doi: 10.1111/sji.12388

Pilot Study of Natural Killer Cells in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis and Multiple Sclerosis

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Received 26 April 2015; Accepted in revised form 5 September 2015

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Abstract

Patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) and multiple sclerosis (MS) suffer from debilitating fatigue which is not alleviated by rest. In addition to the fatigue-related symptoms suffered by patients with CFS/ME and MS, dysfunction of the immune system and, in particular, reduced natural killer (NK) cell cytotoxic activity has also been reported in CFS/ME and MS. The purpose of this pilot study was to compare NK cellular mechanisms in patients with CFS/ME and MS to investigate potential dysfunctions in the NK cell activity pathway. Flow cytometry protocols assessed $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{+/-}$ NK cell expression of adhesion molecules, NK activating and inhibiting receptors, NK cell maturation and lytic proteins. All participants in this study were female and included 14 patients with CFS/ME, nine patients with MS and 19 non-fatigued controls. The patient groups and the non-fatigued controls were not taking any immunosuppressive or immune-enhancing medications. In the MS cohort, KIR2DL5 was significantly increased on $CD56^{bright}CD16^{+/-}$ NK cells and expression of CD94 was significantly increased on $CD56^{dim}CD16^{+}$ NK cells in comparison with the controls. Co-expression of CD57 and perforin was significantly increased on $CD56^{dim}CD16^{+}$ NK cells from patients with CFS/ME compared to the MS and non-fatigued control participants. The results from this pilot study suggest that NK cells from patients with CFS/ME and MS may have undergone increased differentiation in response to external stimuli which may affect different mechanisms in the NK cell cytotoxic activity pathway.

Introduction

Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is associated with disabling levels of fatigue. Patients suffer from a state of permanent exhaustion which is also accompanied by a myriad of symptoms associated with autonomic, neurological, endocrine and immune systems [1, 2]. Symptom severity may vary on a daily or weekly basis and is not alleviated by rest [3, 4]. Persistent fatigue is a characteristic of CFS/ME and approximately 65-95% of patients with multiple sclerosis (MS) also experience unremitting fatigue [5, 6]. Exacerbation of symptoms following physical or cognitive activity has been described in both CFS/ME and MS, as have memory and cognitive difficulties, gastrointestinal disturbances and irregular sleep patterns [5]. The symptoms suffered by patients with CFS/ME and MS may present as a relapsing-remitting course, and both CFS/ME and MS share a significant female preponderance [7, 8]. Approximately six females are affected to every one male for CFS/ME and 2.3–3.5 females to every one male for MS [7, 9].

Dysfunction of the immune system may contribute to the pathogenesis of CFS/ME and MS. In particular, reduced natural killer (NK) cell cytotoxic activity is a consistent finding in CFS/ME and relapsing-remitting patients with MS [10–12]. $CD56^{dim}CD16^{+}$ NK cells elicit cytotoxic activity to remove target cells infected by viruses, bacteria or cells that have been malignantly transformed [13]. NK cell cytotoxic activity is a tightly regulated process which consists of a number of ordered steps including adhesion to the target cell, NK cell activation by surface receptors and release of lytic proteins to induce apoptosis of the target cell [14–16].

SCANDINAVIAN JOURNAL OF Immunology AN INTERNATIONAL MONTHLY JOURNAL

Whilst the consequences of reduced NK cell activity may be attributed to the persistence of viral infections reported in some patients with CFS/ME, contrasting evidence in MS suggests that the activity of NK cells can either exacerbate or attenuate disease activity [3, 7, 17]. The opposing effects of NK cells reported in patients with MS may be mediated by the different subsets of NK cells eliciting either cytotoxic or cytokine effector functions [18]. Reduced cytotoxic activity of peripheral NK cells from patients with MS has been correlated with clinical exacerbations of disease activity [12]. This finding has been replicated in the experimental autoimmune encephalomyelitis (EAE) mouse model where depletion of NK cells was associated with increased disease activity [19]. It has also been suggested that NK cells have an immunoregulatory role that promotes the remission state in relapsing-remitting MS [12, 20]. Through cytotoxic activity and the production of type 2 cytokines such as IL-5 and IL-13, NK cells may lyse and suppress T helper 1 autoimmune cells which mediate the inflammatory process in the CNS of patients with MS [20, 21]. The potential immunoregulatory role of NK cells in MS maintaining the remission state highlights the importance of optimal NK cell effector function.

