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A Comparison of Immune Functionality in Viral versus Non-Viral CFS Subtypes

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Abstract

Participants with CFS were grouped into viral and non-viral onset fatigue categories and assessed for differential immunological marker expression. Peripheral Blood Mononuclear Cells were assessed for differential phenotypic expression of surface adherence glycoproteins on circulating lymphocytes. The flow cytometric analysis employed fluorescent monoclonal antibody labeling. The viral in comparison to the non-viral group demonstrated significant elevations in several Th1 type subsets including: the percentage and number of CD4+ cells, the percentage and number of CD2+CD26+ cells, the percentage and number of CD2+CD4+CD26+ cells, the percentage and number of CD4+ CD26+ cells, and the percentage of Th2 naïve cells (CD4+ CD45RA+CD62L+). Of the remaining significant findings, the non viral group demonstrated significant elevations in comparison to the viral group for the following Th1 type subsets: the percentage of CD8+ cells, the percentage of T-cytotoxic suppressor cells (CD3+8+), and the percentage and number of Th1 memory cells (CD8+CD45RA-CD62L-). The viral group demonstrated a pattern of activation that differed from that of the group with a non-viral etiology, as evidenced by an elevated and out of range percentage and number of CD4+ cells, the percentage of CD2+CD26+, and the percentage of Th2 naïve cells (CD4+CD45RA+CD62L+). Both groups demonstrated reduced and out of range Natural Killer Cell Cytotoxicity and percentage of B-1 cells (CD5+CD19). In addition, both groups demonstrated an elevated and out of range percentage of CD2+CD8+CD26+, percentage of the Th1 memory subset (CD4+CD45RA-CD62L-), the percentage of Th1 memory and naïve cells (CD8+CD45RA-CD62L-, CD8+CD45RA+CD62L-), the percentage and number of Th2 memory cells (CD4+CD45RA-CD62L+), and the percentage of Th2 memory and naïve cells (CD8+CD45RA-CD62L+, CD8+CD45RA+CD62L+). These findings imply that the homeostatic mechanism responsible for the regulation of the Th1 (cell mediated) and Th2 (humoral) immune responses is disturbed in CFS. The implications of these findings are discussed.

Keywords

Chronic Fatigue Syndrome; Immune Functionality; Viral Onset

Chronic fatigue syndrome (CFS) has been linked with exposure to several viruses and other infectious agents, with many onsets reported following primary infection with acute Mononucleosis, Lyme disease, and Q fever among others (Komaroff, 2000). Additionally, acute infections by enteroviruses may persist in patients resulting in manifestations of CFS (Chia & Chia 2008). Infection with or the reactivation of these viruses and possibly others

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(e.g., HSV-1, HHV-6, Epstein Barr virus, and Cytomegalovirus) may be involved the relapsing and remitting pathogenesis of CFS (Englebienne & De Meirleir, 2002).

Hickie, Davenport, Wakefield, Vollmer-Conna, Cameron, and Vernon et al. (2006) followed 253 patients from the point of acute infection with Coxiella burnetii (Q fever), Ross River virus (epidemic polyarthritis), and Epstein-Barr virus (glandular fever), and assessed health status at regular intervals over a 12 month period. Of these patients, the percentage who went on to develop CFS was the same for each of the three infectious diseases (11% at 6 months post infection), suggesting that the onset of CFS in this group was not associated with any one particular pathogen, but rather with host response. Vernon, Whistler, Cameron, Hickie, Reeves and Lloyd (2006) found that those who developed post-infective fatigue had gene expression profiles suggestive of an altered host response during acute mononucleosis, when compared with individuals who recovered. Genes known to be regulated in EBV infection were differentially expressed in post-infective fatigue cases.

