CD8 T cell exhaustion, increased CD4+CD8+ T-cells and aberrant cytokines in myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS)

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ABSTRACT

Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex disorder affecting numerous organ systems and biological processes. Published data seems to suggest that ME/CFS may be preceded by infection, and the chronic manifestation of illness may represent an altered host response to infection, or an inability to resolve inflammation. Previous studies focused on perturbation in cytokines and metabolism have shown that CD8+ T responses are decreased in ME/CFS. Here, we hypothesize that in ME/CFS an aberrant response to an immunological trigger like infection may result in a permanently dysregulated immune system, leading to a state of immunosuppression. We examined the frequency, functional and phenotypic status of CD8+ and CD4+CD8+ T-cells to determine whether their frequency and cytokine production was altered in chronic ME/CFS patients (ME/CFS) as compared to healthy donors (HDs). We examined the T-cell receptor (TCR) repertoire of the CD4+CD8+ population looking for evidence consistent with an antigen driven response whether it will be a viral or auto-antigen. We observed altered expression of exhaustion markers like CTLA4 and 2B4, decrease in CD8 T-cell number, and function, particularly CD107ab and IFNg production. This was associated with a compensatory increased frequency of activated CD4+CD8+ T cells in ME/CFS patients as compared to healthy controls. Both the CD8 and CD4+CD8+ T cell populations were spontaneously producing aberrant cytokines, subdividing into two types of ME/CFS: (1) FoxP3+ cells producing IL9 (female donors), (2) IL17-producing cells (male donors). TCR analyses suggested an antigen-driven response. These results are consistent with immunosuppression mediated via exhaustion of CD8 T-cells as observed either in chronic viral infections or tumor environments. The observed exhaustion was associated with a compensatory increase in activated CD4+CD8+ that make unusual cytokines known to interact with the nervous system. These findings identify potential biomarkers and mechanisms driving the immunopathogenesis of ME/CFS leading to future therapies (Funding: Ramsay Award, Solve ME/CFS Initiative).

RESULTS

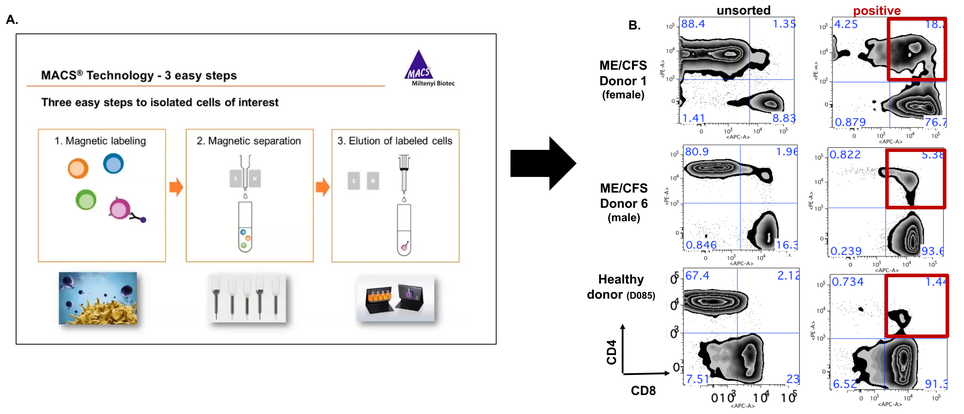


Figure 1. Magnetic separation allows for enrichment of distinct subset of CD8+T cells, CD4+CD8+, isolated from human PBMC. (A) Diagram demonstrates the workflow of isolation of CD8+T cells (MACS Miltenyi Biotec). (B) Representative FACS plots of two cell populations: unsorted PBMC and magnetic separated CD8+T cells (cells were gated on CD3+ cells). Increased frequency of subset of T cells which co-express CD4+ and CD8+ markers in CD8+T cells sorted on magnet (positive population) as compared to its frequency in unsorted PBMC.

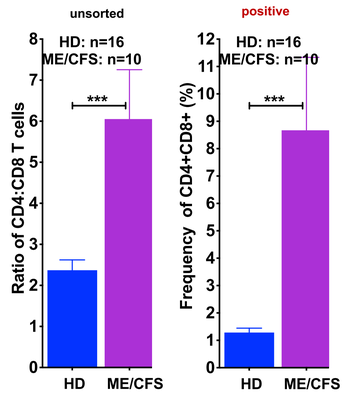


Figure 2. Increased CD4:CD8 ratio in unsorted PBMC and increased frequency of distinct subset of T cells which co-express CD4+ and CD8+ molecules in sorted CD8 T cell fraction of ME/CFS donors PBMC compared to healthy donors (HD).