As reduced NK cell cytotoxic activity has previously been reported in both CFS/ME and MS, the aim of this pilot study was to investigate cellular mechanisms required for NK cell effector function. Adhesion molecules, surface receptors and lytic proteins were measured to determine whether mechanisms which may contribute to reduced NK cell cytotoxic activity are similar or different in female patients with CFS/ME and MS.

Materials and methods

Study participants and inclusion criteria. Participants were enrolled from clinics at Griffith University and the Gold Coast University Hospital, and the non-fatigued controls were enrolled from a database at the National Centre for Neuroimmunology and Emerging Diseases. As a greater proportion of females are affected by CFS/ME or MS, all participants in this study were female [7, 9]. Patients with CFS/ME met the International Consensus Criteria (ICC) according to a symptom checklist in the questionnaire [1] and the patients with MS met the revised McDonald criteria [22]. The control subjects were non-fatigued and screened for exclusionary conditions such as epilepsy, thyroid conditions, psychosis, diabetes, cardiac disorders, smoking, pregnant or breastfeeding and immunological, inflammatory or autoimmune diseases. The patients with CFS/ME and MS included in this study were not taking any immunosuppressive, immune-enhancing or disease-modifying medications. Participants who did not meet the criteria for CFS/ME, MS or non-fatigued controls were excluded from the study. This study was conducted with approval from Griffith University Human Research Ethics Committee (MSC/18/13/HREC) and prior to participating, all subjects provided informed consent.

Blood collection. A total of 5 ml EDTA and 6 ml lithium heparin blood samples were collected from the antecubital vein of each participant. The blood was collected in the morning to avoid the influence of circadian variation, and the samples were analysed within 5 h of collection. Queensland Pathology measured all participant blood parameters including erythrocyte sedimentation rate (ESR) and a full blood count of red and white blood cells on a Sysmex XE-5000.

NK cell phenotypes. Bright and dim NK cell phenotypes from CFS/ME, MS and non-fatigued controls were measured on NK cells isolated from whole-blood heparin using RosetteSep Human NK cell enrichment cocktail (Stem Cell Technologies, Vancouver, BC, Canada). In summary, whole blood was incubated with RosetteSep which preferentially isolates NK cells by cross-linking all other cells with red blood cells except for NK cells. Following density gradient centrifugation with Ficoll–Hypaque (GE Health Care, Uppsala, UP), the NK cells were collected and flurochrome-conjugated monoclonal antibodies CD56-PE-Cy7, CD16-BV711 and CD3-BV510 (BD Biosciences, San Diego, CA, USA) were used to identify CD3-negative NK cells as $CD56^{+}$(total NK cells), $CD56^{bright}CD16^{-/dim}$ or $CD56^{dim}CD16^{+}$ NK cells [18]. Total NK cells and the two NK cell phenotypes were analysed on a LSR-Fortessa X20 flow cytometer (BD Biosciences, San Diego, CA, USA) according to the forward and side scatter properties of the NK lymphocytes and the surface density expression of CD56 and CD16.

