

# Novel characterisation of mast cell phenotypes from peripheral blood mononuclear cells in chronic fatigue syndrome/myalgic encephalomyelitis patients

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## Abstract

**Background:** Mast cells (MCs) mediate inflammation through neuropeptides and cytokines, along with histamine and reactive oxygen species (ROS). Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is an illness characterized by an unexplained disabling fatigue with multiple physiological impairments as well as dysregulated cytokine profiles.

**Objective:** To determine mast cell phenotypes in isolated human PBMCs, in healthy controls and in CFS/ME patients. Second, determine receptor expression of RAGE and its ligand high mobility group box 1 protein (HMGB1).

**Method:** Moderately severe CFS/ME patients (n=12, mean age 39.25 ± SD3.52 years), severe CFS/ME patients (n=6, mean age 43.00 ± SD4.02 years) and healthy controls (n=13, mean age 42.69 ± SD3.87 years) were included in this study. CFS/ME patients were classified according to the 2011 International Consensus Criteria. LSRFortessa X-20 Flow cytometry was used for the identification of phenotypic peripheral mast cell population in PBMCs using an exclusion marker Lin2 cocktail (anti-CD3, anti-CD14, anti-CD19, anti-CD20 and anti-CD56) and inclusion markers (CD117, CD34, FCεRI, chymase, HLA-DR and CD154) following comparative investigation. HMGB1 and soluble RAGE expression in plasma was measured by sandwich ELISA assay.

**Results:** There was a significant increase in CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>chymase<sup>+</sup> mast cell populations in moderate and severe CFS/ME patients compared with healthy controls. There was a significant increase in CD40 ligand and MHC-II receptors on differentiated mast cell populations in the severe CFS/ME compared with healthy controls and moderate CFS/ME. There were no significant differences between groups for HMGB1 and sRAGE.

**Conclusions:** This preliminary study investigates mast cell phenotypes from PBMCs in healthy controls. We report significant increase of naïve MCs in moderate and severe CFS/ME patients compared with healthy controls. Moreover, a significant increase in CD40 ligand and MHC-II receptors on differentiated mast cells in severe CFS/ME patients. Peripheral MCs may be present in CFS/ME pathology however, further investigation to determine their role is required.

**Keywords:** Chronic Fatigue Syndrome; Mast Cells; Receptor for advanced glycation end products; RAGE; Ligand High Mobility Group Box 1 protein (HMGB1); Myalgic Encephalomyelitis

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## Introduction

Mast cells (MCs) are found in the blood circulating as an immature form, prior to migrating to vascularised tissues, where they differentiate into functional cells such as connective tissue (or serosal) and mucosal MCs.<sup>1</sup> Importantly, mature cells do not ordinarily circulate in peripheral blood<sup>2</sup> as they are released as immature cells into the blood periphery where they migrate throughout the body to tissues such as the skin, mucosa, brain and airways to undergo maturation.<sup>3</sup> Stem-cell factor, also known as c-kit, is the ligand for the receptor responsible for the main survival and developmental factor

for MCs. Additionally, immune modulators, such as growth factors, cytokines and chemokines are able to affect MCs and their phenotypes.<sup>2,4,5</sup> MC phenotypes that have been well recognised are defined according to their protease content.<sup>6</sup> MC activation and degranulation can occur from FCεRI (IgE cross-linking) or independent mechanisms such as, other immunoglobulin receptor (FCγR), cytokines, chemokines, neuropeptides and through receptors involved in mast cell interaction with surrounding immune cells (MHC-II, CD40L, OX40). MC phenotype is determined by the level of surface expression of the key differentiating CD markers such as CD117 (c-kit), FCεRI and CD33, where bright and dim populations can be seen, along with the surface expression of CD34. The CD34 marker is expressed during the immature stages of the MC.<sup>7,8</sup> Mast cells that are differentiated, mature or activated, express FCεRI and HLA-DR.<sup>9</sup> Activated MCs produce and release several pro-inflammatory mediators from intracellular stores which alter the inflammatory environment to mobilise immune cells to the site of the pathogen infection and to draining lymph nodes.<sup>2</sup> Importantly, human MC phenotypes have been characterised in bone marrow and tissues from pathological diseases. They have not been well characterised from peripheral blood.