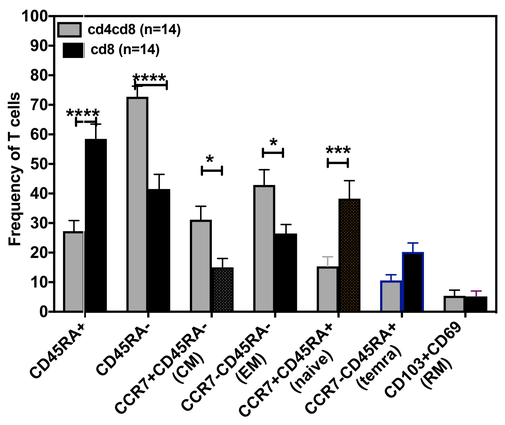


Figure 3. CD4+CD8+ T cells from both ME/CFS and HD are predominantly antigen-experienced cells expressing phenotypic markers of either central or effector memory phenotype. Multivariant ANOVA with adjusted *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. (ME/CFS and HD data was combined as they did not differ in this finding.) TEMRA (terminally differentiated T cells; RM

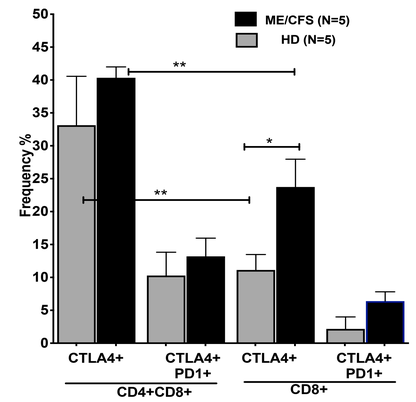


Figure 4. CD4+CD8+ T cells of both ME/CFS and HD express increased levels of the T cell inhibitory molecule CTLA4 as compared to CD8 T ceiis. ME/CFS CD8 T cell express more CTLA4 than HD. Multivariant ANOVA with adjusted *p<0.05, **p<0.01.

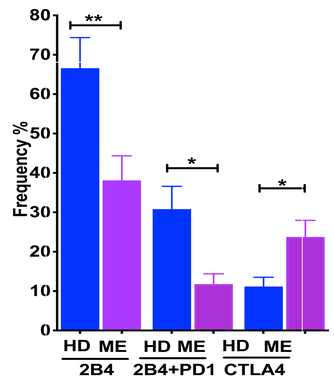


Figure 5. Altered expression of exhaustion/activation markers on ME/CFS patient CD8 T cells. The frequency of CD8 T cells expressing 2B4 or co-expressing 2B4 and PD1 is decreased, but expression of CTLA4 is increased in ME/CFS donors (n=6) compared to HD (n=5). ME is ME/CFS donor. HD is healthy donor. Multivariant ANOVA with adjusted *p<0.05, **p<0.01.

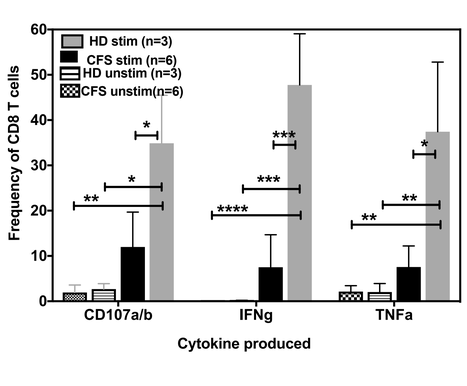


Figure 6. Functional exhaustion of CD8 +T cells in ME/CFS donors compared to HD. Intracellular cytokine assay (ICS) shows decreased ability of CD8 T cells of ME/CFS donors to produce IFNg, TNF or express CD107a/b following stimulation with PMA. Multivariant ANOVA with adjusted *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

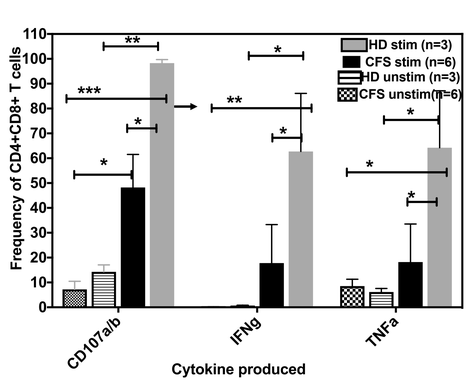


Figure 7. Functional exhaustion of CD4+CD8+ T cells in ME/CFS donors compared to HD. Intracellular cytokine assay (ICS) shows decreased ability of CD4+CD8+ T cells of ME/CFS donors to produce IFNg, or TNF or express CD107a/b following stimulation with PMA/ionomycin. Multivariant ANOVA with adjusted *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

