

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis and the Potential Role of T Cells

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Abstract

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a multifactorial disorder defined by symptom-specific criteria and characterised by severe and prolonged fatigue. CFS/ME typically affects a variety of bodily systems, including the immune system. Patients with CFS/ME exhibit significantly reduced Natural Killer (NK) cell activity suggesting immune which may be hallmarks of changes in the adaptive immune system, potentially including T cell subsets and function. The principal purpose of T cells is to regulate immune responses and maintain immune homeostasis. These regulatory measures can often be compromised during illness and may present in a number of diseases including CFS/ME. This review paper examines the role of T cells in CFS/ME and the potential impact of T cells on CFS/ME immune profiles with an evaluation of the current literature.

Keywords: Chronic Fatigue Syndrome, Fatigue, T cells, T lymphocyte, Cytokines

Introduction

The purpose of T cells is to regulate the immune responses of both innate and adaptive immune cells by maintaining immunological homeostasis, which may often be compromised during illness. Some immunological disorders have also been associated with deficiencies or dysfunction in subtypes of T cells, such as regulatory T cells (Tregs) (1, 2). Dysfunction in T cells and their pro- or anti-inflammatory cytokines can reduce the ability of these cells to maintain cytokine homeostasis, promote autoimmunity or respond to pathogens (3-7). Imbalances in Th1/Th2/Th17 cytokine profiles have been related to autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis (6, 8-12).

Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a serious illness with consistent immune perturbations (13-20). Patients diagnosed with CFS/ME primarily experience persistent fatigue, physically and mentally, for a period of at least 6 months. Other symptoms include headaches, dizziness, muscle pain, pallor, abdominal pain, nausea and swollen lymph nodes (21, 22). CFS/ME patients may in some cases present with altered susceptibility for infections, indicative of chronic low-grade inflammation and potential dysregulation in T cells (23). Currently, many T cell studies in CFS have inconsistent results and it remains to be determined if these cells have a possible role in the pathology of CFS/ME patients (13-20), hence this review aims to examine T cells in CFS/ME.

T Cells

T cells are lymphocytes of the adaptive immune system that play an important role in cell-mediated immunity as they respond to antigens released during inflammation or tumour invasion after being recruited by soluble proteins presented by dendritic cells (DCs), macrophages and neutrophils (24). T cell subsets can be identified based on the expression of surface markers and specific cytokine secretion (25-28).

All T cells originate from the bone marrow and populate the thymus as hematopoietic progenitor cells (HPCs) which differentiate into immature thymocytes (27, 29). Thymic lymphoid progenitors can develop into either $\alpha\beta$ or $\gamma\delta$ T cells as a result of TCR chain rearrangement, with the majority of heterodimers forming the $\alpha\beta$ T cell lineage (~98%) (30). $\alpha\beta$ T cells then develop into $CD3^+CD4^+$ or $CD3^+CD8^+$ T cells which selectively recognise and bind to molecules MHC class II and I respectively or NKT cells (7, 31, 32).

CD4⁺ T Cells

CD4⁺ T cells coordinate the activity of both innate and adaptive immune systems (25). Naive CD4⁺ effector T cells differentiate into distinct lineages following activation by NK cells and DCs and differentiate into Tregs, Th1, Th2 and Th17 subsets (25).

Th1 effector CD4⁺ T cells are responsible for cell-mediated immunity and identified predominantly based on their production of pro-inflammatory cytokines, IFN- γ , LT- α /TNF- β and IL-2 (3, 5, 7, 12). IFN- γ stimulates macrophages to phagocytose pathogens (33-36) and IL-2 importantly regulates and induces the differentiation and proliferation of T cells, memory T cells and NK cells (33-36).