Previous investigations have characterised MC phenotypes from bone marrow and tissue in pathological diseases using predominate methods that are poorly reproducible, expensive to produce, biological properties differ from *in vivo* i.e human primary cell culture model or primary cell lines. Thus, there is a need for development of a relevant, reproducible and inexpensive *in vitro* model to represent MC found *in vivo* morphological and biological properties. Therefore, identification of MC from peripheral blood using flow cytometric methods, facilitate and elucidate MC phenotype and function in healthy and diseases.

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a debilitating heterogeneous disorder associated immune, neurological, musculoskeletal, gastrointestinal and autonomic symptoms. The aetiology of CFS/ME is unknown, however, immune dysfunction has been consistently implicated in the pathomechanism of the illness.<sup>10</sup> Atypical levels of regulatory T cells (Treg) and pro-inflammatory and anti-inflammatory cytokines, generation of nitric oxide and hypersensitivity responses suggest that the pathophysiology of CFS/ME involves the activation of inflammatory pathways.<sup>6,11-13</sup> Nuclear factor kappa beta (NF-κB) is a transcriptional factor that plays a role in inflammatory pathways, cell proliferation, differentiation, and survival. Previous investigators have reported significant increases in NF-κB in CFS/ME patients.<sup>14</sup>

The Receptor for Advanced Glycation End products (RAGE) is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules.<sup>15,16</sup> Its ligands include the high mobility group box 1 protein (HMGB1) and AGEs, which are known to play a major role in the amplification of chronic inflammation and are associated with inflammation.<sup>17,18</sup> Hence it was important to know if self-perpetuating inflammatory signals were being promulgated by mast cells activated by RAGE in CFS/ME.

The purpose of this investigation was to identify MC phenotypes in isolated human PBMCs using flow cytometry and the soluble receptor for RAGE, as well as its ligand high mobility group box 1 protein (HMGB1), in healthy controls and CFS/ME patients.

## Methods

### Subjects

CFS/ME patients were aged matched with healthy controls (**Table 1**). Thirteen healthy controls (aged  $42.69 \pm 3.87$  years), twelve moderate CFS/ME (aged  $39.25 \pm 3.52$ ) and six severe CFS/ME patients (aged  $43.00 \pm 4.03$ ) participated in this study. CFS/ME patients were defined in accordance with the International Consensus Criteria<sup>10</sup> and excluded if they had any disease that would exclude CFS/ME diagnosis, were smokers, pregnant or breast feeding. Severity of fatigue and grouping of CFS/ME patients was determined using Dr David Bell's disability scale (DBDS)<sup>19</sup> Moderate CFS/ME were classified as patients scoring 30% or higher on the Dr Bell's Disability Score (DBDS). Severe CFS/ME were classified as patients scoring less than 30% on the DBDS and according to this scale, are considered housebound or bedridden. All healthy control participants did not meet CFS/ME criteria. All participants were of Caucasian descent and residents of Australia at the time of blood collection. Participants gave written consent prior to blood collection and this study was conducted under the approval of Griffith University Ethics Reference number (MSC/23/12/HREC).

30 mL of whole blood was collected from all participants where 10 mL of whole blood was collected into EDTA tubes to investigate full blood count, electrolytes, high sensitive C reactive protein and erythrocyte sedimentation rate. There were no significant differences between groups for these blood parameters (**Table 1**).

10 ml EDTA whole blood was collected for mast cell phenotypic immune assay and 8 ml of CPT sodium citrate (BD Bioscience, Franklin Lakes, NJ) for HMGB1 and sRAGE ELISA assays.

### Mast cell phenotypic immune assay

Peripheral blood mononuclear cells (PBMCs) isolation was performed using Ficoll-Paque PLUS density centrifugation (GE healthcare, Parramatta NSW) to help remove granulocytes (such as, neutrophils, basophils and eosinophils). PBMCs were stained with trypan blue stain (Invitrogen, Carlsbad, CA) for cell viability count and adjusted in a final concentration of  $1 \times 10^7$  cells in 150 μL. Cells were labelled with the mononuclear antibodies for 30 minutes, excepted CD34 were incubated for 45 minutes. Intracellular staining of chymase was performed with BD fix and perm per manufacture's instruction (BD Bioscience, San Jose, CA). Labelled cells were resuspended with stained buffer (BD Bioscience, San Jose, CA) prior to flow cytometric analyses. Isotypes was used for negative control (**Table 1**).