Several studies have documented findings of decreased natural killer cell cytotoxicity and reduced perforin expression in both NK cells and cytotoxic T cells (Fletcher, Maher, & Klimas, 2002a; Fletcher, Maher, & Klimas, 2002b; Patarca-Montero, Antoni, Fletcher, & Klimas, 2001). For example, a study by Landay, Jessop, Lennette, and Levy (1991) described activation of the immune system in CFS as evidenced by reductions in natural killer cell (NK) populations, and increased expression of the HLA-DR and CD38 receptors on NK cells. Other irregularities have also been found, such as immune activation and chronic inflammation, as evidenced by B cell dysfunction, and antibody-mediated NK cell modulation of T cell activity (Ablashi et al., 2000).

Maes, Coucke, and Ategis (2007) found elevations in serum IgM and IgA which were shown to be directed against gram-negative enterobacteria endotoxins. An increased translocation of lipopolysaccaharide (LPS) of endotoxins from the gut due to increased permeability, may accompany CFS. This translocation of LPS triggers a systemic immune response characterized by chronic central neural inflammation. When LPS becomes systemic, microglia is activated and can lead to the chronic production of proinflammatory cytokines, possibly resulting in cognitive difficulties, sleep disturbances, etc. (Qin, Wu, Block, Liu, Breese, Hong, Knapp, & Crews, 2007). If left untreated, enterobacteria may have a role in the maintenance of a Th2 type autoimmune response. Enteroviruses may also play a role in the pathogenesis of CFS.

The onset of CFS may also be precipitated by non-viral incidents resulting in diffuse damage to the central nervous system, such as acute head trauma and toxic chemical exposure. Pall and Saterlee (2001) found that exposure to pesticides, chlorinated hydrocarbons and other volatile organics may lead to elevations in nitric oxide/peroxynitrite through stimulation of muscarinic acetylcholine receptors, which may play a role in the development of CFS. Therefore, a viral infection may represent one of several agents having the potential to induce symptoms of CFS. The present study investigated differences in the expression of cluster of differentiation markers between a sample of patients with diagnosed CFS who had either a viral or non viral etiology.

Method

Participants

A total of 114 participants were recruited and enrolled based on physician referral. All participants were positively identified as meeting criteria for CFS (Fukuda et al., 1994; Reeves et al. 2003) and had been diagnosed by a physician (See Jason et al., 2007 for more details of this selection process). All persons involved in the research study were at least 18

years of age, not pregnant, able to speak English, and were physically and willfully able to travel to the research center. IRB approval was obtained to conduct this study. Participants were assigned to either the viral or non-viral onset subgroups based on self reported illness phenotypes, or indication that their CFS onset occurred due to a viral illness versus another non- viral precipitant. Viral illnesses included Cytomegalovirus, infectious mononucleosis due to Epstein Barr virus or HHV-6.

All patients in this study were screened by a licensed physician. Medical examinations included a general evaluation and neurological-physical evaluation, as well as a more in-depth medical and neurological history. The evaluation also included a structured clinical instrument; a modified version of the CFS questionnaire developed by Komaroff et al. (1996). This instrument assesses the symptoms, medical history, and signs of CFS to rule out the presence of any other possible medical condition. We administered the Medical Outcomes Study Short Form-36 (SF-36), which is a 36-item, broadly-based, self report measure of functional status related to physical functioning, role physical functioning, role emotional functioning, social functioning, bodily pain, general health, vitality, and mental health. Higher scores on this scale indicate better functioning (Ware, Snow, & Kosinski, 2000). Fukuda et al. (2004) symptoms were assessed (i.e., fatigue, post exertional malaise, sleep dysfunction, pain, neurological/cognitive manifestations, autonomic manifestations, neuroendocrine manifestations, immune manifestations) on a scale of 0 to 100, where 0 = no problem and 100 = the worst problem possible.

Other medical information that was deemed relevant to the study was collected to avoid medical confounds for CFS diagnosis, including exposure to HIV/AIDS, tuberculosis, hepatitis, and other non-HIV/AIDS sexually transmitted diseases. Information on prescribed and illicit drug use was also assessed and recorded. A history of all CFS like symptoms was reported as a final measure. Laboratory tests ran on blood samples included a complete blood count with differential and platelet count, chemistry screen (which assesses thyroid, renal, and liver functioning), hepatitis B, Lyme disease screen, erythrocyte sedimentation rate, arthritic profile (which included rheumatoid factor and antinuclear antibody), HIV screen, and urinalysis. The project physician performed a detailed medical examination to detect evidence of diffuse cardiac or pulmonary dysfunction, adenopathy, synovitis, neuropathy, myopathy, or hepatosplenomegaly.

