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Characterisation of B cell Subsets and Receptors in Chronic Fatigue Syndrome Patients

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Abstract

Limited immunological changes have been previously reported in B cell phenotype in Chronic Fatigue Syndrome (CFS) patients, so there is no clear established role of B cells in the pathophysiology of CFS patients. The aim of this study was to evaluate B cells subsets including naive, memory naive, memory switched, memory non-switched, double negative, transitional, plasmablasts, HLA-DR$^+$, plasma and regulatory B cells (B_{reg}) in CFS patients compared with non-fatigued controls. B cell activation markers (CD81, CD21) and surface receptors (CD79a/b, IgM, IgD, IgA, IgE) were also examined in CFS patients compared with non-fatigued controls. 46 CFS patients (age=50.00 ± 2.00 years) and 34 non-fatigued controls (age=49.00 ± 2.16 years) participated in the study. The percentage of BCR IgM$^+$ B cells was significantly increased in the CFS group compared with non-fatigued controls (p=0.037). Similarly, there was a significant decrease in the CD1d$^+$ B cells in the CFS group compared with non-fatigued controls (p=0.046). No additional differences in B cell phenotypes, activation markers and surface receptors were found in the CFS patients compared with the non-fatigued control group. The differences observed in the B cell phenotype of CFS patients compared with non-fatigued controls may explain some of the disturbances in the immune homeostasis, however whether this is causal or the consequence of immunological imbalances previously reported in CFS patients requires further investigation.

Keywords: Chronic fatigue syndrome; B cells; Phenotype; Surface receptors

Introduction

Chronic Fatigue Syndrome (CFS) is a complex, heterogeneous disorder that is characterized by prolonged and occasionally relapsing fatigue that persists for periods of 6 months or longer [1,2]. Although, fatigue is the main symptom reported in CFS, cognitive debility, unrefreshing sleep, muscle and joint pain and flu-like symptoms are also symptoms of CFS. This debilitating illness with unknown etiology is further complicated by the lack of a diagnostic test, specific biological marker or a clear path for treatment. In addition to fatigue, combinations of multiple physiological and neurological impairments have been associated with CFS. Research into some immune cells has generally produced inconsistent results in CFS, and subsequently the pathogenesis of CFS remains relatively unclear.

Previous studies have described immunological disturbances in CFS [3-5] however some of the findings are divergent. Currently, there is no consistency in the immunological findings with the exception of natural killer (NK) cell activity, which has recurrently shown to be reduced in CFS patients [6-10]. B cell studies in CFS patients have also produced some inconsistent results, more specifically in regards to total B cell numbers, B cell subsets and B cell function [5,11,12]. Recently, B cell depletion through the administration of Rituximab has been shown to significantly improve CFS symptoms in 67% of CFS patients [13,14] suggesting that B cells may play a key role in the pathogenesis and symptom progression of CFS. However, other studies have found no differences in B cells in CFS patients [15,16]. Thus the role of B cells in CFS may be inconsistent. More recent studies have assessed peripheral B cell subsets [5,11], where numbers of transitional, naïve and plasmablasts cells were altered in CFS patients [11].

The possible commonalities between CFS and other autoimmune diseases are salient and have previously been reported [17]. Studies have also shown reduced levels of serum immunoglobulins G (IgG) and its subclasses in CFS patients [18]. Additionally, a reduction in the number of CD19$^+$/IgM$^+$ B cells in CFS has been reported. [19]. Hence, understanding the role of the B cell compartment in CFS patients was the main aim of this study.