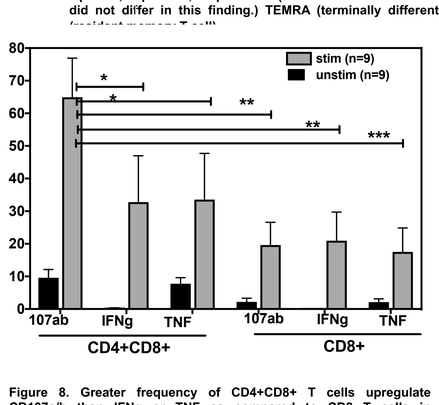
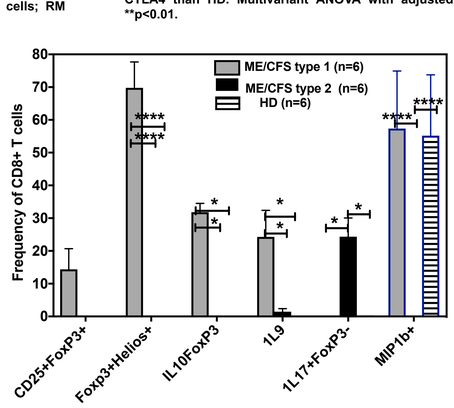


Figure 8. Greater frequency of CD4+CD8+ T cells upregulate CD107a/b than IFNg or TNF as compared to CD8 T cells in Intracellular cytokine assay (ICS) following stimulation with PMA/ionomycin. This suggests CD4+CD8+ T cells may be more cytotoxic even than CD8 T cells. Multivariant ANOVA with adjusted *p<0.05, **p<0.01, ***p<0.001. (ME/CFS and HD donor combined as results did not differ in this finding.)



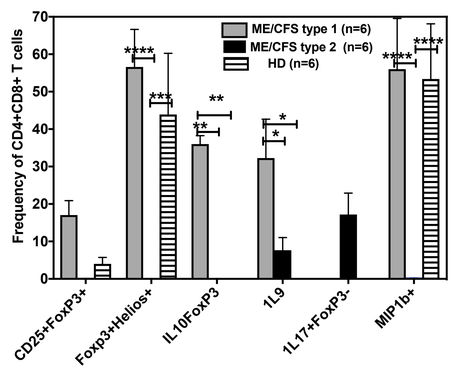


Figure 9. increased frequency of (A) CD8 and (B) CD4+CD8+ T cells spontaneously producing aberrant cytokines in ICS of ME/CFS donors compared to HD without any PMA stimulation. This suggests that these cells have already been activated in vivo. Type 1 ME/CFS donors (all female) had greater frequency of Treg-like CD4+CD8+ and CD8 T cells (express Foxp3 and Helios) with spontaneous production of IL-10, IL-9 and MIP1b. Type 2 ME/CFS donors (all male) have fewer Treg-like cells and increased spontaneous production of IL-17. Multivariant ANOVA with adjusted *p<0.05, **p<0.01, ***p<0.001.

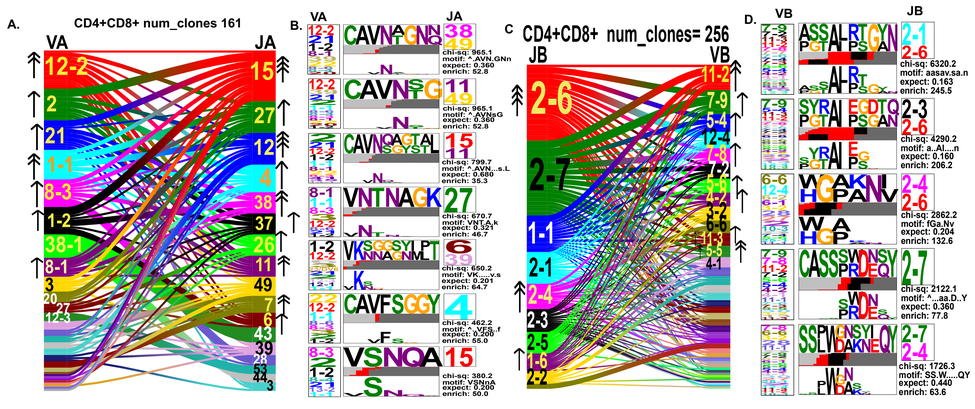
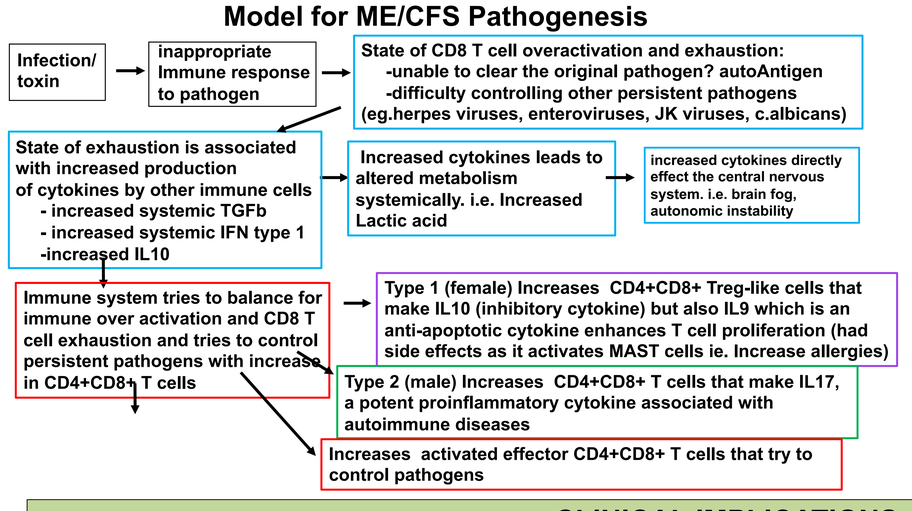


