COMPREHENSIVE REVIEW / revue générale

Chronic fatigue syndrome and the immune system: where are we now?

Fane K F Mensah[a,*], Amolak S Bansal[b], Brian Ford[b], Geraldine Cambridge[a]

[a]Department of Rheumatology Research, Division of Medicine, University College of London, London, United Kingdom

[b]Department of Immunology, Epsom and St Helier University Hospitals NHS Trust, London, United Kingdom

Corresponding Author:

Fane K F Mensah

Department of Rheumatology Research, Division of Medicine, University College London, Rayne Building 5 University street, London WC1E 6JF, UK

Email: f.mensah@ucl.ac.uk

Telephone number: 0044(0)2031082164

Fax: 0044(0)2076799143

Chronic fatigue syndrome and the immune system: where are we now?

Abstract

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is characterized by multiple symptoms including fatigue, headaches and cognitive impairment, which have a significantly adverse effect on the normal functioning and well-being of the individual. These symptoms are often triggered or worsened following physical or mental exertion. ME/CFS has long been thought of as having a significant immunological component, but reports describing changes in immune function are often inconsistent between study groups. Although the wide range of physical, neurocognitive and autonomic symptoms reported have seriously hampered attempts to understand pathophysiological pathways, investment in biomedical research in ME/CFS is finally increasing with a number of novel and promising investigations being published. The onset of ME/CFS may often be linked to (viral) infections which would be consistent with a variety of alterations in NK cell function as described by a number of different groups. Consistency in cytokine data has been lacking so far, although recently more sophisticated approaches have led to more robust data from large patient cohorts. New hope has also been given to sufferers with the possibility that therapies that deplete B cells can result in clinical improvement. To understand the pathogenic mechanism in this complex condition it is important to consider repeated analysis in different cohorts. In this review, we will discuss the potential of different components of the immune system to be involved in the pathogenesis of ME/CFS.

Le syndrome de fatigue chronique et le système immunitaire: où en sommes-nous maintenant?

Résumé

L'encéphalomyélite myalgique / syndrome de fatigue chronique (EM/SFC) se caractérise par des symptômes multiples, dont la fatigue, des maux de tête et des troubles cognitifs, qui ont un effet négatif significatif sur le fonctionnement normal et le bien-être de l'individu. Ces symptômes sont souvent déclenchés ou aggravés suite à un effort physique ou mental. L'EM/SFC a longtemps été considérée comme ayant une importante composante immunologique, mais les études témoignant des modifications des réponses immunitaires dans ce contexte sont souvent discordantes entre les différents groupes de recherche. Bien que la vaste gamme de symptômes physiques, neurocognitifs et autonomes rapportés par les patients ait sérieusement entravé les tentatives de comprendre les mécanismes physiopathologiques impliqués, l'investissement dans la recherche biomédicale concernant l'EM/SFC augmente finalement, avec un certain nombre de publications nouvelles et prometteuses. La survenue d'une EM/SFC peut être liée à des infections (virales) qui détermineraient différentes altérations de fonctionnement des cellules NK comme décrit par beaucoup de groupes différents. La cohérence des données rapportées sur les cytokines a fait défaut jusqu'à présent, bien que récemment certaines approches plus sophistiquées aient conduit à obtenir des données plus robustes dans de grandes cohortes de patients. Un nouvel espoir a également été donné aux patients avec la possibilité que les thérapies qui provoquent une déplétion en cellules B pourraient entraîner une amélioration clinique. Pour comprendre les mécanismes physiopathologiques de ce syndrome complexe, il est important de considérer les données qui ont été reproduites dans différentes cohortes. Dans cette revue, nous discuterons du potentiel des différentes composantes du système immunitaire à être impliquées dans la pathogenèse de l'EM/SFC.

Introduction

Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a condition characterized by multiple symptoms including fatigue, headaches, cognitive impairment, myalgia, arthralgia and postural instability [3, 55]. These symptoms are often worsened by physical or mental exertion. There is at present no proven effective therapy for sufferers and the chronicity of the condition has a significant and often long-term adverse effect on the normal functioning and well-being of patients [31, 58]. In the absence of a specific laboratory test, the diagnosis of ME/CFS presently rests on the exclusion of any medical or psychiatric causes of fatigue in someone with new onset persistent tiredness for over six months. Additional symptoms detailed in the Canadian, Centre of Disease Control (CDC) and Fukuda diagnostic criteria are also included to aid diagnosis [15, 16, 25].