Methods

Participant recruitment

CFS patients (n=46, age=50.00 ± 2.00 years) were recruited for the study from the National Centre for Neuroimmunology and Emerging Diseases (NCNED) patient database. Non-fatigued controls (HC) (n=34, age=49.00 ± 2.16 years) were also recruited using the NCNED database of control participants. HC group was composed of individuals with no history of CFS, smokers, autoimmune disease, psychosis, depression, epilepsy, heart disease, pregnant or breastfeeding. CFS patients were excluded if they were smokers, pregnant or breastfeeding or had been diagnosed with autoimmune diseases, psychosis, depression, epilepsy or heart disease. All participants completed a consent form and a CFS questionnaire based on the 1994 CDC [1] case definition. At the time of the study the CFS patients were taken one or more of the following medications

including anticholinergic, antihistamine, anti-convulsants, antidepressants, anticoagulants, anti-inflammatory, benzodiapenes, opioids, opioid analgesics, proton pump inhibitors, steroids, triptans, vitamins and supplements.

This study obtained ethical approval from the Griffith University Human Research Ethics Committee (MSC/22/13/HREC).

Blood collection

Venous blood samples (5 mL) were collected and processed within 4 hours of collection from the antecubital vein into ethylenediamine tetra acetic acid (EDTA) tube.

B cell phenotype

Total B cells and their subsets in the periphery were characterized as Naive, memory naive, memory switched, memory nonswitched, double negative, transitional 1, 2 and 3, plasmablasts, HLA-DR$^+$, plasma B cells, Regulatory B cells (B_{reg}), activation markers and surface receptors were also examined. All samples were examined using monoclonal antibodies (Table 1).

To determine the number and distribution of B cell phenotypes, whole blood samples were stained for 30 minutes with the following antibodies CD138 (FITC), IgM (APC), CD19 (BV421), CD27 (BV605), IgD (AF700), CD38 (BV711), HLA-DR (PER-CP-Cy 5.5) and CD10 (PE-Cy7). To determine the distribution of regulatory B cells and activation markers samples were stained with CD19 (BV421), CD27 (BV605), CD1d (PE), CD21 (PE-Cy7), CD5 (AF700), and CD81 (APC-H7). While the B cell receptors were stained with CD19 (BV421), CD79a (Per-CP-Cy 5.5), CD79b (FITC), CD154 (PE-Cy7), IgD (AF700), IgE (APC), IgM (BV605) and IgA (PE). Following staining, red blood cells were lysed for 10 minutes using FACSLyse (BD Biosciences, San Jose, CA) and then washed twice, using phosphate buffered saline (PBS) (Gibco, Life Technologies, Victoria). Cells were fixed and analysed using flow cytometry, with the lymphocyte gate specific for the B cell protein CD19$^+$ [5,11,20].

B cell surface receptors were assessed, specifically B cell receptors CD79a and CD79b which play a key role in modulating immune responses [20,21]. To quantify these receptors, isolated B cells were stained with the appropriate antibody panels for each receptor and analysed via flow cytometer with the lymphocyte gate specific for CD19$^+$ B cells. The gating strategy used is presented in Figure 1.