NK cell adhesion molecules, activating and inhibiting receptors. $CD56^{bright}CD16^{-/dim}$ and $CD56^{dim}CD16^{+}$ NK cell expression of adhesion molecules and activating and inhibiting receptors was measured on isolated NK cells. Monoclonal antibodies for CD56, CD16 and CD3 identified NK cell phenotype expression of the adhesion molecules CD2-FITC, CD18-BV421, CD11a-PE, CD11b-APC-Cy7 and CD11c-APC (BD Biosciences, San Diego, CA). Natural cytotoxicity receptors (NCRs) including NKp30-PE, NKp46-BV650 (BD Biosciences) and NKp80-FITC (Miltenyi Biotec, Cologne, BG) were assessed on $CD56^{bright}CD16^{-/dim}$ and $CD56^{dim}CD16^{+}$ NK cells. 2B4-APC (BD Biosciences) from the signalling lymphocytic activating molecule (SLAM) family of receptors and killer immunoglobulin-like receptors (KIRs) including CD94-PerCP-Cy5.5, KIR2DL1-PE (BD Biosciences), KIR3DL1/DL2-PE, KIR2DL5-APC, KIR2DS4-PE, KIR3DL1-FITC, NKG2D-APC, KIR2DL2/DL3-APC and KIR2DL1/DS1-FITC (Miltenyi Biotec) were also measured on the two NK cell phenotypes. NK cell expression of the adhesion molecules and receptors was determined by flow cytometric methods.

NK cell maturation and intracellular staining for lytic proteins. NK cell maturation and lytic proteins were measured on peripheral blood mononuclear cells (PBMCs) isolated from

EDTA whole blood by density gradient centrifugation with Ficoll–Hypaque (GE Health Care). NK cell maturation was measured by the surface expression of CD57-PE-CF594 (BD Biosciences) on $CD56^{bright}CD16^{-/dim}$ and $CD56^{dim}CD16^{+}$ NK cells. Intracellular staining of isolated PBMCs (1×10^7cells/ml) measured NK cell lytic proteins including perforin, granzyme A and granzyme B. NK cells were stained with monoclonal antibodies for CD56, CD16 and CD3, permeabilized and fixed with BD Cytofix (BD Biosciences), washed with perm wash buffer (BD Biosciences) and incubated with monoclonal antibodies for perforin-PE, granzyme A-FITC and granzyme B-BV421 (BD Biosciences). $CD56^{bright}CD16^{-/dim}$ and $CD56^{dim}CD16^{+}$ NK cell expression of CD57, perforin, granzyme A and granzyme B was determined using the flow cytometer.

Statistical analysis. spss (IBM Corp, Version 21, Armonk, NY, USA) and GRAPHPAD Prism (GraphPad Software Inc., Version 6, La Jolla, CA, USA) were used for statistical analysis of the data. The Shapiro–Wilk test was used to assess for a Gaussian distribution in each data set, and a one-way ANOVA or the Kruskal–Wallis test was used to determine significant differences in participant full blood counts and NK cell parameters between the three groups. Tukey's or Dunn's multiple comparisons test was used to identify which groups (CFS/ME, MS or non-fatigued control) were significantly different. Significance was set at $P < 0.05$, and the data are presented as either mean ± SEM or median ± SEM.

Results

A total of 14 patients with CFS/ME (mean age [years] ± standard error of the mean (SEM) = 49.14 ± 2.50), nine patients with MS (mean age [years] ± SEM = 48.00 ± 5.36) and 19 non-fatigued controls (mean age [years] ± SEM = 48.37 ± 2.21) were included in this study. The clinical course of the nine patients with MS cohort included three relapsing-remitting, one primary-progressive, two secondary progressive and three clinically isolated syndrome. Patients with MS had an average disease duration of 12.3 years and an average multiple sclerosis severity score of 3.25.

Lymphocytes, monocytes and ESR significantly increased in patients with MS

White and red blood cell parameters for the three groups are shown in Table 1. In comparison with the CFS/ME and control groups, patients with MS had a significant increase in lymphocytes, monocytes and ESR ($P < 0.05$ as indicated by the bolded values in the table). No other significant differences between the groups were identified from the full blood count results.

NK phenotype distribution in MS, CFS/ME and non-fatigued controls

Measurement of NK cell phenotypes including total, dim and bright NK cells in CFS/ME, MS and non-fatigued controls revealed no significant differences (Table 2). The total number of NK cells was higher in the CFS/ME and MS groups in comparison with the non-fatigued controls, although this difference was not significant. No significant differences were observed in the distribution of $CD56^{dim}CD16^{+}$ NK cells between the three groups. Although patients with CFS/ME had a lower number of $CD56^{bright}CD16^{-/dim}$ NK cells compared to the non-fatigued controls and patients with MS had an increased number of $CD56^{bright}CD16^{-/dim}$ NK cells compared to the controls, this difference was not significant.

