation and autoimmunity is said to be present in ME/CFS [35]; whilst the current evidence suggests an association between certain risk alleles, it is still yet to consider the heterogeneity of the patient population presented in these studies. Namely the presence of comorbid ADs, and the nuanced genetic signatures within subgroups of ME/CFS patients. Together, these findings underscore the multifaceted nature of ME/CFS and the need for further comprehensive genetic studies to elucidate its pathogenesis. Future research is imperative to uncover the functional significance of these risk alleles and their contribution to the immunoregulatory nature of ME/CFS and its underlying mechanisms. The demarcation of ME/CFS-specific genetic signatures from those that are associated with comorbidities will be a pivotal distinguishing factor that should be considered in future research.

Limitations

A limitation to this systematic review is that it was restricted to specific articles that had the terms 'autoimmunity' or 'autoimmune' in the title and abstract of the paper. The aim for this systematic review was to evaluate the actual extent autoimmune mechanisms may have in ME/CFS pathogenesis. Therefore, to avoid confusion, other terms such as immune dysregulation and immune system dysfunction were excluded due to their potential flaw in distinguishing self-reactivity from aberrative immune mechanisms. Moreover, their exclusion was also due to their overtly broad nature, which we do acknowledge may have resulted in possibly applicable articles not being captured. Therefore, future reviews could benefit from expanding the search criteria to capture studies investigating general immune dysfunction alongside autoimmunity to better distinguish their potential contribution in ME/CFS pathogenesis.

Notably, we have highlighted the inclusion of studies involving ME/CFS patients with comorbid ADs to potentially be problematic. Various publications utilised comorbidities as either a tool for direct comparison, or for indirect associations. This, in itself, introduces complexity due to the heterogeneity of the patient population, making it challenging to isolate specific factors unique to ME/CFS. The presence of comorbid ADs can confound results and complicate the interpretation of immune or genetic alterations. Furthermore, the presence of ADs may impact AAb and genetic risk allele profiles, affecting the ability to distinguish contributions to ME/CFS alone. Clinical implications, including treatment responses, can vary significantly between ME/CFS patients with and without comorbid autoimmune conditions. Therefore, findings should be cautiously applied to ME/CFS patients without comorbidities. Future research should focus on homogenous patient populations to better understand the specific immunological and genetic aspects of ME/CFS.

A further limitation to not only this point, but also others investigating ME/CFS and utilising diagnostic criteria, is that of testing for ADs prior to patient selection. There exist several diagnostic criteria for ME/CFS, all of which allow for the inclusion of FM, and other diseases in their diagnostic inclusion criteria. The Fukuda definition and others have been overtly criticised due to its over inclusive nature. Its criteria are polythetic, which inevitably leads to great heterogeneity among the group of patients diagnosed according to these criteria. For instance, two patients could have very little symptom overlap, yet both be diagnosed with ME/CFS. This raises concerns for broad ME/CFS research, as well as that for investigating autoimmune mechanisms specific to ME/CFS. According to the Fukuda, CCC and IOM, patients meeting the ME/CFS diagnosis are not necessarily excluded if they have an accompanying comorbidity, such as FM, IBS, and more [1, 36]. This issue presents a unique problem for the ME/CFS population, as delineation between conditions becomes problematic as the lack of specific biomarkers in ME/CFS. Therefore, future research should focus on eliminating any possibility of their selected patient cohort having a previous ADs through a more vigorous inclusion and exclusion criteria.

Conclusion

Exactly how the immunoregulatory mechanisms impact ME/CFS pathogenesis and contribute to the exacerbation of patient symptomology is poorly understood. Here, we highlight that the proposed autoimmune mechanisms investigated in patients suffering with ME/CFS is variable and inconsistent. Whilst there exists evidence suggestive of immune deregulatory phenomena and autoantibodies in ME/CFS patients, these findings cannot deduce ME/CFS to an autoimmune disease. These findings underscore the pressing need for further research to unravel the precise immunological and genetic factors at play in ME/CFS. With direct emphasis on distinguishing autoimmunity from broader immune dysregulation, such targeted efforts will ultimately pave the way for more effective diagnostic and therapeutic strategies in the ongoing quest to understand and manage this enigmatic condition.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Not applicable.

Author contributions
JB, NEF, and SMG designed the study. JB and JD conducted the literature search and extracted the data. JB, NEF, and JD performed the quality assessment. JB performed data analysis and interpretation. JB and NEF drafted the article. NEF and SMG supervised the study and critically reviewed the manuscript for important intellectual content. JB, NEF, and SMG contributed to the interpretation of the data, revision of the manuscript and provided final approval of the version to be published.

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Data availability
Data generated or analysed during this study are provided within the manuscript or its accompanying supplementary files. Additional files will be provided upon request.

Declarations

Ethics approval and consent to participate
The authors declare that there are no ethical conflicts pertaining to this research project to disclose. As this research is a systematic review and did not include direct recruitment of participants, no consent was required.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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