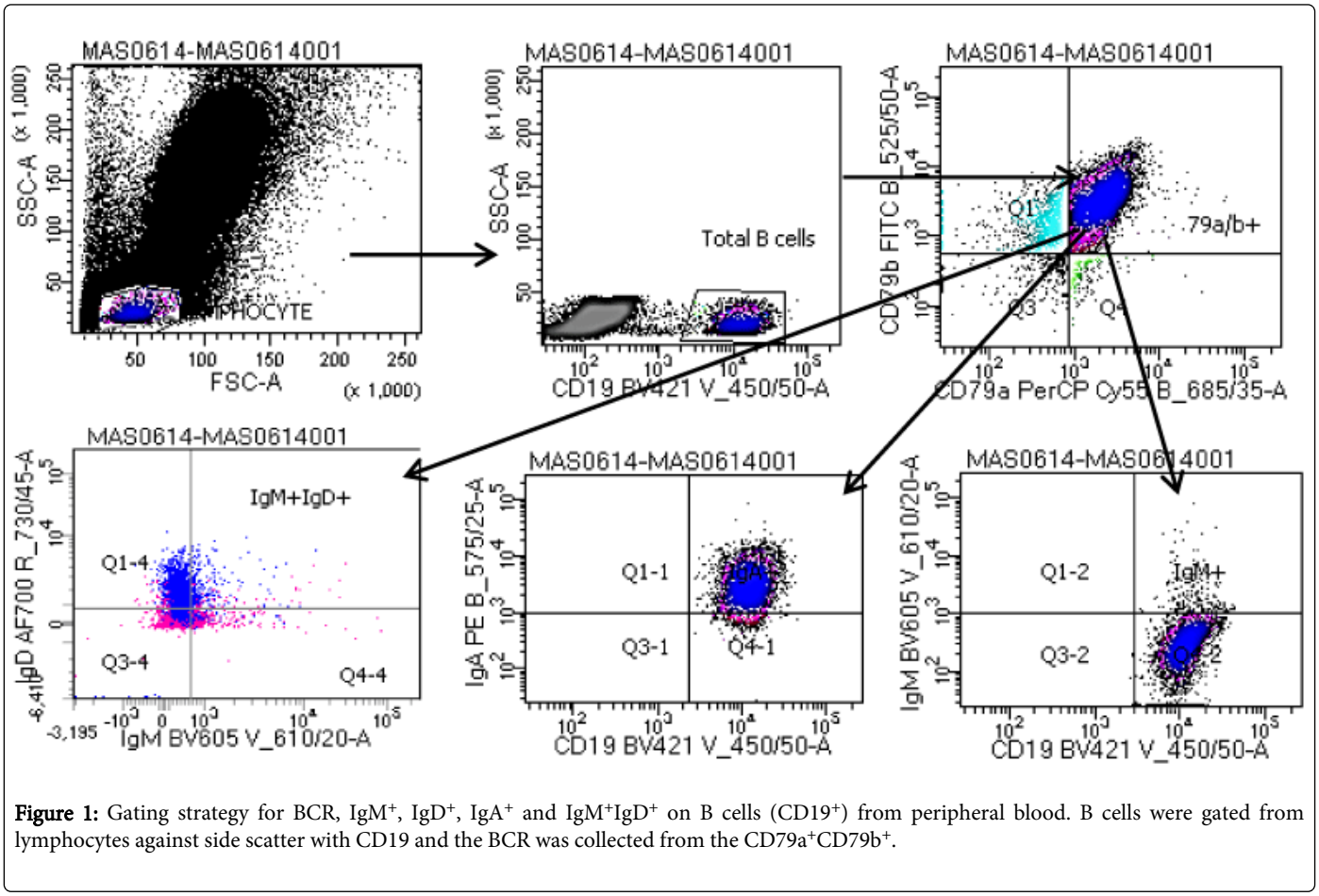

Figure 1: Gating strategy for BCR, IgM$^+$, IgD$^+$, IgA$^+$ and IgM$^+$IgD$^+$ on B cells (CD19$^+$) from peripheral blood. B cells were gated from lymphocytes against side scatter with CD19 and the BCR was collected from the CD79a$^+$CD79b$^+$.

Statistical analysis

All data was analysed using the SPSS (version 18.0, SPSS Inc., Chicago, USA) and Graph Pad Prism (version 6.0, Graph Pad Software, Inc., San Diego, USA) statistical tools. Normality was evaluated using the Kolmogorov-Smirnov tests. ANOVA was used to determine significance for normally distributed data while the Mann Whitney U test was the non-parametric test used to determine measures of significance with Post-hoc Bonferroni. Data were significant were p-values were less than or equal to 0.05. All results in figures and tables are presented as either median or mean ± standard error of the mean (SEM).