The problem is compounded by the fact that diagnosing this condition is primarily one of exclusion. Even comparing subjects with acute onset ME/CFS with those whose symptoms had a gradual onset confirms significant differences in premorbid personality, prognosis and response to treatment [46]. It is presently estimated that ME/CFS has a prevalence in the population of 0.2–0.4%, with an overall prevalence varying from 0.1% to 2.5%, depending on the criteria applied [1, 20, 54]. A recent Norwegian study has noted distinct peaks in the age prevalence of ME/CFS. As such there is a first peak between 10 to 19 years' age and a second peak in the age group between 30 to 39 years (Figure 1) with a higher prevalence in women than in men, as is consistent with previous studies [6, 54].

Many patients with ME/CFS describe a history of viral infections prior to the onset of their illness [2, 35]. This has also been suggested to underlie some of the immunological abnormalities described in patients with ME/CFS [4, 7, 17, 38]. However, strong evidence for persistent or chronic infection is presently lacking in the majority of patients. ME/CFS has also long been thought as having a significant immunological component. However, it is still not clear whether changes in immunological parameters in patients with ME/CFS are the cause or the result of the condition. The inconsistency of the results of biomedical research in ME/CFS, coupled with the wide range of physical, neurocognitive and autonomic symptoms reported have thus seriously hampered attempts to understand pathophysiological pathways in ME/CFS. In this review, we will summarise the present state of knowledge related to immunological findings in patients with ME/CFS.

Cellular cytotoxicity and ME/CFS

The most consistent findings of changes in immune system components in ME/CFS patients have come from studies of natural killer (NK) cell function. The NK cell is a type of cytotoxic lymphocyte and is part of the innate immune system. NK cells play a key role in the earliest stages of recognition of virally infected cells and host rejection of transformed cells. NK cells can also secrete cytokines which can influence other cells of the adaptive immune system. The onset of many cases of ME/CFS have been linked to (viral) infections, in particular those caused by Epstein Barr virus (EBV), cytomegalovirus (CMV) and human herpes viruses type 6 and 7 [4, 17, 26, 35]. Reductions in NK cell numbers and function have therefore been suggested as predisposing ME/CFS 'signatures'. Alternatively, this could have occurred as the result of a viral infection and not be directly related to ME/CFS.

Reduced numbers and *in vitro* cytotoxic activity of NK cells (CD16$^+$CD56$^+$) have been reported in ME/CFS patients by several groups [8, 40, 45]. Two other studies have reported reduced levels of the activation marker CD69 on both T cells and NK cells in patients with ME/CFS [41, 48]. In line with these findings Tirelli *et al* found that the albeit reduced NK cell population expressed an increased number of adhesion (CD11b, CD11c and CD54) and activation (CD38) markers [61]. This was confirmed recently in a publication by Brenu *et al*, where they found a significant decrease in CD56^{bright} NK cells [12]. They also found that levels of perforin were significantly increased in NK cells from ME/CFS patients but that there was a decrease in granzyme A and granzyme K (inducers of programmed cell death) expression compared to NK cell populations in healthy controls.

The molecular basis of diminished NK cytotoxicity was also tested *in vitro* by Maher *et al* where a reduction in NK cell associated perforin (cytolytic protein of cytolytic granules) levels was observed in samples from ME/CFS patients, compared to healthy controls [43]. Interestingly, reduction of granzyme or perforin levels is also commonly associated with herpes virus infections [50]. More recent work in ME/CFS has focused on the molecular basis of NK cell dysfunction. As such, Fletcher *et al* evaluated the natural killer function and the expression of the activation marker dipeptidyl peptidase-4 (DPP4)/CD26. DPP4/CD26 is a transmembrane glycoprotein expressed on the surface of lymphocytes, and plays a role as an activation marker which was shown to be increased in ME/CFS patients [32]. Natural killer cell

cytotoxicity (NKCC) levels were lower in cases than controls, while abnormalities in samples assayed for NKCC showed increased cell surface expression, decreased number of molecules on NK cells, and decreased levels of serum DPP4/C26 [22]. The authors suggested that these abnormalities could have particular relevance to the possible role of infection in the initiation and/or persistence of ME/CFS.