Table 1 White and red blood cell parameters from full blood for CFS/ME, MS and non-fatigued controls. Data are presented as median ± SEM and significance was set at $p<0.05$.

				P values			
	CFS/ME (n = 14)	MS (n = 9)	Control (n = 19)	Overall	CFS/ME & MS	CFS/ME& Control	MS&Control
White blood cells (10^9/l)	5.62 ± 0.35	6.8 ± 0.76	5.87 ± 0.22	0.066	0.064	>0.999	0.226
Lymphocytes (10^9/l)	1.70 ± 0.11	2.42 ± 0.27	1.84 ± 0.10	**0.007	**0.007	0.734	*0.023
Monocytes (10^9/l)	0.32 ± 0.01	0.46 ± 0.04	0.29 ± 0.02	***<0.0001	**0.002	0.462	****<0.0001
Neutrophils (10^9/l)	3.44 ± 0.27	3.71 ± 0.54	3.56 ± 0.21	0.388	0.597	>0.999	0.687
Eosinophils (10^9/l)	0.12 ± 0.02	0.21 ± 0.03	0.12 ± 0.02	0.0502	0.078	>0.999	0.082
Basophils (10^9/l)	0.02 ± 0.006	0.03 ± 0.004	0.03 ± 0.004	0.650	>0.999	>0.999	>0.999
Platelets (10^9/l)	263.79 ± 19.12	265.78 ± 15.83	260.47 ± 12.86	0.973	0.998	0.987	0.974
Red blood cells (10^{12}/l)	4.49 ± 0.07	4.47 ± 0.10	4.47 ± 0.05	0.966	0.976	0.969	0.999
Haemoglobin (g/l)	132.93 ± 2.74	132.67 ± 2.20	135.37 ± 1.80	0.631	0.997	0.698	0.715
Haematocrit	0.400 ± 006	0.40 ± 0.005	0.40 ± 0.004	0.690	0.687	0.983	0.751
Mean cell volume (fl)	89.43 ± 0.84	88.67 ± 1.20	89.74 ± 0.80	0.741	0.860	0.964	0.720
ESR (mm/hr)	11.50 ± 3.47	14.00 ± 5.28	10.00 ± 1.20	*0.020	>0.999	0.085	*0.047

Table 2 NK cell phenotype distribution for CFS/ME, MS and non-fatigued control participant groups. Data are presented as median ± SEM and significance was set at $p<0.05$.

				P values			
	CFS/ME (n = 14)	MS (n = 9)	Control (n = 19)	Overall	CFS/ME & MS	CFS/ME & Control	MS & Control
Total NK	68.95 ± 5.79	82.30 ± 4.45	58.76 ± 5.25	0.085	>0.999	0.534	0.098
$CD56^{dim}CD16^{+}$	51.60 ± 4.42	50.31 ± 3.69	51.20 ± 2.98	0.668	>0.999	>0.999	>0.999
$CD56^{bright}CD16^{-/dim}$	4.29 ± 0.74	7.83 ± 1.48	5.75 ± 0.41	0.147	0.1753	0.538	>0.999

KIR2DL5 and CD94 expression significantly increased in MS cohort

Patients with MS had significantly increased amounts of KIR2DL5 on $CD56^{bright}CD16^{-/dim}$ NK cells compared to the non-fatigued controls (Fig. 1, A). $CD56^{dim}CD16^{+}$ NK cells from the MS cohort expressed significantly increased amounts of the receptor CD94 compared to the non-fatigued controls (Fig. 1, B. Refer to Figs S1 and S2 for representative gating strategies for $CD56^{bright}CD16^{-/dim}$ and $CD56^{dim}CD16^{+}$ NK cell measurement of KIR2DL5 and CD94). The MS cohort also had an increased amount of CD94 on $CD56^{dim}CD16^{+}$ NK cells compared to CFS/ME; however, this difference was not significant. Measurement of additional KIR, NCR and SLAM surface receptors revealed no significant differences (Table S1).