B cell Phenotype	CD Markers
Total B cells	$CD19^+$
Naïve B cells	$CD19^+CD27^-IgD^+$
Mature Naïve B cells	$CD19^+CD27^-CD10^-CD38^-$
Switched Memory B cells	$CD19^+CD27^+IgD^-$
Non-switched Memory B cells	$CD19^+CD27^+IgD^+$
Double negative B cells	$CD19^+CD27^-IgD^-$
T1/2 B cells	$CD19^+CD27^-CD10^+CD38^+IgD^+$
T3 B cells	$CD19^+CD27^-CD10^-CD38^+IgD^+$
Plasmablasts	$CD19^{DIM}CD27^{HIGH}IgD^-CD38^{HIGH}$
Plasma B cells	$CD19^{DIM}CD27^{HIGH}IgD^-CD38^{HIGH}CD138^+HLA\text{-}DR^+$
IgM^+ B cells	$CD19^+CD27^+IgD^-IgM^+$
$HLA\text{-}DR^+$ B cells	$CD19^+CD27^+IgD^-CD38^+CD138^-HLA\text{-}DR^+$
Regulatory B cells (Bregs)	$CD19^+CD27^{LOW}CD21^+CD5^+CD1d^{HIGH}$
B cell activation markers	$CD19^+CD27^{LOW}CD21^+CD5^+CD1d^{HIGH}CD81^+$
B cell receptor complex (BCR)	$CD19^+CD79\alpha^+CD79\beta^+$
Surface IgM	$CD19^+CD79\alpha^+CD79\beta^+IgM^+$
Surface IgD	$CD19^+CD79\alpha^+CD79\beta^+IgD^+$
Surface IgA	$CD19^+CD79\alpha^+CD79\beta^+IgA^+$
Surface IgE	$CD19^+CD79\alpha^+CD79\beta^+IgE^+$
*CD - Cluster of Differentiation, Ig - Immunoglobulin; HLA - Human Leucocyte Antigen; T1/2 - transitional 1 and 2; T3 - transitional 3.	

Table 1: Antibodies combinations for B cell subsets.

Results

There was no difference in age between the two groups, 76% of the CFS group were females while 60% of the non-fatigued group were females (Table 2).

	CFS Patients	Non-fatigued Controls	
N	46 (76% Female)	34 (60% Female)	
Age	50.00 ± 2.0	49.00 ± 2.16	0.38
White Cell Count	5.90 ± 0.24	6.28 ± 0.28	0.31

Table 2: Characteristics of CFS and non-fatigued groups.

B cell phenotypes

There was no difference in the number or percentage of total B cells between CFS and control groups (Figure 2). There were no differences in naïve, mature naïve, switched or non-switched between CFS and control groups (Table 3). Activation markers showed no significant difference between CFS and non-fatigued groups. $CD5^+$ B cells was similar in both groups with no significant difference between groups (data not shown). $CD1d^+$ B cells were significantly decreased in the CFS patients in comparison to the non-fatigued controls (Figure 3).

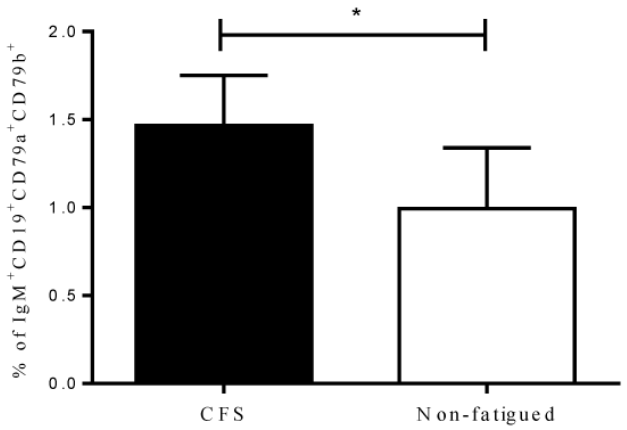

Figure 2: Total B cells in CFS patients and non-fatigued controls. The percentage of total B cells was not significantly different between the two groups of participants. CFS group is represented in black while the non-fatigued control group is represented in white, error bars indicate SEM.