Curriu *et al* observed higher expression of the killer activation receptor (KAR) NKp46 (natural cytotoxicity receptor on NK-cells) and lymphocyte activation marker CD69, but lower expression of the IL2 receptor CD25 on NK-cells, identifying a possibly specific signature of NK cell phenotype in ME/CFS patients [18]. Genomic polymorphisms of killer cell immunoglobulin-like receptors (KIRs) has also been described with an excess of KIR3DS1 combined with increased levels of ligand-free KIRDL1, which may hamper the recognition of target structures of pathogens by NK-cells [51]. Studies by Marshall Gradisnik *et al*, focused on NK cytotoxicity, single nucleotide polymorphisms (SNPs) and genotypes in transient receptors potential (TRP) and acetylcholine receptors (AChRs) in ME/CFS patients. Here they observed a significant reduction in NK cell mediated lysis of target cells compared with a (unfatigued) control group. They found that particular SNPs and genotypes localised to TRP ion channels and AchRs were present with increased frequencies in isolated NK cells from patients with ME/CFS. Further, they suggested that these SNPs and genotypes may be involved in changes in NK cell function and the development of ME/CFS pathology through dysregulation of calcium metabolism in AchRs and TRP ion channel signalling [44].

In summary, there appears to be some evidence for skewing towards particular NK cell genotypes but many of the changes in NK function could also reflect environmental stressors which may differ between patients. Serial studies of NK cell function, as with other immunological studies are clearly needed.

Cytokines

Cytokines are a broad category of small proteins that are important in cell signalling, predominantly as communicators between cells of the immune system, and primarily important in modulating the balance between humoral and direct cell mediated immune responses. However, cytokines can become dysregulated during and/or after immune responses especially those associated with inflammation. While pro-inflammatory cytokines

such as IL1, TNFα and type 1 interferon promote systemic inflammation, anti-inflammatory cytokines such as IL4, IL10 and TGFβ can inhibit the synthesis of pro-inflammatory cytokines. The finding of raised cytokine levels has been linked to different diseases, for example high levels of TNFα have been found in rheumatoid arthritis and inflammatory bowel disease [11, 59]. Also, cytokine profiles can indicate the source of cytokine production which may indicate different compartments of the immune system (B cells, T cells NK cells etc.) which may be involved in disease expression or progression.

Recently, several different research groups have been focusing on cytokine levels in serum and cerebrospinal fluid of ME/CFS patients, compared with those in healthy controls. Not surprisingly perhaps, a majority of these findings have not shown to be consistent with one another [19, 21, 28, 29, 52, 62]. This is probably due to differences in laboratory methodologies, patient and control group selection, phase of illness as well as the timing of sampling. Some however have found their way to redesign their approach.

Rather than comparing to healthy controls, Hickie *et al* for example prospectively evaluated cytokine production (post infection) in patients with proven EBV infection who had developed ME/CFS compared with those who did not develop ME/CFS post EBV, but no significant differences were found [26]. Interestingly, a study by Brodrick *et al* of adolescent patients developing ME/CFS following infectious mononucleosis (IM) compared with controls who recovered normally, showed an increase in IL-8 and a decrease in IL-5 and IL-23 cytokines [14]. The most significant difference in this study was the level of IL-23, which was lower in ME/CFS patients. IL-23 is essential for the full and sustained differentiation of the inflammatory Th17 T cell subset, but as most cytokines are usually delivered in the context of immune micro-environments, it is doubtful that a lower serum level of this cytokine would impact specific T follicular helper cell generation [63]. *In vitro* studies using patient lymphocytes and specific stimuli could however be used to explore these findings. Another approach by Russell *et al* was aimed at exploring the shift in discriminatory cytokines across ME/CFS subjects in relation to duration of illness. In this study cytokine expression was observed in samplings stratified on the basis of age for female ME/CFS subjects; (1) 18 years or younger, ill for 2 years or less (n = 18), (2) 18–50 years of age, ill for 7 years (n = 22), and (3) age 50 years or older (n = 28), ill for 11 years on average. Control subjects were matched for age and body mass index. A co-expression pattern of increased IL-1α and IL-8 in the context of decreased IL-6 was shown to be characteristic for early course illness compared to

age and BMI-matched controls. In contrast, elevated IL-1α and IL-6 co-expressed in the context of lower than average IL-8 was a more abundant pattern in mid and late course ME/CFS subjects. These 3 markers could be set as a triple screen and by adjusting their contribution according to illness duration, sub-groups produced ME/CFS classification accuracies of 75–88 % [57]. The authors suggest that these cytokines may serve as a robust biomarker independent of age in screening of ME/CFS patients.