Distribution of NK Cell adhesion molecules

Measurement of adhesion molecules on $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-/dim}$ NK cells from CFS/ME, MS and non-fatigued controls revealed no significant differences between the groups (Table 3). Although not significant, expression of CD11b was increased on $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-/dim}$ NK cells from patients with CFS/ME and MS compared to the non-fatigued controls. CD11c expression in contrast was lower in CFS/ME and MS compared to the non-fatigued controls; however, these differences were not significant.

Co-expression of CD57 and perforin significantly increased in CFS/ME

Patients with CFS/ME compared to the MS and non-fatigued control participants had a significant increase in the co-expression of CD57 and perforin on $CD56^{dim}CD16^{+}$ NK cells (Fig. 2. Refer to Figs S3 and S4 for representative flow cytometry plots). Co-expression of CD57 and perforin on $CD56^{bright}CD16^{-/dim}$ NK cells showed no significant differences between the cohorts. Intracellular measurement of additional lytic proteins including granzyme A and granzyme B revealed no significant differences between the groups (Table 4).

Discussion

Reduced NK cell cytotoxic activity has previously been reported in CFS/ME and MS. This study compared cytotoxic effector mechanisms of NK cells between CFS/ME and MS to investigate potential mechanisms which may contribute to reduced NK cell activity in these two patient cohorts. Significant differences in full blood count parameters from the MS cohort were observed, and consistent with previous literature on female MS patients,

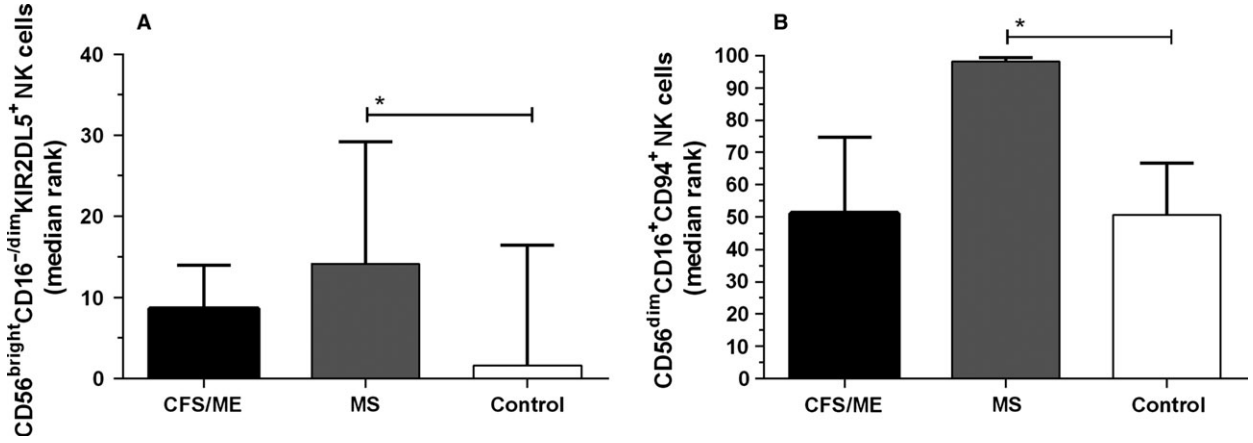

Figure 1 Expression of KIR2DL5 on $CD56^{bright}CD16^{-/dim}$ NK cells and CD94 on $CD56^{dim}CD16^{+}$ NK cells from CFS/ME, MS and non-fatigued controls. In comparison with the controls, KIR2DL5 expression was significantly increased ($P < 0.05$) on $CD56^{bright}CD16^{-/dim}$ NK cells from patients with MS (A). CD94 expression on $CD56^{dim}CD16^{+}$ NK cells from patients with MS was also significantly increased ($P < 0.05$) compared to the controls (B). Data are presented as median ranked values with interquartile ranges.