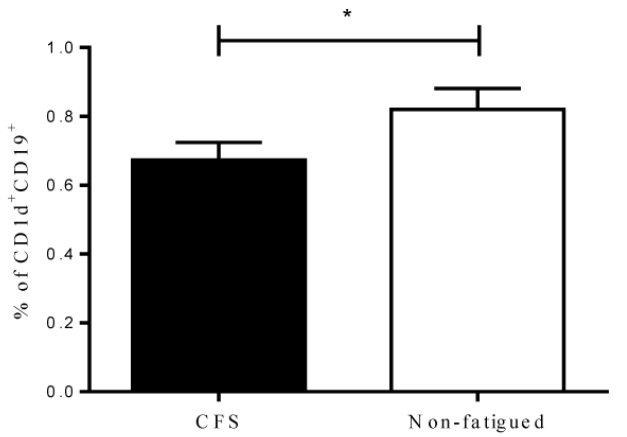

Figure 3: Levels of CD1d in CFS patients and non-fatigued controls on B cells. There was a significant decrease in the % of $CD1d^+$ B cells in the lymphocytes of CFS/ME patients compared with Non-fatigued group (p=0.046). CFS group is represented in black while non-fatigued control group is represented in white, error bars indicate SEM.

B cell receptors

Surface receptors, IgA^+, IgD^+, IgE^+ and $CD40L^+$ were not significantly different between CFS and control groups. However, the $CD19^+CD79a^+CD79b^+IgM^+$ subset of B cells was significantly higher in the CFS patients compared with the non-fatigued group ($P \leq 0.05$) (Figure 4).

B cell Phenotype (cells/μL)	CFS Patients	Non-fatigued controls	P-value
Naive	86.62 ± 21.14	113.81 ± 23.05	0.43
T1/2	10.61 ± 2.75	16.45 ± 3.52	0.56
Mature naive	11.42 ± 2.75	14.85 ± 5.23	0.42
T3	59.24 ± 15.22	72.29 ± 16.05	0.51
Non-switched	4.85 ± 2.30	4.03 ± 2.53	0.53
Double negative	223.31 ± 30.78	236.72 ± 29.78	0.44
switched	137.37 ± 12.97	113.21 ± 14.73	0.47
Plasmablast	96.66 ± 9.03	88.56 ± 11.05	0.49
HLA-DR plasma cells	90.61 ± 8.46	78.45 ± 9.79	0.52
Plasma cells	1.45 ± 0.23	1.84 ± 1.9	0.61
B cell Receptors (%)	**CFS Patients**	**Non-fatigued controls**	**P-value**
Total $CD79\alpha^{+}\beta^{+}$ (BCR)	58.99 ± 4.46	58.72 ± 5.92	0.74
$CD40L^{+}$	4.01 ± 0.60	4.68 ± 1.23	0.65
BCR IgE^{+}	3.05 ± 1.30	2.62 ± 1.87	0.9
BCR IgA^{+}	54.02 ± 4.02	50.47 ± 5.24	0.83
BCR IgD^{+}	7.24 ± 3.21	9.76 ± 3.24	0.48

*CD - Cluster of Differentiation, Ig – Immunoglobulin; HLA – Human Leucocyte Antigen; T1/2 – transitional 1 and 2; T3 – transitional 3, BCR – B cell receptor complex.

Table 3: Distribution of B cell phenotypes and receptors in CFS patients and non-fatigued controls.

Discussion

This study evaluated B cell phenotypes and their surface receptors in CFS patients compared with healthy controls. The CFS patients evaluated in this study showed no difference in naïve, mature naïve, T1/2, T3 B cells, plasmablast or plasma cells compared with controls.

Our results indicate a significant increase in the percentage of BCR IgM^{+} B cells ($CD19^{+}CD79a^{+}CD79b^{+}IgM^{+}$) in the CFS group and this may suggest that signaling through the surface IgM may be increased in the CFS patients. Membrane bound IgM is a necessary component of the BCR complex for mature B cell survival [22]. BCR IgM^{+} has been shown to activate signaling pathways involving Btk, Syk, ERK1/2 and p38 phosphorylation. BCR IgM^{+} also activates a negative feedback loop that controls the magnitude and extent of the phosphorylation of these signaling motifs thus fostering optimal B cell signaling [23]. Ligation of IgM has been shown to reduce terminal differentiation of B cells [24]. In the present study, although, the terminally differentiated B cells were lower in the CFS patients, they were not significantly different.