Hornig *et al* used multivariate analysis of data from two large, multicentre cohort studies of ME/CFS to assess the relationship between immune signatures with diagnosis, illness duration, and other clinical variables. Importantly, controls were frequently matched on key variables known to affect immune status, including geographic site and season of sampling in addition to age and sex. Their findings unveiled a plasma signature of cytokines in patients' serum with a decrease in CD40L and increase in IFNα levels in patients with a disease duration of 3 years or less compared to those with >3 years history and also with healthy controls [29].

These findings were suggested to be the consequences of immune triggering following an infection. A stronger correlation of cytokine alterations with illness duration than with measures of illness severity was found, suggesting that the underlying immunopathology/ dysregulation of ME/CFS is chronic and progressive but may not explain all the symptoms. In another study, Houghton *et al* measured the plasma levels of cytokines, chemokines and growth factors in ME/CFS patients of long duration compared with age and sex matched healthy controls. Highly significant reductions in the levels of circulating IL16, IL7 and Vascular Endothelial Growth Factor (VEGF-A) were observed in ME/CFS patients [34]. Although a decrease in IL7 was also found in a publication by Lombardi *et al* in 2011, this publication was based on Xenotropic murine leukaemia virus-associated (XMRV) ME/CFS patients, an association which has been retracted in ME/CFS [39].

In conclusion, consistency in cytokine data has been lacking so far, and therefore it is difficult to attribute their role to either the cause or chronicity of the disease. Although different approaches have led to more robust data in big cohorts, it is even more important that future studies encompass repeated analysis in similar and different cohorts at different time-points.

B cell depletion therapy in ME/CFS

More recently, new hope has been given to sufferers with the possibility that therapies removing B cells (lymphocytes responsible for antibody production) can result in clinical improvement.

Norwegian oncologists from the Haukeland University in Bergen have conducted clinical trials of the B cell depleting agent rituximab in ME/CFS patients. Rituximab is a chimeric monoclonal antibody directed towards CD20, a cell surface marker widely expressed on B cells from early to late differentiation, but absent on plasma cells (Figure 2). After their unpublished observation of a patient showing unexpected and markedly improved ME/CFS symptoms while being treated with immunosuppression for non-Hodgkin's lymphoma, Fluge *et al* reasoned that the mechanism inducing the symptoms of ME/CFS may have involved B cells. They followed up their hypothesis by the open label treatment of a further two ME/CFS patients with rituximab and again, the patients remarkably showed signs of improvement in all their symptoms [26]. Depleting B cells might not only decrease the number of harmful (auto)antibodies produced by daughter plasmablasts or plasma cells, but also may act by depleting virus infected B cells in a direct manner. Depletion of B cells infected with Epstein Barr virus (EBV), the virus known to cause infectious mononucleosis (IM) could be considered as an effective mechanism. EBV infected B cells have been described as a possible predisposing factor in patients with ME/CFS [30]. Interestingly, EBV has also been suggested to play a role in other autoimmune diseases like rheumatoid arthritis (RA) and Sjögren's syndrome, but given the ubiquitous presence of EBV within human B cells, it is difficult to assign definitive cause and effect to this virus [49, 64].

Encouraged by these promising results, Fluge and Mella conducted a double-blind and placebo-controlled study of B cell depletion therapy with a single treatment of rituximab (two infusions two weeks apart). Here they found lasting improvement in operator and self-reported fatigue scores, as well as other symptoms, in 10 out of 15 patients (67%) in the rituximab group and two out of 15 (13%) in the placebo group [23]. The response was found to be 'delayed' with clinical improvement starting from 2-7 months after rituximab treatment but beyond the primary endpoint of 3 months. Such a pattern of discordance between the actual removal of peripheral B cells, which takes days to a week and clinical response is well described in patients with Rheumatoid arthritis treated with rituximab [53]. It is thought to

reflect the time needed to reduce the levels of pathogenic autoantibodies (half-life of IgG~24 days). In the placebo-controlled trial of ME/CFS patients treated with rituximab, the even longer delay to clinical response may also have been contributed to by study design, in which 'improvement' in fatigue scores had to be maintained for >6 weeks to score 'positive' which would extend over primary endpoint in some cases.