### Flow cytometry

LSR Fortessa™ X-20 flow cytometry was employed to analyse mast cell phenotype. All samples were collected

**Table 1. Participant demographics and pathology**

	Healthy controls (n=13)	Moderate CFS/ME (n=12)	Severe CFS/ME (n=6)	P Value
Age (years)	42.69 ± 3.87	39.25 ± 3.52	43.00 ± 4.03	0.469
Sex (% female)	61.50%	91.70%	50%	0.038
Dr Bell's Score (%)	93.08 ± 7.48	52.50 ± 7.23	25.00 ± 13.17	0.000
<b>Pathology</b>				
White Cell Count (x 10 <sup>9</sup> /L)	6.48 ± 0.82	6.25 ± 0.59	6.02 ± 0.51	0.962
Lymphocytes (x 10 <sup>9</sup> /L)	1.99 ± 0.171	1.82 ± 0.16	2.01 ± 0.25	0.745
Monocytes (x 10 <sup>9</sup> /L)	0.31 ± 0.03	0.34 ± 0.02	0.36 ± 0.04	0.557
Neutrophils (x 10 <sup>9</sup> /L)	4.04 ± 0.728	3.91 ± 0.49	3.40 ± 0.29	0.877
Eosinophils (x 10 <sup>9</sup> /L)	0.13 ± 0.03	0.16 ± 0.03	0.23 ± 0.08	0.252
Basophils (x 10 <sup>9</sup> /L)	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.905
Platelets (x 10 <sup>9</sup> /L)	240.31 ± 15.61	278.67 ± 19.71	230.17 ± 20.66	0.144
Haemoglobin (g/L)	140.69 ± 3.17	135.42 ± 2.26	135.83 ± 3.76	0.242
Haematocrit	0.42 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.449
Red Cell Count (x 10 <sup>12</sup> /L)	4.82 ± 0.01	4.52 ± 0.06	4.55 ± 0.15	0.300
Mean Corpuscular Volume (fL)	87.38 ± 1.66	89.75 ± 0.70	90.33 ± 1.84	0.316
Sodium (mmol/L)	138.08 ± 0.57	137.25 ± 0.40	138.33 ± 0.49	0.275
Potassium (mmol/L)	4.16 ± 0.09	4.11 ± 0.08	3.83 ± 0.17	0.208
Chloride (mmol/L)	102.54 ± 0.43	102.25 ± 0.46	102.50 ± 1.34	0.889
Bicarbonate (mmol/L)	27.31 ± 0.59	27.42 ± 0.50	27.83 ± 1.47	0.806
Anion Gap (mmol/L)	8.23 ± 0.30	7.50 ± 0.34	7.83 ± 0.60	0.324
Erythrocyte sedimentation rate (mm/Hr)	11.31 ± 2.41	15.50 ± 3.47	10.50 ± 2.61	0.218
High sensitive C-Reactive Protein	2.635 ± 0.83	4.08 ± 1.65	0.50 ± 0.22	0.174

\* means significance,  $P < 0.05$

at 200,000 events. All antibodies were purchase from BD Bioscience, unless otherwise stated. Cells exclusion markers were Lin2 cocktail (anti-CD3, anti-CD14, anti-CD19, anti-CD20 and anti-CD56) to exclude lymphocytes, monocytes, eosinophil and neutrophils and inclusion markers and protein to help identify mast cells were CD117, CD34, FCεRI, and chymase. Mast cells were further characterised by interaction markers HLA-DR (MHC-II), CD154 (CD40L).<sup>20-22</sup> Mast cells subsets were defined as mast cell progenitor (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>chymase<sup>-</sup>),<sup>9,23,24</sup> mature mast cell (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>) activated mast cell (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>HLA-DR<sup>+</sup>CD40L<sup>+</sup>).