Table 3 NK cell adhesion molecules on $CD56^{dim}CD16^{+}$ and $CD56^{bright}$ NK cells from CFS/ME, MS and non-fatigued controls. Data are presented as median ± SEM and significance was set at $p<0.05$.

				P values			
	CFS/ME (n = 14)	MS (n = 9)	Control (n = 19)	Overall	CFS/ME & MS	CFS/ME & Control	MS & Control
$CD56^{dim}CD16^{+}$							
CD11a	100 ± 0.23	100 ± 0.06	100 ± 0.04	0.613	>0.999	>0.999	>0.999
CD11b	96.60 ± 1.98	93.56 ± 2.31	91.90 ± 3.08	0.357	>0.999	0.501	>0.999
CD11c	75.55 ± 6.78	75.15 ± 7.84	78.95 ± 3.08	0.764	>0.999	>0.999	>0.999
CD18	100.0 ± 0.18	99.90 ± 0.03	100.0 ± 0.04	0.483	0.686	>0.999	>0.999
CD2	75.65 ± 2.26	75.12 ± 3.58	74.56 ± 2.87	0.961	0.993	0.957	0.991
$CD56^{bright}CD16^{-/dim}$							
CD11a	100 ± 0.16	99.53 ± 0.17	100 ± 0.05	0.243	0.638	>0.999	0.287
CD11b	94.50 ± 3.35	96.10 ± 0.99	93.66 ± 1.403	0.883	>0.999	>0.999	>0.999
CD11c	74.63 ± 4.43	83.70 ± 3.61	88.20 ± 2.34	0.274	0.959	0.339	>0.999
CD18	99.75 ± 0.20	100.0 ± 0.16	100.0 ± 0.06	0.142	0.708	0.153	>0.999
CD2	90.22 ± 1.08	92.70 ± 1.86	91.21 ± 0.90	0.630	>0.999	>0.999	>0.999

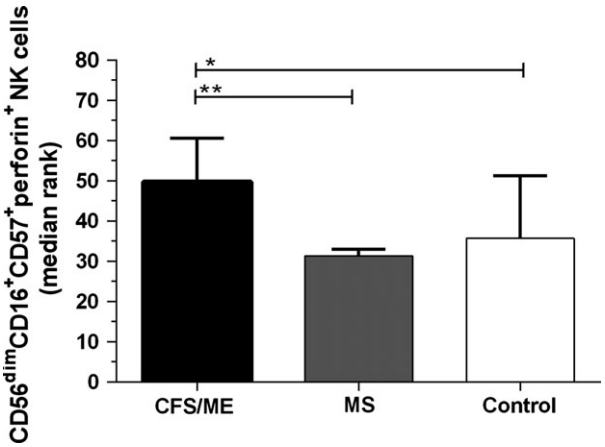

Figure 2 CFS/ME, MS and non-fatigued control co-expression of CD57 and perforin on $CD56^{dim}CD16^{+}$ NK cells. Compared to both the MS and non-fatigued control cohorts, patients with CFS/ME had a significant increase in the co-expression of CD57 and perforin on $CD56^{dim}CD16^{+}$ NK cells (*$P < 0.05$ and ** $P < 0.01$). Data are presented as median ranked values with interquartile ranges.

ESR results were significantly increased compared to CFS/ME and non-fatigued controls [23]. This result suggests that there is significant peripheral inflammation in this cohort of untreated patients with MS. Additional significant increases were reported for lymphocyte and monocyte results in the MS cohort compared to CFS/ME and the non-fatigued controls; however, previous literature has reported no significant differences [24, 25]. The small sample size may contribute to differences observed in white blood cell parameters in this MS cohort.