To investigate whether a prolonged effect of rituximab in ME/CFS patients could be achieved, an open-label phase II study maintenance treatment was conducted. In this single-centre study, 29 patients were included for treatment with rituximab (500 mg/m^2) two infusions two weeks apart, followed by maintenance rituximab infusions after 3, 6, 10 and 15 months, and with follow-up for 36 months. Eighteen out of 28 patients receiving rituximab maintenance showed clinical significant responses (64%) with a mean response of 105 weeks [24]. From these studies, it was concluded that in a subset of ME/CFS patients prolonged B cell depletion with rituximab maintenance treatment was associated with sustained clinical response, but further research is needed to understand the mechanism.

Although, rituximab seems to be effective in a subset of patients the question remains why and who will respond? The prolonged effect occurred when peripheral B cells were relatively absent from the periphery and new B cells prevented from exiting the bone marrow. Response to rituximab-based therapy in autoimmunity is usually associated with the presence of pathogenic autoantibodies produced by short-lived plasmablasts generated from either naïve B cells constantly entering the circulation, or from the rapid differentiation to autoantibody production from memory B cells. Protective immunity is largely retained as long term memory resides in long-lived plasma cells which are not depleted by rituximab. The delay in improvement following induction of depletion may also suggest that in ME/CFS patients' antibodies and their parent B cells might be involved.

Autoantibodies and B cell phenotype in ME/CFS

In ME/CFS, there are a number of reports describing the presence of autoantibodies to neuroendocrine receptors [33, 42, 60, 65]. Anti-muscarinic and anti-adrenergic antibodies have been described in two studies in a proportion of ME/CFS patients [36, 60]. Autoantibodies against β1 and β2 adrenergic receptors were described in postural orthostatic tachycardia (POTS), which is of relevance for ME/CFS as a small subgroup concurrently suffers

from POTS [27]. A study by Loebel *et al* in collaboration with Fluge *et al* showed significant decline in elevated β2 and M4 receptor autoantibodies in clinically responding patients receiving rituximab maintenance treatment and thus prolonged B cell depletion [37]. This is a potentially exciting discovery as β2 adrenergic receptors are involved in the maintenance of blood vessel tone in a number of different tissues and therefore uncontrolled blockade or stimulation could potentially result in a number of diverse clinical consequences. Whether the autoantibodies described however have any functional consequences on β2 adrenergic signalling cascades is not yet known. From the study of other clinical conditions where antibodies were directed to microbial agents or auto antigens, possible targets for autoantibodies in ME/CFS patients could include molecules involved in cellular metabolism or perhaps interacting with other immune cells resulting in inappropriate or chronic cytokine production.

The possibility that B cells and (auto)antibodies may play a role in the genesis and perpetuation of ME/CFS has therefore stimulated interest in (parent) B cell function and phenotype. Klimas *et al* have described an elevated proportion of CD20 and of CD21 positive B cells, confirmed by Tirelli *et al* with an additional increase in absolute numbers of CD19 and CD5 positive B cells (immature B cells) [32, 61]. CD19 and CD21 are co-receptors of the B cell receptor complex, CD21 is also a receptor for EBV [56]. CD19 along with CD20 (a glycosylated phosphoprotein) are surface markers expressed on the surface of B-cell subsets, and are therefore widely used to distinguish B cells from other lymphocytes.

More recently, Bradley *et al* showed significant increases in transitional and naive B cell subsets compared with healthy controls, but in contrast, naive B cells were reportedly decreased and memory B cells increased in another study by Brenu *et al* [10, 13].

Phenotype studies have therefore not been consistent; this might be due to methodology (as different methods of B cell classification can be used) or patient sampling (whole blood phenotype or phenotype on isolated PBMCs).