#### HMGB1 and sRAGE sandwich ELISA

Sodium citrate blood tubes were centrifuge at 1800g for 20 minutes at 20°C to collect 3ml of plasma. Plasma was stored at -20°C until analysed. Sandwich ELISA assay for HMGB1 (IBL International GMBH, Hamburg, Germany) and sRAGE (R&D Systems, Minneapolis, USA) was performed as per manufacturer's instructions. Briefly, 50µl of standards and plasma from healthy controls, moderate and severe CFS/ME groups were plated in duplicate on a 96 microtiter plate that was pre-coated with either human anti-sRAGE or anti-HMGB1 monoclonal antibody. Prior to labelling HMGB1 and sRAGE with anti-HMGB1, 2 conjugated with peroxidase or anti-sRAGE conjugated with horseradish peroxidase, the plate was washed with manufacturer's buffer using an automatic plate washer. Reactions were stopped with 100µl of sulfuric acid prior to measuring the optical density with POLARstar Omegaversion OMEGA 1.20. The optical density was measured at 450nm, with wavelength correction set to 540nm and 600nm for sRAGE and HMGB1 plate, respectively. The average OD for each sample was determined, and was corrected for the OD of the blank well. Linear standard curves

were analysed to determine samples sRAGE and HMGB1 concentration (**Figure 1**)

#### Statistical analyses

Data was analysed using SPSS Version 22. Shapiro-wilk normality test was used to determine the distribution of data. Demographics of participants was normally distributed and ANOVA was used to test for significance. Kruskal-Wallis test was performed to determine the statistical significance of pathology test, mast cell phenotype, HMGB1 and soluble RAGE between groups. Mann-Whitney U tests determined the significance between groups for both non-parametric and parametric data and reported as  $p < 0.05$ .

#### Results

There were no significant differences between groups for gender, age and pathology results (**Table 1**). There was a significant difference between groups for the Dr Bell's Fatigue Severity Score where the mean score for the healthy control group was 92% compared to the mean score for the moderate and severe CFS/ME groups at 53% and 25%, respectively (**Table 1**).