Flow Cytometric Analysis

The peripheral blood of subjects meeting criteria for inclusion was drawn prior to completion of psychological instrumentation. Drawn peripheral blood was transported in sodium heparin tubes to the University of Miami clinical immunology laboratory, and processed by Dr. Mary Ann Fletcher. All reagents and instrumentation came from Beckman Coulter Corporation, Hialeah, FL. The Center for Disease Control and Prevention's recommendations for flow cytometric analysis was adhered to in performing these analyses. For each case and control, 100μl of whole blood was lysed, fixed, and stained with optimal concentrations of fluorochrome conjugated antibodies using the Q-Prep and analyzed by four-color flow cytometry. The percent of cells positively identified as a lymphocyte subset of interest was multiplied by the lymphocyte count taken from the automated complete blood count (CBC), and used to calculate the absolute count for each of the lymphocyte subsets measured. For the following subsets, Total count of T cells (CD3+ T-cells), helper/inducer (CD3+CD4+) T-Cell, cytotoxic (CD3+CD8+) T-cell, B-cell (CD19+), and natural killer cells (CD3-CD56+), results were reported as percentages of total lymphocytes. Subsets including CD45RA and CD62L were reported as total percentages of either CD4 or CD8 cell populations. For the CD45 bright and CD45 negative population, light scatter and back gating on fluorescence were used to determine total counts of lymphocyte, granulocyte,

and monocyte cell populations. Immunoglobulin isotype controls were used as a reference measure for negative events. Spectral compensation was assessed and established daily. In order to control for quality optimization for lymphocyte recovery, lymphosum, and purity of the gate of analysis were carried out. Subsets of the helper/ inducer, effector/ suppressor, and cytotoxic lymphocytes were assessed and reported.

Natural Killer Cell Cytotoxicity (NKCC)

The bioassay for NKCC was performed using whole blood in a chromium release assay as previously described (Fletcher, et al, 2002a). The NK sensitive erythroleukemic K562 cell line was used as the target cell. The assay was done in triplicate at four target-to-effector cell ratios with 4-hour incubation. The % cytotoxicity at each target-to-effector ratio and number of CD3-CD56+ (NK) cells per unit of blood was used to express the results as % cytotoxicity at a target-to-effector cell ratio of 1:1.

Results

Socio Demographic Differences

There were no differences between the viral and non-viral groups with regard to race, gender, age, marital status, socio-economic status, or length of illness. The percentage of participants that reported an employment status of “full time” however, differed by etiology type, with significantly fewer individuals who had a viral onset (9%) being employed on a full time basis versus those who had a non-viral onset (26%) [$\chi^2(1,N=107)=5.31$, $p=.02$]. This finding suggests that individuals with a viral etiology may be more impaired than those with a non-viral etiology. The groups also differed on their reporting of the presence of individuals living within their home who have had severe fatigue, extreme tiredness, or exhaustion for a period of six months or longer [$\chi^2(1, N=107)=3.89$, $p=.049$]. Significantly more individuals in the viral group (24%) reported cohabitating with individuals having severe fatigue for six months or longer than did members of the non viral group (10%). These findings demonstrate that in the viral group, living in close proximity to others with fatiguing illnesses (possibly resultant of viral exposure) may play a role in the onset or maintenance of the illness.