Th17 cells also secrete pro-inflammatory cytokines, IL-17A, IL-17F, IL-21, IL-22, IL-26 and TNF- α (3, 7, 37) and enhance host protection against extracellular bacteria, fungi and microbes as well as improving the clearance of intracellular pathogens (5). Th17 cells also secrete chemokines CCL2, CCL3 and CCL20 to allow for the migration of monocytes, T cells and neutrophils towards necessary sites for inflammatory responses (3, 7, 37). IL-17 from Th17 cells is involved in the development of immune-related diseases, such as Autoimmune Arthritis and Multiple Sclerosis, and is also amplified in patients with asthma and rheumatoid arthritis (34, 36). Regulation of pro- and anti-inflammatory cytokines is important for immune-related responses and cytokine shift either towards a Th1/Th17 or Th2 cytokine profile may underlie certain disorders, including CFS/ME (4, 9, 35).

Th2 cells are responsible for extracellular pathogen immunity, producing anti-inflammatory cytokines, including IL-4, IL-5, IL-9, IL-10, IL-13 and IL-25 (3, 7, 27). IL-5 and IL-9 are important in immune response to allergic reactions while IL-4 and IL-10 regulate inflammatory responses (7). Shifts towards a Th2 mediated immune response may encourage chronic inflammation, as observed in disorders such as Multiple Sclerosis, Rheumatoid Arthritis and Gulf War Illness (6, 9-11, 13, 18).

T Regulatory Cells

Tregs are a subset of CD4⁺ T cells, distinguished by their functional ability to suppress immune responses and prevent autoimmunity (27, 38, 39). There are two main CD4⁺ Tregs, iTregs which develop from naïve CD4⁺ T cells in peripheral lymphoid tissues and intrathymic nTregs (27, 39). Foxp3 is an important transcription factor in iTregs and nTregs, which regulate pro-inflammatory factors by suppressing both IL-2 and IFN- γ (37, 40). IL-4, IL-10 and TGF- β induce the generation of iTreg cells from naïve CD4⁺ T cells (26). The iTreg subset of T cells includes further subsets such as type 1 Tregs (Tr1) and Th3 cells which variably express Foxp3 (26, 28). iTregs mediate inhibitory function by producing suppressive anti-inflammatory cytokines, IL-5, IL-10, TGF- β and IFN- γ

(predominantly IL-10 and TGF- β) (26, 38). Impairments in Treg development and function, including diminished Foxp3 expression, can also be related to autoimmune diseases (41).

CD8⁺ T Cells

CD8⁺ T cells are functionally important for both innate and adaptive immunity (42). CD8⁺ T cells protect the body from foreign or invading microorganisms by recognising diverse antigens presented by MHC I peptides. This stimulates CD8⁺ T cell proliferation, cytokine (IFN- γ and TNF) and chemokine (IL-8) secretion and lysis of infected cells (42, 43). CD8⁺ T cells also produce lytic proteins (such as granzyme B and perforin) (43).

Cytotoxic CD8⁺ T cells express high quantities of granzymes, perforin, cytokines and chemokines (42-45). The cytotoxic pathways of CD8⁺ T cells allow defence against virus-infected or transformed cells through MHC I recognition (46, 47). Perforin and granzymes exocytose from CD8⁺ T cells to induce apoptosis of target cells (46). Perforin is a membrane-disrupting protein, secreted during an immune response, which enters the membrane of a target cell, allowing granzymes to enter. Once inside, granzymes cleave caspases and degrade the DNA of target cells, promoting an apoptotic cascade (46). Aside from perforin and granzyme secretion, target-cell death receptors, such as Fas (CD95) can also induce caspase-dependent apoptosis in target cells (46). The role of CD8⁺ T cells and their maintenance of inflammation may be associated with autoimmune disease (47, 48), incidentally, increases in CD8⁺ T cells, perforin and granzyme B, may be related to diseases such as Lupus (48).

T Cells in CFS/ME

CFS/ME is a diverse multisystem illness with varied symptom severity that can substantially affect a person's way of life (21). There are substantial costs associated with CFS/ME worldwide and there is no known cure, successful treatments and or useful diagnostic method. Most patients with CFS/ME are incapable of maintaining full-time occupations while the more severe cases require constant daily assistance (49).