#### Identification of human mast cells in peripheral blood

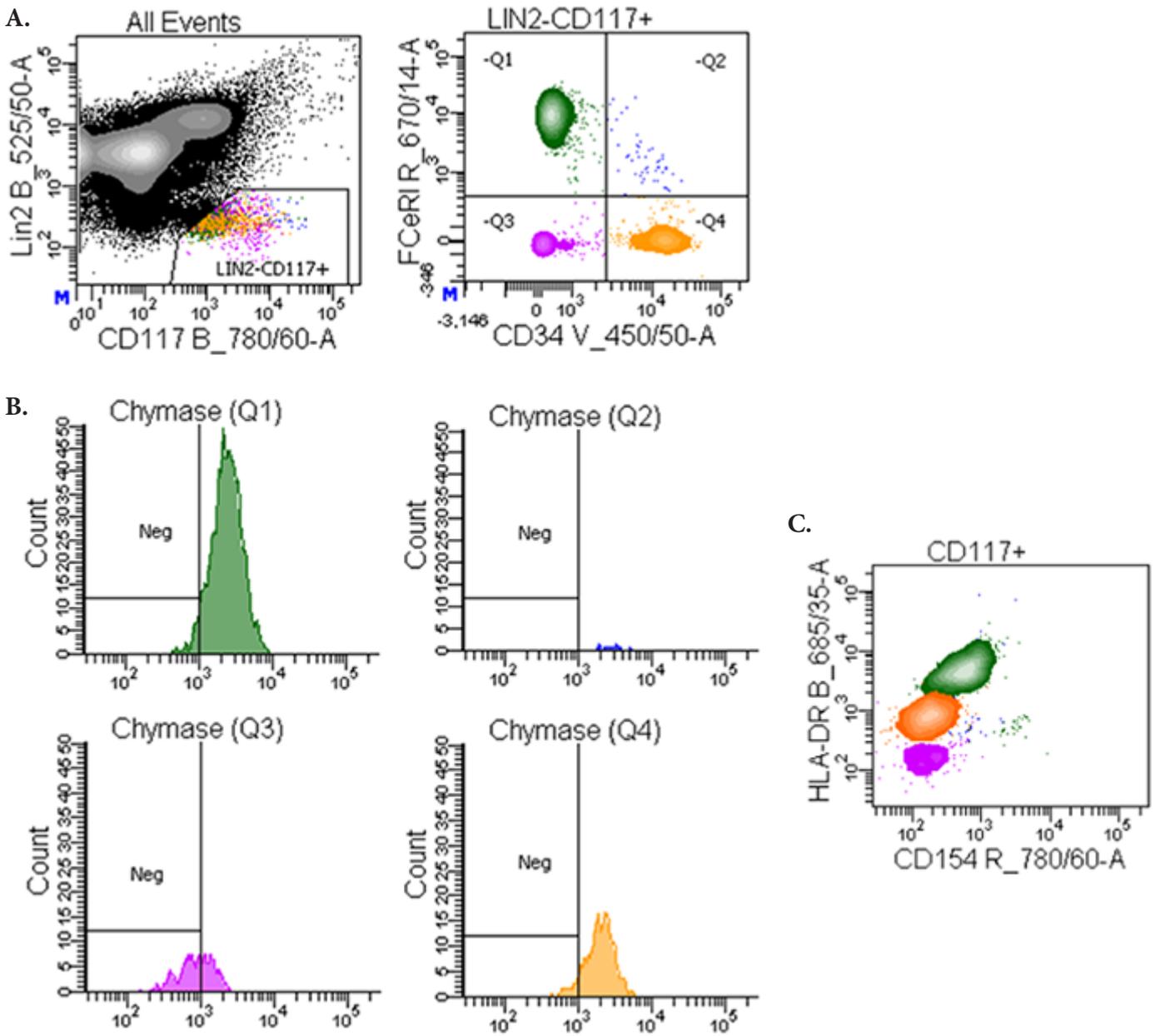
Lin2 monoclonal antibody cocktail (anti-CD3, anti-CD14, anti-CD19, anti-CD20 and anti-CD56) was used to labelled immune cells (such as, T cells, B cells, natural killer cells, monocytes, eosinophil and neutrophils) is represented in black of the contour plot (**Figure 1A**). Cells labelled with a myeloid receptor, anti-CD117 were gated as CD117<sup>+</sup> committed mast cells propagator (**Figure 1A**). CD117<sup>+</sup> mast cells were further labelled with anti-CD34 and anti-FCεRI to differentiate four

different phenotypes, including FCεRI<sup>+</sup> CD34<sup>-</sup> (Q1), FCεRI<sup>+</sup> CD34<sup>+</sup> (Q2), FCεRI<sup>-</sup> CD34<sup>-</sup> (Q3), and FCεRI<sup>-</sup> CD34<sup>+</sup> (Q4). The four phenotypes also showed low and bright expression for intracellular chymase (Figure 1B) and interaction receptors CD40L and major histocompatibility complex class II (MHC-II) (Figure 1C). FCεRI<sup>+</sup> CD34<sup>-</sup> represented in green (Q1) demonstrated higher expression of CD40L and MHC-II. Whereas FCεRI<sup>+</sup> CD34<sup>+</sup> (Q3) shown in blue and FCεRI<sup>-</sup> CD34<sup>+</sup> shown in orange (Q4) population demonstrated low/dim expression of MHC-II receptors and negative expression for

CD40L. Both MHC-II and CD40L receptors showed negative expression for FCεRI<sup>-</sup> CD34<sup>-</sup> (Q3) displayed as the pink population (Figure 1C).