Subsets found to be Activated in CFS Viral vs. Non-Viral Etiologies

An independent samples t test was employed in the comparison of the mean density of surface adherence glycoproteins present on circulating lymphocytes of participants with a viral etiology and compared against those with a non viral etiology (See Table 1). An alpha level of .05 was used as significance criterion for each of the tests performed. To determine the percent difference between the means, we took the mean of the viral group, subtracted it by the mean of the non viral group, and then divided the difference by the mean of the non viral group. As an example, for the percentage of CD4+ cells: (56.39- 50.66)/(50.66) = 11%). The viral group demonstrated significant elevations in the following Th1 type subsets relative to the non viral group: the percentage and number of CD4+ cells (11% and 20% respectively), the percentage and number of CD2+CD26+ cells (9% and 16% respectively), the percentage and number of CD2+CD4+CD26+ cells (17% and 26%), the percentage and number of CD4+ CD26+ cells (17% and 26%); and the following Th2 type subsets: CD4+CD45RA+CD62L+ cells (52%). Of the remaining significant findings, the non viral group demonstrated significant elevations in the following Th1 type subsets relative to the viral group: the percentage of CD8+ cells (-15%), the percentage of T-cytotoxic suppressor cells (CD3+8+) (-15%), and the percentage and number of CD8+ CD45RA- CD62L-cells (-18% and -28% respectively). There were no significant findings for the Th2 type subsets on which the non viral group demonstrated elevations relative to the viral group.

Elevated Patterns

The viral group demonstrated a pattern of activation that differed from that of the group with a non-viral etiology, as evidenced by an elevated and out of range (in bold within Table 1, with an arrow up meaning higher and an arrow down meaning lower) percentage and number of CD4+ cells, the percentage of CD2+CD26+, and the percentage of Th2 naïve cells (CD4+CD45RA+CD62L+). Both groups demonstrated reduced and out of range Natural Killer Cell (CD3-CD56+) Cytotoxicity (reduced in 80.7% of the sample as a whole) and percentage of B-1 cells (CD5+CD19+) (reduced in 71.1% of the sample as a whole).

In addition, both groups demonstrated an elevated and out of range percentage of CD2+CD8+CD26+ (elevated in 83.3%), percentage of the CD4 anchored Th1 memory subset (CD4+CD45RA-CD62L-)(elevated in 71.9% of the sample), , the percentage of CD8 anchored Th1 memory and naïve cells (CD8+CD45RA-CD62L-, CD8+CD45RA+CD62L-) (elevated in 93.9% and 62.3%) , the percentage and number of CD4 anchored Th2 memory cells (CD4+CD45RACD62L+)(elevated in 95.6% and 62.3%), and the percentage of CD8 anchored Th2 memory and naïve cells (CD8+CD45RA-CD62L+, CD8+CD45RA+ CD62L +) (elevated in 97.4% and 93.0%).

CD4/CD8 Ratio

The CD4/ CD8 % pos ratio of the group with a viral etiology was within normal range, but was approaching the upper limit with a value of 2.36 for the viral group as compared with a value of 1.81 for the non viral group. A significant difference between subject groups in the percentage of CD4+ cells and CD4+ cells/ μl, such that those with a viral etiology had counts that were out of normal range, and demonstrated a statistically significant increase in the number and percentage of CD4+ cells when compared to those with a non-viral etiology (11% and 20% respectively). Differences between subject groups were also observed for the percentage of CD8+ cells; however unlike the CD4+ cells, these cells fell within normal range. A predominance of CD4+ activity over that of CD8+ is suggestive of inflammation and increased immune reactivity.

SF-36 Subscale Group Differences

Significant differences between the viral and non viral groups were also observed for scores on the Physical Functioning [$t(105)= 2.09$, $p=.039$] and Social Functioning [$t(104)= 2.18$, $p=.031$] subscales of the SF-36 (Short Form 36), such that the Viral group demonstrated more impairment on both the physical functioning and social functioning measures than did the non viral group (See Table 2).

Discussion

The purpose of this study was to determine if there was phenotypic evidence of prior viral infection in individuals with CFS. Between group comparisons demonstrated in the viral onset group a pattern of increased cell marker expression for CD4 positive cells, and increased percentages of CD2+CD26+cells, and Th2 naïve cells (CD4+CD45RA+CD62L+). These findings are suggestive of increased immune surveillance. In healthy individuals, the CD4 anchored Th2 naïve subset is expected to steadily decline as part of immunosenescence. These age-related reductions in the naïve subset are believed to be advantageous in that their decline prevents self reactivity (Dowling & Hodgkin, 2009; Aronson, 1991), and to occur concomitant with increases in memory T cell lineages. However, in this sample the viral group demonstrated elevations in this and the CD4+ and CD2+CD26+ subsets, which is suggests a ongoing process of systemic inflammation.