The estimated prevalence rate of CFS/ME is 0.2-0.7% (49) with women being more greatly affected by CFS/ME than men by up to 80% (16, 50). Of those diagnosed with CFS/ME, approximately 83% report gradual onset of the disorder while 17% experience sudden onset, highlighting potential subgrouping based on the onset of CFS/ME (50). Immune investigations in CFS/ME have identified variations in immune cell numbers, significantly reduced lymphocyte cytotoxic activity, decreased neutrophil respiratory burst, fluctuations in cytokine distribution with particular shifts in Th1/Th2 related cytokines and altered expression of immune related genes (13-20, 51, 52).

Many studies have examined T cells in CFS/ME, particularly overall T cell numbers, CD4⁺ and CD8⁺ T cells, with inconsistent results. Some studies found decreases in the number of CD4⁺ T cells (18, 53), while others concluded that there were increases [55]. Similar variations in results have been discovered regarding the CD8⁺ subset of T cells where decreases in the number of CD8⁺ T cells were found in some studies (53-55) while another found increases in CD8⁺ T cell subsets (56).

Only one study has examined cytokine production in isolated CD4⁺ T cells in CFS/ME patients and it was found that IFN- γ was significantly reduced in CFS/ME patients (4). In CFS/ME patients, variable levels of Th1 cytokines and IFN- γ may potentially explain the constant infections and increased inflammation experienced by CFS/ME patients (18, 35). The potential increase in secretion or presence of IFN- γ specifically may lead to autoimmune related immune responses (4, 10, 18, 21, 35).

Isolated CD4⁺ T cells and subsets may provide definitive results thereby reducing the interference from other potential producers of cytokines. Most CFS/ME cytokine studies did not use this technique hence the cytokine data is representative of the whole PBMC population and a number of studies on these cytokines have been measured in CFS/ME patients (IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, TNF- α , TNF- β , IFN- γ and TGF- β), producing inconsistent results (4, 16, 57, 58). These studies have highlighted potential dysregulation in the CFS/ME cytokine profile by demonstrating significant shifts in cytokines in CFS/ME. Although majority of these studies measured cytokines in PBMCs, irrespective of this, cytokine production in CFS/ME may be indicative of the cytokine profile of T cells in CFS/ME and potential shifts between Th1/Th2/Th17 regulations.

IL-2 levels have been inconsistent in CFS/ME participants. IL-2 plays an important role in the maintenance of natural immunological self-tolerance with impairments in IL-2 leading autoimmune gastritis, early onset diabetes and T-cell mediated autoimmune diseases such as thyroiditis and severe neuropathy (16, 59). IL-2 also contributes to the induction of NK cytotoxic activity (60, 61), therefore alterations in pro-inflammatory IL-2 levels may potentially correlate with consistently significant reduced NK numbers and activity in CFS/ME (15, 16, 23, 48, 50-52).

The levels of cytokines TGF- β and IL-6 are sometimes raised in patients with CFS/ME (40, 52, 62). Elevated levels of TGF- β and IL-6 in CFS/ME patients promote the production of Th17 cells by inducing STAT3, necessary for Th17 cell differentiation (63). Th17 cells produce IL-17 which contributes to disease pathogenesis by acting as a potent pro-inflammatory mediator and is inconsistent in CFS/ME (64). IL-17 enhances autoimmune inflammation by acting on APCs to signal IL-1, IL-6, IL-23 and TGF- β , factors for pathogenic Th17 development and resulting in exacerbation of autoimmunity (54, 65). An increased expression of

IL-17 cytokines (such as IL-17A), has been linked to a number of autoimmune, immune and inflammatory related diseases, including Rheumatoid Arthritis, Lupus and Asthma (16, 54). Similarly, a decrease in Th17 and particularly IL-17 may be related to a reduced host protection mechanism and clearance of pathogens (5) .