**Increased levels of mast cell committed progenitors in moderate and severe CFS/ME patients**

Mast cells expressing CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>-</sup> that did not express intracellular chymase were compared between the control, moderate and severe CFS/ME patients. CD117<sup>+</sup> CD34<sup>+</sup>FCεRI<sup>-</sup>chymase<sup>-</sup> cells were significantly elevated in both



**Figure 1. Identification and flow cytometry gating of human mast cells in peripheral blood.** A. All PBMC events were plotted with Lin2 (anti-CD3, anti-CD14, anti-CD19, anti-CD20, anti-CD56) and CD117 antibodies to identify CD117<sup>+</sup> cells. CD117<sup>+</sup> cells were then gated for FCεRI and CD34 and the four phenotypes FCεRI<sup>+</sup> CD34<sup>-</sup> (Q1), FCεRI<sup>+</sup> CD34<sup>+</sup> (Q2), FCεRI<sup>-</sup> CD34<sup>-</sup> (Q3), and FCεRI<sup>-</sup> CD34<sup>+</sup> (Q4) are represented. B. CD117<sup>+</sup> cells subsets expressed differential levels of intracellular chymase. C. CD117<sup>+</sup> cells subsets showed differential expression of HLA-DR and CD154 depending on the FCεRI and CD34 phenotype. HLA-DR is highly expressed in FCεRI<sup>+</sup> CD34<sup>-</sup> (Green) > FCεRI<sup>-</sup> CD34<sup>+</sup> (orange)/ FCεRI<sup>+</sup> CD34<sup>+</sup> (Blue) > to FCεRI<sup>-</sup> CD34<sup>-</sup> (Pink). Additionally, FCεRI<sup>+</sup> CD34<sup>-</sup> (Green) showed high expression for CD154 receptor.

the moderate and severe CFS/ME patients compared with the healthy control group (Figure 2). No difference was observed between further subsets (Figure 1).

**Upregulation of peripheral mast cell interaction receptors in severely affected CFS/ME patients**

Surface markers CD154 and HLA DR receptors were compared between controls, moderate and severe CFS/ME patients. There was a significant increase in CD154 (CD40L) and HLA DR (MHC-II) receptors for the severe CFS/ME group compared to the healthy control and moderate CFS/ME group (Figure 3).

**sRAGE and its ligand HMGB1 in CFS/ME patients**

There was no significant difference between groups for HMGB1 and sRAGE (Figure 4).

**Discussion**

The present paper reports, for the first time, the identification of MC phenotypes in PBMCs from healthy individuals participating as controls. Flow cytometric methods

consisting of a cocktail of inclusion and exclusion cell markers, along with cell surface markers CD154 and MHC II (HLA-DR), were used for the first time. We were able to identify MC progenitor (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>chymase),

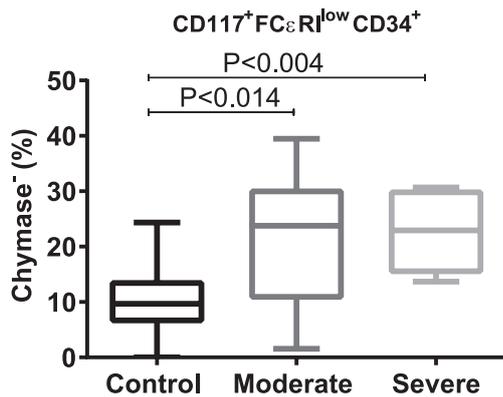


Figure 2. Box-whisker plot for mast cells in control, moderate and severe CFS/ME patients. CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>chymase mast cells are represented as percentage. The horizontal line within the box represents as medium and the outer horizontal lines of the box represents 25% and 75% percentile. The error bars denotes the minimum and maximum range.