Proliferation of further CD4+ T cells is typically stimulated by IL-12 secretion by macrophages. In response, CD4+ cells secrete IFN γ and IL-2, which leads to the release of additional Type 1 cytokines. This contributes to the development of an inflammatory immune response. Increased proliferation of CD4+ cells is suggestive of immune reactivity and inflammation, and has been found in association with delayed type or Type IV hypersensitivity (Hilgers & Frank,1996), which appears to be one of several incitants associated with the onset of CFS (Maes et al., 2005). Klimas et al. (1990) and Fletcher et al. (2009) reported elevations in the CD2+ subset containing the dipeptidyl peptidase IV isoenzyme CD26+ activation marker in patients with CFS. In the present study, we found elevated counts of the CD2+CD26+ subset in the group with a viral etiology, and normal counts in the non viral group, indicating an activation of the lymphocyte compartment. Abnormal expression of this marker has also been associated with the development of cancer and other autoimmune diseases.

The viral in comparison to the non-viral group demonstrated significant elevations in several Th1 type subsets including: the percentage and number of CD4+ cells, the percentage and number of CD2+CD26+ cells, the percentage and number of CD2+CD4+CD26+ cells, the percentage and number of CD4+ CD26+ cells; and the percentage of Th2 naïve cells (CD4+ CD45RA+CD62L+). Of the remaining significant findings, the non viral group demonstrated significant elevations in comparison to the viral group for the following Th1 type subsets: the percentage of CD8+ cells, the percentage of TCytotoxic suppressor cells (CD3+8+), and the percentage and number of Th1 memory cells (CD8+CD45RA-CD62L-). These findings may be suggestive of a differential pattern of activation in the non-viral group. The CD8 anchored Th1 memory subset is up regulated and out of normal range in both groups, however there is also a significant difference between groups where the non viral group has a mean that is elevated relative to the viral group, which when presented with the additional finding of elevations in this and the CD8+ and CD3+8+ subsets , is indicative of a stronger pathogenic response in the non viral group, and conversely, the possibility of a tendency toward compromised responsivity in the viral group. This indicates a strong pro-inflammatory response in those individuals with non-viral onset, as compared to a more Th2 response set in those with viral onset. These findings may account for some of the divergent literature on immune function in those with CFS, with differential immunologic responses dependent on exposure to a viral epitope.

Both groups demonstrated reductions in Natural Killer Cell (CD3- 56+) cytotoxicity, and reductions in the percentage of B-1 cells (CD5+ CD19+), such that performance on each of these parameters was not within normal range and down regulated. Also of interest, both groups demonstrated a nearly identical pattern of activation as evidenced by elevations in the proportion of the following subsets, such that the counts were not within normal range and up regulated: the percentage of CD2+CD8+CD26+, percentage of the Th1 memory subset (CD4+CD45RA-CD62L-), the percentage of Th1 memory and naïve cells (CD8+CD45RACD62L-, CD8+CD45RA+CD62L-), the percentage and number of Th2 memory cells (CD4+CD45RACD62L+), and the percentage of Th2 memory and naïve cells (CD8+CD45RA-CD62L+, CD8+CD45RA+CD62L+). The elevation in the proportion of these subsets may result both age related decline in immune system (Saule et al., 2006) as evidenced by the elevated proportion of Th1 and Th2 memory cell subsets (CD4+CD45RA-CD62L-, CD4+CD45RA-CD62L+, CD8+CD45RA-CD62L-, CD8+CD45RA-CD62L+) , and increased immunosurveillance as evidenced by elevated proportions of CD8 anchored Th1 and Th2 naïve cells (CD8+CD45RA+CD62L- and CD8+CD45RA+CD62L+).