Activation of NK cell effector function is stringently regulated by surface receptors and the significant increase in KIR2DL5 on $CD56^{bright}CD16^{-/dim}$ NK cells and CD94 on $CD56^{dim}CD16^{+}$ NK cells may contribute to the reduced NK cell activity reported in patients with MS [12, 14, 20]. The KIR2DL5 receptor relays inhibitory signals, and increased expression of the KIRs on peripheral NK cells has been associated with decreased cytotoxic activity and production of IFN-γ [26, 27]. The cytoplasmic tails of the inhibitory receptors consist of immunoreceptor tyrosine-based inhibitory motif (ITIM) and following receptor ligation, tyrosine phosphatases suppress NK activity by dephosphorylating the protein substrates required for the tyrosine kinases on the activating receptors, preventing the calcium influx required for NK cell cytotoxic activity and cytokine production [14, 28]. CD94 is a homodimer structure which associates with NKG2- A, B, C, E or H to form heterodimers capable of regulating NK activity through the transmission of activating or inhibitory signals [29, 30]. CD94 is known as a regulatory molecule and alterations in the expression of CD94 on $CD56^{dim}CD16^{+}$ NK cells from the MS cohort may affect the ability of NK cells to induce cytotoxic lysis of target cells due to changes in signalling thresholds [29]. As the regulation of NK cell activity is governed by an intricate balance of both activating and inhibitory signals, the significant increases in KIR2DL5 and CD94 in the MS cohort may contribute to changes in threshold levels [29]. In particular, increased inhibitory signals from KIR2DL5 may set a higher activation threshold, masking important activating signals for NK effector function in patients with MS [14, 31]. Inhibition of NK cell activity through enhanced expression of specific KIRs on NK cells from patients with MS has been associated with an increased susceptibility to infections from the herpesvirus [26, 27].

Exposure to environmental agents such as viral infections may increase the expression of the KIR receptors as a mechanism to avoid elimination by NK cells [27]. Patients with MS may also have a genetic predisposition to alterations in KIR expression as carrier frequencies of KIR genes including KIR2DL5 are decreased in patients with MS [32, 33]. Expression of CD94/NKG2A on NK cells has been examined in mouse models of MS as a

Table 4 Lytic proteins and cell maturation CD57 on $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-/dim}$ NK cells from CFS/ME, MS and non-fatigued controls. Data are presented as median ± SEM and significance was set at $p<0.05$.

				P values			
	CFS/ME (n = 14)	MS (n = 9)	Control (n = 19)	Overall	CFS/ME & MS	CFS/ME& Control	MS&Control
$CD56^{dim}CD16^{+}$							
Perforin	67.19 ± 3.73	51.66 ± 4.58	63.43 ± 5.03	0.134	0.122	0.825	0.253
Granzyme A	80.02 ± 3.23	77.93 ± 4.98	85.40 ± 5.81	0.986	>0.999	>0.999	>0.999
Granzyme B	76.91 ± 3.57	65.41 ± 4.94	85.70 ± 4.107	0.088	0.404	>0.999	0.083
CD57	74.75 ± 2.44	63.92 ± 5.13	70.10 ± 3.94	0.134	0.245	0.265	>0.999
$CD56^{bright}CD16^{-/dim}$							
Perforin	2.64 ± 0.66	1.40 ± 1.33	1.3 ± 1.49	0.619	>0.999	>0.999	>0.999
Granzyme A	31.68 ± 4.95	29.04 ± 4.13	29.76 ± 3.27	0.908	0.916	0.934	0.993
Granzyme B	11.36 ± 1.96	11.09 ± 2.28	14.58 ± 2.24	0.453	0.997	0.532	0.568
CD57	25.86 ± 3.31	16.78 ± 2.44	19.50 ± 2.03	0.247	0.301	0.885	>0.999

potential target for treatment [21]. It has been suggested that genetic disruption of CD94/NKG2A removes NK cell inhibition, enhancing cytotoxic lysis of pathogenic autoreactive $CD4^{+}$ T cells and microglia in the central nervous system, favouring the remission state of MS and reducing the disease severity [21].