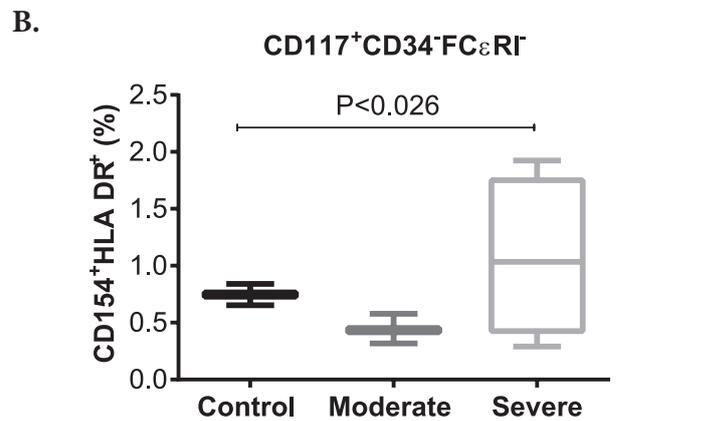
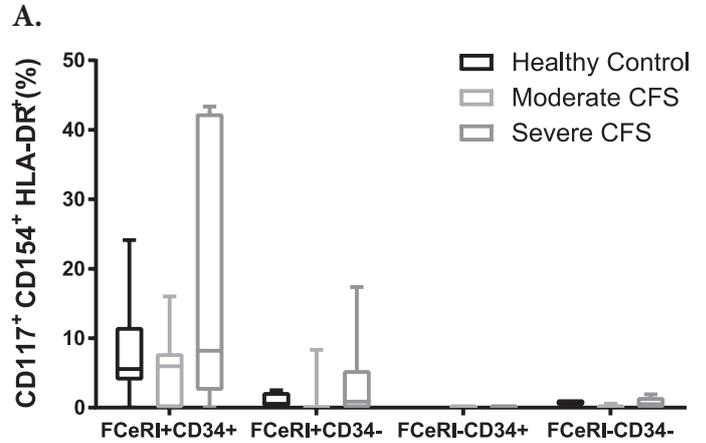


Figure 3. Immune profile of interaction markers expressed on mast cell surface. Box-whisker plots for CD154<sup>+</sup>HLA-DR<sup>+</sup> is shown as percentage of parent CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup> cells in controls, moderate and severe CFS/ME groups. The horizontal line within the box is represented as medium and the outer horizontal lines of the box are represented as 25% and 75% percentile. The error bars denotes the minimum and maximum range.

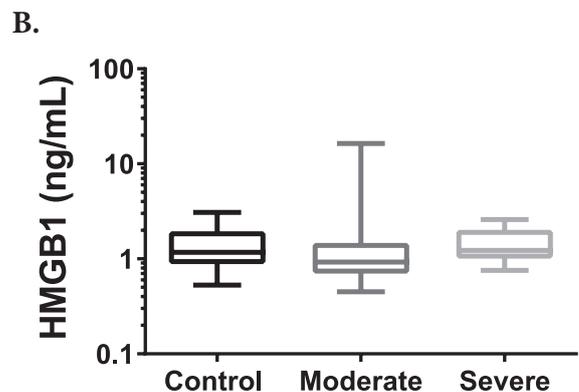
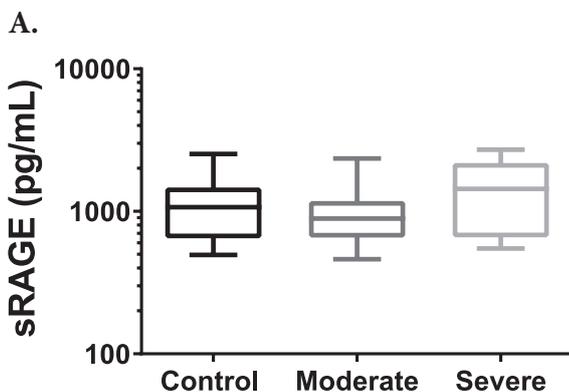


Figure 4. Comparison of plasma soluble RAGE (sRAGE) and HMGB1 in control, moderate and severe CFS/ME groups. A. sRAGE concentration was measured as pg/ml between different severity groups. B. HMGB1 concentration was measured as ng/ml between different severity groups.

mature MCs (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>-</sup>) and activated MCs (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>+</sup>HLA-DR<sup>+</sup>CD40L<sup>+</sup>) in human PBMCs from healthy individuals. In the comparative investigation we also report novel findings of a significant increase in CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>-</sup>chymase<sup>-</sup> MC populations in moderate and severe CFS/ME patients compared with healthy controls. Also we are the first to report a significant increase CD40 ligand and MHC-II receptors on differentiated MC populations for severe CFS/ME patients compared with the healthy control group and moderate CFS/ME patients.

A particular strength of this study is that it supports the identification of MC phenotypes and receptor expression in isolated human PBMCs using flow cytometric techniques without the need for morphological and cell culture based characterization.

