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 suggests 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-cell responses are decreased in ME/CFS. Here, we hypothesize that in ME/CFS an aberrant response to an immunological trigger, 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 be a virus or auto-antigen. We observed altered expression of exhaustion markers like CTLA4, and a decrease in CD8 T-cell number, and function, particularly CD107a and IFNγ production. The shift will be associated with a compensated 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), and (2) IL7-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 therapeutics (Funding: Ramsay Award, Solve ME/CFS Initiative).

RESULTS

1. Enrichment analysis of exhausted/infiltration markers on ME/CFS patient CD8 T cells. The frequency of CD8 T cells expressing in ME/CFS donors compared to healthy donors, and the change in frequency of expression of CD8 T cells in CD4+CD8+ donor subset. CD8 T cells showed increased frequency of CD8 T cells (vs HD) and of CD4+CD8+ cells in ME/CFS donors in HD. Multivariate ANOVA with adjusted p-values in HD.

2. Invariant skewing of cytokine responses in CD4+CD8+ T-cells of ME/CFS donors by deep sequencing in polyclonal but shows unique features consistent with antigen-driven expansion. Ribbon plots show patterns of TCR Vβジャンル in sorted CD4+CD8+ T-cells in TCRVJ (A) and TCR VB (B) directly ex vivo, as revealed TCR deep sequencing. Polyclonal of gene segments utilize and gene-gene sharing, and illustrate four major vectors (B1, B2, B3, and C) of antigen-driven response. Multiple class HLA alleles, and antigen-driven response of TCRVJ segments (B1, B2, B3, and C) driven by antigen-driven expansion. The upper left corner highlights the TCRVJ segments with the most skewed patterns among the different TCRVB segments. The frequency of TCRVJ segments was found to be significantly increased in ME/CFS donors compared to healthy donors by multivariate analysis with adjusted p-values for HD.

3. Functional exhaustion of CD8+ T cells in ME/CFS donors compared to HD, intracellular cytokine assay (ICA) shows decreased frequency of CD8 T cells of ME/CFS donors compared to healthy donors. Multivariate ANOVA with adjusted p-values for HD.

4. Increased CD4+CD8+ T cells that make IL17, a potent proinflammatory cytokine associated with autoimmune diseases.

Model for ME/CFS Pathogenesis

CLINICAL IMPLICATIONS

- **Potential biomarkers:** low CD8, altered CD4+CD8 ratio, high CD4+CD8+ frequency. CD8 functional studies for exhaustion
- **Check point inhibitors** (anti-PD-1, 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, HHV, 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 IFNα and TGFβ. 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 aberrant immune activation in ME/CFS**