Reduced Natural Killer cell cytotoxicity and age related reduction in perforin expression is a common feature of aging and immunosenescence, (Mocchegiani & Malavolta, 2004; Rukavina, et al., 1998). Reduced cytotoxicity is expected to adversely affect the efficacy

with which the innate and adaptive immune systems respond to viruses and other pathogenic agents, as NKCs are known mediators of both of these systems (Taniguchi et al., 2003). In patients with CFS, there may be an increased susceptibility to infection by bacterial polysaccharides which contain carbohydrate antigens, as evidenced by down regulated B-1 cells with phenotype CD5+ CD19+ in both groups.

In this sample, we found that immune activation was a characteristic common to both groups as evidenced by hyperplasia, or an increased proliferation of several lymphocyte subsets. This activation was more pronounced in the group with a viral etiology, which additionally demonstrated elevations in the proportion of CD4+, CD2+CD26+, and Th2 naïve (CD4+CD45RA+CD62L+) cell subsets. The present findings support the premise that reductions in the efficacy with which Natural Killer cells are able to eliminate target cells, concomitant with elevations in activated T-cell subsets (Table 1), may contribute to the maintenance of inflammation and immune activation (Maher, Klimas, & Fletcher, 2005). Reductions in the CD5+CD19+ subset in both groups is also suggestive of compromised integrity of the stomach/ intestinal tract and increased susceptibility to bacterial infection, which may contribute to the maintenance of symptoms.

Overall, our findings are compatible with data indicating that latent viral infection is associated with various subsets of memory T cells (Guerreiro et al., 2010). The decrease in naïve T cells is consistent with immunosenescent changes in which the repertoire of T cells is believed to be dominated by virus specific T cell lineages. Virally initiated effectors may also be able to escape apoptotic mechanisms and eventually differentiate into memory cells (Lohning et al., 2008). It is then apparent that viral exposure could interact with genetic predisposing characteristics to elicit a course of chronic inflammation in association with suppression of select cell subsets in individuals with CFS. This then argues for a differential course in the development of CFS, with viral exposure contributing to disease development in a subset of individuals. These findings demonstrate the need for continued study of viral exposure in individuals with CFS; for while these pathways may not include all cases, it is apparent that for a significant subset a viral onset is apparent.

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Table 1

Significant Differences Between Subject Groups on Th1 and Th2 Type Lymphocyte Subsets