Our research findings report a significant increase in mast cell progenitor (MCPs) (CD117<sup>+</sup>CD34<sup>+</sup>FCεRI<sup>-</sup>chymase<sup>-</sup>) in both moderate and severe CFS/ME patients compared with controls. MCPs circulate in the blood and lymphatics and migrate to peripheral tissues upon inflammation.<sup>26</sup> MCPs have extensive proliferation potential and can differentiate into both connective and mucosal mast cells depending on the factors present in the tissue microenvironment. Mature mast cells are derived from MCPs under the influence of stem cell factor (SCF) secreted locally by fibroblasts, stromal cells and endothelial cells.<sup>27</sup> Mast cell maturation and biological expression in the tissue microenvironment is influenced by cytokines specifically, IL-4, IL-10, IL-17, TNF-α, and IFN-γ, which has been reported to be elevated in CFS/ME patients, suggesting that these cytokines may influence the tissue microenvironment and consequently the proliferation and differentiation of MCPs into mature mast cells in CFS/ME patients.<sup>28,29</sup>

Mast cells express a broad array of cell surface receptors and ligands which mediate the communication with immune cells belonging to both innate and acquired immunity, as well as with non-immune tissue-specific cell types.<sup>21</sup> Our novel discovery of significant increase in CD154 (CD40L) and HLA DR (MHC-II) receptors for the severe CFS/ME group compared with the healthy control and moderate CFS/ME group suggests MCs, macrophages as well as glial cells expressing the CD40L<sup>30</sup> potentially may play a role in mediating immune dysregulation of B and T cell responses as well as contributing to neuroinflammation in CFS/ME patients.<sup>21</sup> MHC-II expression on MC effectively supports T cell proliferation and effector functions, subsequently leading to expansion of Tregs<sup>21,31</sup> as well as B cell proliferation. Importantly, previous investigators have reported increased Treg cell numbers, B cell phenotypes and inflammation in CFS/ME patients.<sup>32</sup>

RAGE is known to play a role in MC activation, having been identified on both serosal and mucosal mast cells.<sup>33</sup> Mechanisms proposed in RAGE activation of MC include reactive oxygen species production (ROS) and histamine release that may be involved in sensitivity, allergic reactions and immune-inflammation. Multiple chemical sensitivity for example, is a co-morbidity of CFS/ME.<sup>10</sup> Secretory RAGE and its ligand, HMGB1, was not significantly different between groups. Further investigation into RAGE receptor expressed on

MC may provide understanding in MC inflammatory response in CFS/ME patients.

Mast cell activation has been associated with blood brain barrier (BBB) dysfunction such as neuroinflammation. Neuroinflammation on PET scans has been assessed in CFS/ME patients.<sup>6</sup> During neuroinflammation, mast cells can respond to neuropeptides, such as vasoactive intestinal polypeptide (VIP) in an FCεRI independent manner for degranulation and chemokine production.<sup>34</sup> We have reported increased in VIP receptor (VPAC2) on CD4<sup>+</sup> T cells and decreased IL-10 in cerebrospinal fluid from CFS/ME patients.<sup>35,36</sup> Further, compared with controls, CFS/ME patients had significantly increased serum IFN-γ, IL-5 and IL-10 compared with controls.<sup>37</sup> Elevation in IFN-γ is noteworthy, being a strong cytokine for antigen presentation and expression of MHC-II on mast cells. Detection of peripheral MC may possibly elucidate further pathways of neuroinflammation in the brain of CFS/ME patients.

## Conclusion

We have discovered activated mast cells, for the first time, in CFS/ME patients as well as identifying mast cell phenotypes in blood of healthy controls. Moreover, the putative distribution of activated mast cells in almost all tissues and organs in the body requires further investigation to establish whether they have a role in the pathomechanism in CFS/ME. Importantly, neuroinflammatory processes and their sequelae involving the blood brain barrier and brain parenchyma should be investigated further in these patients.

## Author's contributions

- SMG, TN, DRS designed and developed all experiments.
- TN, DG, JC, AC, PS and SJ assisted with the analysis of the data.
- SMG, DRS, SJ, and TN assisted with the preparation and final manuscript.
- SMG, DRS, PS, SJ, AC and TN assisted in the revisions of this manuscript.

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