Figure 10. TCRVA and TCRVB repertoire of sorted CD4+CD8+ T-cells of ME/CFS donor 1 by deep sequencing is polyclonal but shows unique features consistent with antigen-driven expansion. Ribbon plots show patterns of TCR V-J pairings in sorted CD4+CD8+ T-cells in TCRVA (A) and TCR VB (C) directly ex vivo as revealed TCR deep sequencing. Preference of gene segment usage and gene-gene pairing are illustrated using four vertical stacks (one for each V and J segment) connected by curved paths whose thickness is proportional to the number of TCR clones with the respective gene pairing. Genes are colored by frequency within the repertoire with red>green>blue>cyan>magenta>black. The arrows indicate significantly increased usage of those V or J regions compared to naïve random repertoire consistent with antigen-driven expansion. The presence of unique clearly defined CDR3 motifs of TCRVA (B) and BV (D) suggest antigen-driven expansion. The upper CDR3 motif 1 shows the aminos acids which are enriched in comparison to the total CD4+CD8+ population; the lower CDR3 motif 2 shows the amino acids which are enriched compared to a naïve random CD8 T-cell repertoire. Both indicate that these amino acids are important for peptide/MHC contact. Naive repertoire does not generate motifs as it requires clonal expansions. (Analysis Method from Dash et al. Nature. 2017; Our experience with use of method_Kamga et al Plos Pathogens 2019) (Done by Dr. Ghersi)

Model for ME/CFS Pathogenesis



SUMMARY

1. Increased frequency of CD4+CD8+ T cells and low CD8 T cell frequency in ME/CFS donors as compared to healthy controls (potential biomarker).
   The CD4+CD8+ cells are producing cytokines without stimulation and express high levels of CTLA4. (What is driving their activation?)
2. Evidenced of exhausted CD8 T cells in ME/CFS donors:
   - Decreased production of cytokines (IFNg, MIP1b and TNFa) after *ex vivo* stimulation in ME/CFS donors (potential biomarker).
   - Decreased expression of 2B4 on CD8 T cells
   - Increased expression of CTLA4
   - Is there a persistent antigen (virus or autoantigen?)
3. TCRαβ repertoire analysis of CD4+CD8+ T cells demonstrated highly polyclonal response with features indicating an antigen-driven response (persistent virus or autoantigen?)
4. Dysregulated unusual cytokine responses in CD4+CD8+ and subset of CD8 T cells of ME/CFS donors without any stimulation: IL-9 in females or IL-17 in males).

CLINICAL IMPLICATIONS

- **Potential biomarkers:** low CD8, altered CD4:CD8 ratio, high CD4+CD8+ frequency, CD8 functional studies for exhaustion
- **Therapy:**
  - Check point inhibitors (anti-PD1, anti-CTLA4) are being used to reverse CD8 T cell exhaustion in tumor therapy and chronic viral infections.
  - Anti-cytokine therapies such as anti-IL17 is being developed for other autoimmune conditions like inflammatory bowel disease
  - Due to CD8 T cell exhaustion do ME/CFS patients have difficulty controlling their commensal bacteria, funguses (Candida albicans) and viruses such as EBV, HHV6, CMV?
    -would antivirals help, anti-fungal, microbiome therapy help, hyperbaric oxygen
- **Understanding the pathogenesis of ME/CFS**
  - IL9 and IL17 have receptors in the CNS (may contribute to CNS disease)
  - IL9 is a potent mast cell inducer (may contribute to the allergies and mastocytosis in ME/CFS)
  - CD8 T cell exhaustion is known to be associated with increased systemic levels of IFNa/b and TGFb . These cytokine abnormalities associated with CD8 T cell exhaustion lead to the types of metabolic dysregulation observed in ME/CFS.
  - Potentially use TCR sequencing to identify the major antigens whether viral or auto-antigen that are driving or contributing to this abberant immune activation in ME/CFS