Lymphocyte Marker	Non-viral (*n*=62) M (SD)	Viral (*n*=46) M (SD)	*p*	*d*
T Cells (CD3+) % pos	77.46 (7.79)	79.15 (6.36)		
T Cells (CD3+) / μL	1606.27 (464.48)	1749.28 (618.45)		
Natural Killer Cells (CD3-56+) % pos	6.75 (4.34)	6.14 (3.58)		
Natural Killer Cells(CD3-56+) cells/μL	135.24 (90.92)	133.00 (90.96)		
Natural Killer Cell Cytotoxicity	**9.18 (5.90)** ↓	**9.91 (6.28)** ↓		
T-Helper (CD4+)% pos	50.66 (7.85)	**56.39 (9.34)** ↑	.001	-0.666
T-Helper (CD4+)cells/μL	1034.27 (361.72)	**1243.67 (459.79)** ↑	.012	-0.510
T-Cytotoxic (CD3+8+)% pos	24.51 (6.41)	20.75 (7.23)	.005	0.551
T-Cytotoxic (CD3+8+) cells/μL	509.34 (196.91)	465.83 (263.38)		
CD5+ % pos	75.00 (8.44)	77.48 (7.08)		
CD5+ cells/μL	1663.06 (890.47)	1714.60 (613.71)		
CD5+CD19+ % pos	**.79 (.57)** ↓	**.84 (1.15)** ↓		
CD5+ CD19+ cells/ μL	16.47 (12.56)	17.98 (28.72)		
CD2+ %pos	82.35 (6.98)	83.90 (5.51)		
CD2+ cells/μL	1702.24 (465.87)	1852.37 (644.16)		
CD2+ CD26+ % pos	55.36 (12.15)	**60.18 (8.68)** ↑	.024	-0.463
CD2+ CD26+cells/ μL	1140.24 (372.95)	1326.43 (469.89)	.029	-0.442
CD2+CD4+CD26 + %pos	41.10 (9.67)	48.08 (9.40)	.000	-0.731
CD2+CD4+CD26+ cells/ μL	846.90 (284.64)	1068.07 (423.02)	.003	-0.625
CD4+ CD26+ % pos	41.24 (9.69)	48.19 (9.34)	.000	-0.731
CD4+ CD26+ cells/μL	849.68 (285.38)	1070.37 (422.87)	.003	-0.623
CD2+CD8+CD26+ %pos	**12.07 (4.73)** ↑	**10.49 (4.64)** ↑		
CD2+CD8+CD26+ cells/μL	249.89 (118.40)	225.17 (108.77)		
CD8+ %pos	27.93 (6.42)	23.81 (7.39)	.003	0.595
CD8+ cells/μL	577.53 (205.08)	532.80 (290.12)		
CD4+CD45RA+ CD62L- % pos	.59 (.90)	.63 (1.09)		
CD4+CD45RA+ CD62L- cells/μL	5.97 (8.79)	7.46 (11.18)		
CD4+CD45RA- CD62L- % pos	**15.80 (6.59)** ↑	**14.08 (5.92)** ↑		
CD4+CD45RA- CD62L- cells/μL	162.71(86.40)	169.04 (85.52)		
CD8+ CD45RA- CD62L- % pos	**24.79 (10.06)** ↑	**20.37 (8.25)** ↑	.016	0.482
CD8+ CD45RA-CD62L- cells/ μL	150.76 (122.22)	108.65 (70.62)	.039	0.422
CD8+ CD45RA+CD62L- % pos	**16.68 (9.23)** ↑	**18.65 (11.67)** ↑		
CD8+ CD45RA+CD62L-cells/μL	150.76 (122.22)	108.65 (70.62)		
CD4/CD8 Ratio	1.81	2.36		
B cells (CD19+) % pos	14.53 (6.40)	13.50 (5.34)		
B cells (CD19+) cells/μL	313.81 (191.83)	284.22 (114.40)		
CD4+CD45RA + CD62L+ % pos	24.19 (14.82)	**29.71 (17.40)** ↑		
CD4+CD45RA + CD62L+ cells/μL	253.46 (184.24)	386.00 (321.53)	.015	-0.524
CD4+CD45RA- CD62L+ % pos	**59.42 (13.18)** ↑	**55.58 (16.80)** ↑		

Lymphocyte Marker	**Non-viral (*n*=62) M (SD)**	**Viral (*n*=46) M (SD)**	***p***	***d***
CD4+CD45RA- CD62L+ cells/μL	**628.12 (273.97)** ↑	**681.04 (304.60)** ↑		
CD8+CD45RA-CD62L+ % pos	**21.17 (8.80)** ↑	**20.25 (11.66)** ↑		
CD8+CD45RA-CD62L+cells/μL	118.48 (56.36)	106.38 (85.10)		
CD8+CD45RA+CD62L+ % pos	**37.34 (15.07)** ↑	**40.73 (14.87)** ↑		
CD8+ CD45RA+CD62L+ cells/μL	212.58 (120.07)	211.86 (140.20)		

Table 2

SF-36 Subscale Scores and Symptom Differences for Viral versus Non-Viral Etiologies

SF-36 Scale	Non-Viral (*n*=62) M (SD)	Viral (*n*=46) M (SD)	*p*
Physical Functioning	49.43 (23.72)	39.93 (22.71)	.039
Bodily Pain	41.31 (22.42)	35.24 (22.13)	
General Health Functioning	33.17 (16.84)	28.41 (16.63)	
Vitality	18.52 (14.03)	16.56 (15.33)	
Social Functioning	44.67 (24.95)	34.17 (22.68)	.031
Role Emotional	52.22 (41.79)	50.37 (44.73)	
Mental Health Functioning	63.21 (17.17)	62.58 (17.97)	