Th2 T cells are responsible for the secretion of anti-inflammatory cytokines, such as IL-4 and IL-10. When isolated CD4⁺ T cells were analysed in CFS/ME patients, no significant changes were found in IL-4 cytokine levels (4). In whole blood, CFS/ME patients have significantly increased expression of the anti-inflammatory cytokine IL-10. IL-10 stimulates the production and survival of B cells as well as antibody production and down regulating Th1/Th17 pro-inflammatory cytokines (66). Infection is the primary promoter of the production of IL-10 producing cells, suggesting that an increase in IL-10 in CFS/ME patients could reflect the chronic infection typically experienced by CFS/ME patients (17, 67). IL-10 also influences signalling of T cells with B cells and may alter T cell responses necessary to autoimmune diseases (66). Assessment of such cytokines in isolated T cells in CFS/ME patients can provide further insight into dysregulation and cytokine profiles in the disorder.

TGF- β is the only cytokine examined unique to iTregs and has been up regulated in CFS/ME patients (63). TGF- β is primarily an immunosuppressive cytokine which down regulates the inflammatory response through the inhibition of pro-inflammatory cytokines (68, 69). TGF- β deficiencies may promote excessive lymphocyte activation and differentiation, cell adhesion molecule expression, Treg functioning and cell apoptosis, therefore in CFS/ME it is possible that increases in TGF- β may reflect an increase in Treg suppression or Treg related activities in CFS/ME (68). Incidentally, significantly increased levels of CD4⁺CD25⁺FOXP3⁺ cells have been found in CFS/ME patients (70). Regulation and maintenance of immunological tolerance and inflammatory responses can be maintained by Tregs (1, 2, 45). Hence, deficiencies or dysfunctions of Tregs or the subtypes of Tregs may promote auto reactive immune responses resulting in autoimmune diseases (1, 2). Increased FOXP3⁺ is typically observed in various forms of cancer (71).

Significantly reduced cytotoxic activity is an important hallmark of CFS/ME, with many CFS/ME patients demonstrating significant reductions NK cell cytotoxic activity (13, 14, 17, 18). Recent studies have identified significant reductions in the cytotoxic activity of isolated CD8⁺ T cells (14). Although, the underlying causal factor stimulating this effect is unknown, it presupposes that CFS/ME patients are potentially compromised due to failures in this cytotoxic mechanism, possibly relatable to the function of cytotoxic granules and subsequent cytokines in these T cells. Incidentally, reductions in perforin and granzymes have been reported in CFS/ME (14, 46, 72). Perforin and granzymes are lytic proteins that ensure effective lysis of viral or microbial pathogens (73). Reductions in perforin

lead to significant declines in apoptosis of target cells (73, 74). Perforin levels are severely decreased in systemic juvenile idiopathic arthritis, instigating defective cytotoxicity in T cells (75). In contrast, elevated concentrations of perforin are reported in chronic inflammatory disorders with autoimmune features, such as multiple sclerosis and autoimmune thyroid disease. Perforin is significantly increased in inflammatory disorders although the role in these disorders is undefined, it may be indicative of increased cytolytic activity or an immune reaction aimed at removing inflammatory cells (76).

Alterations in the levels of perforin can incidentally affect the release of other lytic proteins, such as granzymes. Granzyme A expression is significantly decreased in CD8⁺ T cells in CFS/ME patients (14). Granzyme A specifically induces the breakage of single-strand DNA and the nuclear lamina. Therefore, decreases in granzyme A in CFS/ME patients may lead to a reduced ability of the cells to induce target cell death (77, 78).

T cell perturbations may potentially be attributing to alterations T cell subtypes, fluctuations in T cell cytokine production, decreases in cytotoxic activity and differential expression of immune related genes in CFS/ME patients.

Conclusion

A number of studies have assessed T cells in CFS/ME, although further studies are required to obtain consistency and validation of results. Assessment of T cell cytokines in CFS/ME patients based on PBMCs is not the most appropriate method of assessing these cells as they are not specific to subsets of T cells that vary in cytokine secretion. Similarly, assessment of CD8⁺ T and CD4⁺ T cells and cytokine profiles, may highlight specific cells that may be affected in CFS/ME patients. In particular, Tregs and their regulatory activities may deserve closer investigation. Subgrouping of CFS/ME patients may be necessary in the future to determine whether T cell subsets and function differs among CFS/ME patients based on their variation of disorder onset or severity.

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Competing Interests

The authors declare that they have no competing interests.

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