Interestingly, earlier studies have characterized CFS as an IgM^{+} related immune disorder [25]. Another study has also confirmed significantly increased IgM mediated response to acetylcholine in CFS patients compared with controls, categorizing it as an autoimmune response that may be responsible for the dysregulation of certain cellular functions [25]. According to Guo et al. IgM enhances anti-Ig-initiated B cell activation and proliferation. Additionally, B cells are responsible for the increase in the production of IgM in response to infection. It is well known that IgM enhances complement activation and has a critical role in the defense of the host before adaptive immune response [26]. Further studies are required to evaluate B cell activation and function in a larger sample of CFS patients.

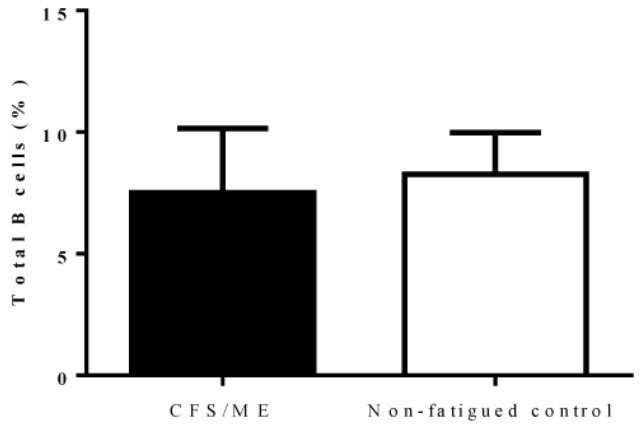

Figure 4: B cell receptor complex in CFS patients and controls. Subset of B cells $CD19^{+}CD79a^{+}CD79b^{+}IgM+$ significantly increased in the CFS group compared with non-fatigued group ($p=0.037$). The CFS group is represented in black while non-fatigued control group is represented in white, error bars indicate SEM.

B cells are also responsible for presenting lipid antigen to CD1d-restricted invariant Natural Killer T (iNKT) cells in the healthy immune system. A previous study reported decreased CD1d expression on B cells in autoimmune diseases such as SLE leading to a reduction in the frequency of iNKT cells in this population. It has a role in maintaining tolerance in autoimmunity [27,28]. There was a significant decrease in the percentage of $CD1d^{+}$ B cells in the lymphocytes of CFS patients compared with Non-fatigued group. The decrease in the $CD1d^{+}$ B cells presented by the CFS group is suggestive of a possible dysfunction in the iNKT cell in this population. Additionally, $CD1d^{+}$ B cells may be induced to produced IL-10 and this has been shown to regulate Th2 immune responses [29,30]. CD1d is generally expressed on most subsets of B cells and a decrease in this marker may affect the regulatory effects of B cells during inflammatory reactions. CD1d is essential for antiviral immune responses and may be reduced on antigen presenting cells in the presence of pathogens such as viruses [31-35]. In CFS recurring viral infections have been suggested to occur and this may be related to a general decrease CD1d expression on immune cells.

The inconsistencies in the results of B cell phenotypes amongst CFS populations are not well understood. Perhaps these inconsistencies can be explained by the differences in the characterization of the various B cell phenotypes. Furthermore, immunoglobulins (total IgG, IgG1, IgG2 and IgG3) have been investigated and shown discrepancies amongst CFS studies [36-38]. Studies with CFS patients require a greater attention to the recruitment and screening of participants to

avoid major heterogeneity and extra confounds within the sample groups, such as the presence of psychiatric disorder or use of drugs that might influence the immune system regulation of participants and mischaracterize the CFS sample.

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