Expression of adhesion molecules on peripheral immune cells has been reported to be 1.5–3 fold higher in patients with MS and is associated with enhanced pro-migratory capabilities [34]. Whilst there were no significant differences in NK cell adhesion molecules in this cohort of patients with MS, mononuclear cells infiltrating MS brain lesions have been found to have upregulated expression of adhesion molecules [34, 35]. Under inflammatory conditions, adhesion molecules have an enhanced affinity for their ligands on normal endothelial cells which can facilitate the extravasation of NK cells to inflammatory sites and into the CNS to mediate their effector functions [34, 35]. The adhesion molecule CD11b has been associated with disease severity of MS with a deficiency of CD11b linked to a delayed disease onset and the development of mild EAE symptoms [36]. Disease activity of MS has also been linked to expression of either high or low CD11c on peripheral NK cells [37]. NK cells with $CD11c^{low}$ differentiate into NK2 cells, reflecting a T helper 2 cytokine profile which favours the remission state. Conversely, $CD11c^{high}$ NK cells produce cytokines reminiscent of T helper 1 cells, which has been linked to relapses occurring earlier and more frequently, particularly in younger females [37]. Due to the importance of the adhesion molecules for NK cell circulation and extravasation into the CNS to elicit their effector function which is linked to MS disease activity, further investigations are required in a larger cohort.

$CD56^{dim}CD16^{+}$ NK cells are considered mature cells capable of inducing cytotoxic lysis of target cells through the release of lytic proteins perforin, granzyme A and granzyme B [38, 39]. Patients with CFS/ME had a significant increase in the co-expression of CD57 and perforin on $CD56^{dim}CD16^{+}$ NK cells which may contribute to the cytotoxic dysfunction reported in patients with CFS/ME. Through NK cell development from $CD56^{bright}$ NK cells to $CD56^{dim}$ NK cells, NK cells undergo a shift acquiring expression of the cytotoxic lytic proteins and CD57 [38, 40, 41]. In addition to maturation acquired expression of CD57, heightened levels of CD57 are also associated with chronic immune activation from pathogens or cytokines [42, 43]. Whilst increased CD57 is traditionally associated with higher cytolytic potential, increased expression of CD57 has also been correlated with a diminished ability of NK cells to produce IFN-γ and degranulate in response to high levels of exogenous cytokines such as IL-12 and IL-18 [41, 44]. Further investigations in a larger CFS/ME group are required to determine whether increased CD57 expression is associated with reduced NK cell cytotoxic activity.

In this study, comparison of NK cell cytotoxic effector molecules in CFS/ME and MS has suggested that NK cell differentiation as a result of exposure to external stimuli may affect different mechanisms in the NK cell cytotoxic activity pathway in these two patient cohorts. As this was a pilot study, further investigations are required to determine whether these findings are consistent in cohorts with a larger sample size. Furthermore, as specific NK cell markers have been attributed to disease activity in MS, comparisons of NK cell effector function with differing severities of CFS/ME and MS may help to further identify mechanisms which contribute to reduced NK cell activity in illnesses with varying clinical features.

Acknowledgments

National Centre for Neuroimmunology and Emerging Diseases, Alison Hunter Memorial Foundation, Mason Foundation (Grant Number MA43120) and Queensland

Government Department of Science, Information Technology, Innovation and the Arts Smart Futures Fund (Grant Number 216702MRE).

Competing Interests

The authors declare that they have no competing interests.

Authorship

TKH performed the experimental protocols for NK cell phenotypes, adhesion molecules, surface receptors, cell maturation and lytic proteins, analysed the data and wrote the manuscript. EWB designed the experiments, set up the protocols for data acquisition on the flow cytometer and drafted the manuscript. SR wrote the ethics documents, sought ethics approval, collected the blood samples from the participants and drafted the manuscript. TN recruited and scheduled all of the CFS/ME and non-fatigued participants for blood collection and drafted the manuscript. SB recruited and provided the MS patient cohort from the Gold Coast University Hospital and conceived the study in collaboration with SMG and DS. DS critically revised the intellectual content and interpretation of data analysis. SMG provided the CFS/ME and non-fatigued control cohorts from the National Centre for Neuroimmunology and Emerging Diseases database, critically revised the intellectual content and interpretation of data analysis and drafted the manuscript. All authors critically reviewed and approved this manuscript for publication.

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