In order to provide a possible platform for studies of B cell function in patients with ME/CFS we have used a large panel of phenotypic markers to define an extended profile of B cell subsets. The aim therefore was to investigate particular phenotypes associated with B cell maturation, differentiation and activation. Although broadly, results in ME/CFS patients and controls were similar, the marker CD24, a sialo-glycoprotein cell adhesion molecule expressed on the surface of most B cell and differentiating neuroblasts was found to have

increased expression, or to be retained, on naïve B cells in ME/CFS patients [47]. In the same publication, we also reported a higher frequency of $CD21^+CD38^-$ memory B cells which showed a negative correlation with the presence disease duration in ME/CFS patients. Percentages of this subset were also higher than in healthy controls. So far, B cell studies have predominately focused on phenotype analysis, but functional studies have rarely been described in ME/CFS patients. In one study, however the specific memory B cell response to EBV has been described by Loebel *et al* [38]. They found a diminished or absent number of EBV nuclear antigen (EBNA) and Viral Capsid Antigen (VCA)- antibody secreting cells in up to 76% of patients. When comparing EBV load in blood immune cells, EBV encoded small nuclear RNAs (EBER) was found more frequently in ME/CFS patients compared to healthy controls. EBER is an immediate-early viral gene of EBV, homologous to other intermediate/early genes in other Herpes viruses [5]. This suggests a more frequent latent persistence. Their conclusions were that that there was a deficient EBV-specific B cell memory response in ME/CFS patients and an impaired ability to control early steps of EBV reactivation. The early response to EBV infection relies on innate mechanisms such as natural antibodies and the rapid deployment of NK cells [9]. Defects at both this early stage and later could also have contributed to reportedly aberrant memory B cell responses in at least a proportion of ME/CFS patients.

Discussion

In summary, the ME/CFS literature taken as a whole suggests mildly raised circulating pro-inflammatory cytokines and a skewing towards impaired cellular immunity especially in NK cells. Interpretation of immunological data in relation to particular symptoms or patterns of symptoms may reveal associations between different findings, e.g. NK cell dysfunction and a cytokine profile with a specific level or pattern of fatigue. The possible intervention with rituximab in a subset of ME/CFS patients has initiated a further interest in B cells and their products and their possible role in this condition. The relationships between disease duration and immune parameters have also been suggested by some studies e.g. Horning *et al* (cytokine signature) and Mensah *et al* (B cell phenotype). Most importantly, longitudinal studies investigating immune function over time together with changes in the severity or symptoms of ME/CFS are needed.

There are major challenges however in applying hypothesis driven biomedical research to a heterogeneous condition such as ME/CFS where the cause is unknown. Added to this is the marked variability in symptom severity from day to day and thus there is bound to be variation in the levels of mediators which have short half-lives. Further complications are evident in the analysis of immune cells which can vary in number depending on time of day and even mild exertion. All this makes it easy to understand why there is little consensus in the literature on immune dysfunction in ME/CFS. It is promising however to see the recent increased numbers of research groups interested in the pathogenesis and possible treatment of patients with ME/CFS. Rather than searching for biomarkers, well-structured studies with defined hypothesis to understand certain findings should be the centre of focus in these studies. It is also important to perform repeated analysis in different cohorts.

The multiplicity of overlapping and non-overlapping symptoms grouped together under the umbrella diagnosis of ME/CFS not only complicates diagnosis but has hampered biomedical research. Recognition of the complexity of ME/CFS and new approaches to stratification however are beginning to reveal underlying mechanisms.

Conflict of interests: The authors have no conflict of interest

References

1.   National Collaborating Centre for Primary Care (UK). Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (or Encephalopathy): Diagnosis and Management of Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (or Encephalopathy) in Adults and Children (NICE Clinical Guidelines, No. 53.). London: Royal College of General Practitioners (UK); 2007National Institute for Health and Clinical Excellence: Guidance. London2007.

2.   Ablashi DV. Viral studies of chronic fatigue syndrome. Clin Infect Dis 1994;18 Suppl 1:S130-3.

3.   Afari N, Buchwald D. Chronic fatigue syndrome: a review. Am J Psychiatry 2003;160:221-36.

4.   Agliari E, Barra A, Vidal KG, Guerra F. Can persistent Epstein-Barr virus infection induce chronic fatigue syndrome as a Pavlov reflex of the immune response? J Biol Dyn 2012;6:740-62.

5.   Baichwal VR, Sugden B. Latency comes of age for herpesviruses. Cell 1988;52:787-9.

6.   Bakken IJ, Tveito K, Gunnes N, Ghaderi S, Stoltenberg C, Trogstad L, et al. Two age peaks in the incidence of chronic fatigue syndrome/myalgic encephalomyelitis: a population-based registry study from Norway 2008-2012. BMC Med 2014;12:167.

7.   Bansal AS, Bradley AS, Bishop KN, Kiani-Alikhan S, Ford B. Chronic fatigue syndrome, the immune system and viral infection. Brain Behav Immun 2012;26:24-31.

8.   Barker E, Fujimura SF, Fadem MB, Landay AL, Levy JA. Immunologic abnormalities associated with chronic fatigue syndrome. Clin Infect Dis 1994;18 Suppl 1:S136-41.

9.   Bhat NM, Kshirsagar MA, Bieber MM, Teng NN. IgG Subclasses and Isotypes of VH4-34 Encoded Antibodies. Immunol Invest 2015;44:400-10.

10.   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.

11.   Brennan FM, McInnes IB. Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest 2008;118:3537-45.

12.   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.

13.   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.

14.   Broderick G, Katz BZ, Fernandes H, Fletcher MA, Klimas N, Smith FA, et al. Cytokine expression profiles of immune imbalance in post-mononucleosis chronic fatigue. J Transl Med 2012;10:191.

15.   Brurberg KG, Fonhus MS, Larun L, Flottorp S, Malterud K. Case definitions for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): a systematic review. BMJ Open 2014;4:e003973.

16.   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.

17.   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.

18.   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.

19.   Devanur LD, Kerr JR. Chronic fatigue syndrome. J Clin Virol 2006;37:139-50.

20.   Dinos S, Khoshaba B, Ashby D, White PD, Nazroo J, Wessely S, et al. A systematic review of chronic fatigue, its syndromes and ethnicity: prevalence, severity, co-morbidity and coping. Int J Epidemiol 2009;38:1554-70.

21.   Fletcher MA, Zeng XR, Barnes Z, Levis S, Klimas NG. Plasma cytokines in women with chronic fatigue syndrome. J Transl Med 2009;7:96.

22.   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.

23.   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.

24.   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.

25.   Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann Intern Med 1994;121:953-9.

26.   Hickie I, Davenport T, Wakefield D, Vollmer-Conna U, Cameron B, Vernon SD, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ 2006;333:575.

27.   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.

28.   Hornig M, Gottschalk G, Peterson DL, Knox KK, Schultz AF, Eddy ML, et al. Cytokine network analysis of cerebrospinal fluid in myalgic encephalomyelitis/chronic fatigue syndrome. Mol Psychiatry 2016;21:261-9.

29.   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(1).pii:e1400121

30.   Katz BZ, Shiraishi Y, Mears CJ, Binns HJ, Taylor R. Chronic fatigue syndrome after infectious mononucleosis in adolescents. Pediatrics 2009;124:189-93.

31.   Keech A, Sandler CX, Vollmer-Conna U, Cvejic E, Lloyd AR, Barry BK. Capturing the post-exertional exacerbation of fatigue following physical and cognitive challenge in patients with chronic fatigue syndrome. J Psychosom Res 2015;79:537-49.

32.   Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. J Clin Microbiol 1990;28:1403-10.

33.   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.

34.   Landi A, Broadhurst D, Vernon SD, Tyrrell DL, Houghton M. Reductions in circulating levels of IL-16, IL-7 and VEGF-A in myalgic encephalomyelitis/chronic fatigue syndrome. Cytokine 2016;78:27-36.

35.   Levy JA. Viral studies of chronic fatigue syndrome. Clin Infect Dis 1994;18 Suppl 1:S117-20.

36.   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.

37.   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.

38.   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.

39.   Lombardi VC, Hagen KS, Hunter KW, Diamond JW, Smith-Gagen J, Yang W, et al. Xenotropic murine leukemia virus-related virus-associated chronic fatigue syndrome reveals a distinct inflammatory signature. In Vivo 2011;25:307-14.

40.   Lorusso L, Mikhaylova SV, Capelli E, Ferrari D, Ngonga GK, Ricevuti G. Immunological aspects of chronic fatigue syndrome. Autoimmun Rev 2009;8:287-91.

41.   Maes M, Mihaylova I, Leunis JC. In chronic fatigue syndrome, the decreased levels of omega-3 poly-unsaturated fatty acids are related to lowered serum zinc and defects in T cell activation. Neuro Endocrinol Lett 2005;26:745-51.

42.   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.

43.   Maher KJ, Klimas NG, Fletcher MA. Chronic fatigue syndrome is associated with diminished intracellular perforin. Clin Exp Immunol 2005;142:505-11.

44.   Marshall-Gradisnik S, Huth T, Chacko A, Johnston S, Smith P, Staines D. Natural killer cells and single nucleotide polymorphisms of specific ion channels and receptor genes in myalgic encephalomyelitis/chronic fatigue syndrome. Appl Clin Genet 2016;9:39-47.

45.   Masuda A, Nozoe SI, Matsuyama T, Tanaka H. Psychobehavioral and immunological characteristics of adult people with chronic fatigue and patients with chronic fatigue syndrome. Psychosom Med 1994;56:512-8.

46.   Masuda A, Munemoto T, Yamanaka T, Takei M, Tei C. Psychosocial characteristics and immunological functions in patients with postinfectious chronic fatigue syndrome and noninfectious chronic fatigue syndrome. J Behav Med 2002;25:477-85.

47.   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.

48.   Mihaylova I, DeRuyter M, Rummens JL, Bosmans E, Maes M. Decreased expression of CD69 in chronic fatigue syndrome in relation to inflammatory markers: evidence for a severe disorder in the early activation of T lymphocytes and natural killer cells. Neuro Endocrinol Lett 2007;28:477-83.

49.   Niller HH, Wolf H, Minarovits J. Regulation and dysregulation of Epstein-Barr virus latency: implications for the development of autoimmune diseases. Autoimmunity 2008;41:298-328.

50.   Odom CI, Gaston DC, Markert JM, Cassady KA. Human herpesviridae methods of natural killer cell evasion. Adv Virol 2012;2012:359869.

51.   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.

52.   Peterson D, Brenu EW, Gottschalk G, Ramos S, Nguyen T, Staines D, et al. Cytokines in the cerebrospinal fluids of patients with chronic fatigue syndrome/myalgic encephalomyelitis. Mediators Inflamm 2015;2015:929720.

53.   Popa C, Leandro MJ, Cambridge G, Edwards JC. Repeated B lymphocyte depletion with rituximab in rheumatoid arthritis over 7 yrs. Rheumatology (Oxford) 2007;46:626-30.

54.   Prins JB, van der Meer JW, Bleijenberg G. Chronic fatigue syndrome. Lancet 2006;367:346-55.

55.   Reid S, Chalder T, Cleare A, Hotopf M, Wessely S. Chronic fatigue syndrome. BMJ 2000;320:292-6.

56.   Roberts ML, Luxembourg AT, Cooper NR. Epstein-Barr virus binding to CD21, the virus receptor, activates resting B cells via an intracellular pathway that is linked to B cell infection. J Gen Virol 1996;77:3077-85.

57.   Russell L, Broderick G, Taylor R, Fernandes H, Harvey J, Barnes Z, et al. Illness progression in chronic fatigue syndrome: a shifting immune baseline. BMC Immunol 2016;17:3.

58.   Shukla SK, Cook D, Meyer J, Vernon SD, Le T, Clevidence D, et al. Changes in Gut and Plasma Microbiome following Exercise Challenge in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). PLoS One 2015;10:e0145453.

59.   Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology 2011;140:1756-67.

60.   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.

61.   Tirelli U, Marotta G, Improta S, Pinto A. Immunological abnormalities in patients with chronic fatigue syndrome. Scand J Immunol 1994;40:601-8.

62.   Tomoda A, Joudoi T, Rabab el M, Matsumoto T, Park TH, Miike T. Cytokine production and modulation: comparison of patients with chronic fatigue syndrome and normal controls. Psychiatry Res 2005;134:101-4.

63.   Torchinsky MB, Blander JM. T helper 17 cells: discovery, function, and physiological trigger. Cell Mol Life Sci 2010;67:1407-21.

64.   Toussirot E, Roudier J. Epstein-Barr virus in autoimmune diseases. Best Pract Res Clin Rheumatol 2008;22:883-96.

65.   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.