

# **Review: Immunology of Chronic Fatigue Syndrome**

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## **ABSTRACT**

A review of the literature on the immunology of CFS reveals that people who have Chronic Fatigue Syndrome (CFS) have two basic problems with immune function that have been documented by most research groups: [1] immune activation, as demonstrated by elevation of activated T lymphocytes, including cytotoxic T cells, as well as elevations of circulating cytokines; and [2] poor cellular function, with low natural killer cell cytotoxicity (NKCC), poor lymphocyte response to mitogens in culture, and frequent immunoglobulin deficiencies, most often IgG1 and IgG3. These findings have a waxing and waning temporal pattern which is consistent with episodic immune dysfunction (with predominance of so called T-helper type 2 and proinflammatory cytokines and low NKCC and lymphoproliferation) that can be associated as cause or effect of the physiological and psychological function derangement and/or activation of latent viruses or other pathogens. The interplay of these factors can account for the perpetuation of disease with remission/exacerbation cycles. Therapeutic intervention aimed at induction of a more favorable cytokine expression pattern and immune status is discussed.

## **INTRODUCTION**

The nervous and immune systems respond to internal and external challenges and communicate and regulate each other by means of shared or system-unique hormones, growth factors, neurotransmitters and neuromodulators. Similar alterations in central catecholamine neurotransmitter levels are associated with immune activity and stressor exposure, alterations that are more pronounced in aged as opposed to younger animals (1). For example, a decreased norepinephrine turnover in the hypothalamus and brain stem of rats occurs at the peak of the immune response to sheep red blood cells (2,3), and increased serotonin metabolism is associated with depressed Arthus reaction and plaque-forming cell response in rats stressed either by overcrowding lasting two weeks or more or by repeated immunobilization for four days (4,5). The long term effects of these acute changes are evidenced by chronic variable stress which facilitates tumor growth (6) and is associated with immune dysregulation in multiple sclerosis (7). The hypothalamic-pituitary-adrenal axis plays a pivotal role in stress-mediated changes, and stimulation of corticotropin-releasing factor in the central nervous system (8,9) has been shown to suppress rapidly a variety of immune responses, an effect which can be blocked by infusion into the brain of alpha-melanocyte-stimulating hormone, a tridecapeptide derived from pro-opiomelanocortin (10).

Besides external stimuli, intrinsic imbalances in neurotransmitter levels affect the immune system either directly by acting on immunocompetent cells or indirectly via induction of hormonal secretions. For instance, depression is associated with neurotransmitter imbalances and with decreased natural killer cell cytotoxic activity (11-14). Moreover, several studies have documented the existence of striking physiologic, neuroendocrine, metabolic, and pharmacologic differences between depressed and normal subjects and between depressed and severely ill subjects (15-20).

The examples mentioned above illustrate the fact that disorders, or persistent noxious stimulation, of the neuroimmunological circuitry can lead to, or result from, neurological, immunological, psychiatric or

multiorgan pathology. The latter link has encouraged a search for neuroimmunological markers with functional or pathological correlates.

Although the cause of CFS remains to be elucidated, many studies summarized herein have provided evidence for abnormalities in immunological markers among individuals diagnosed with CFS. A clear picture has not been achieved because of the noticeable variability in the nature and magnitude of the findings reported by different groups (21,22). Moreover, little support has been garnered for an association between the latter abnormalities and the diverse physical and health status changes in the CFS population. For instance, Buchwald and coworkers (23) concluded that although a subset of CFS patients with immune system activation can be identified, serum markers of inflammation and immune activation are of limited diagnostic usefulness in the evaluation of patients with CSF and chronic fatigue because changes in their values may reflect an intercurrent, transient, common condition, such as an upper respiratory infection, or may be the result of an ongoing illness-associated process. On the other hand, Patarca and colleagues (24,25) have found that CFS patients can be categorized based on immunological findings. It is also worth noting that although the degree of overlap between distributions of soluble immune mediators in CFS and controls has fueled criticism on the validity or clinical significance of immune abnormalities in CFS, the latter degree of overlap is not unique to CFS and is also present, for instance, in sepsis syndrome and HIV-1-associated disease, clinical entities where studies of immune abnormalities are providing insight into pathophysiology (26).

The aim of this report is to comprehensively review the literature on the immunology of CFS, to formulate consensus by majority conclusions when possible, and to discuss how this knowledge may contribute to the understanding of the physiological and psychological function changes seen in CFS. Immunological status findings will be reviewed and discussed at three levels: immune cell phenotypic distributions, immune cell function, and cytokines and other soluble immune mediators.

## **IMMUNE CELL PHENOTYPIC DISTRIBUTIONS**

Analysis of the complex interactions underlying immune responses was greatly facilitated by the development of monoclonal antibodies to various surface proteins on lymphoid cells, which defined functionally distinct subsets (27-29). Such analysis has also demonstrated that each type of lymphoid cell is genetically programmed to carry out defined immunological functions that are predictable on the basis of surface phenotype (29).

Surface-marker phenotyping of peripheral blood lymphoid cells has also allowed insight into the cellular basis of immune dysfunction associated with pathologies of the central nervous system with diverse causes, including viral, autoimmune, and genetic, among others (see, e.g., refs. 30-36). Several reports also documented alterations in the distribution of various lymphoid cell subsets among CFS patients. Certain discrepancies in the findings from different study groups can be attributed to group nonequivalences on diverse parameters such as demographic variables (gender, age, socioeconomic status), medical status variables predating onset of disease, medication use, concomitant substance abuse, nutritional status, and the effects of time of sample collection (diurnal or seasonal variations; 31, 37-44).

### **T Lymphocytes**

CD4<sup>+</sup> T cells (helper-inducer cells) are the principal source of “help” for antibody production by B cells in response to T-cell-dependent antigenic stimulation, as well as inducers of cytotoxic and suppressor T-cell function (CD8<sup>+</sup> cells; 28). Discrepant results have been reported in reference to CD4 and CD8 cell counts in CFS patients. Straus and colleagues (45) reported a statistically higher percentage of CD4<sup>+</sup> lymphocytes with normal numbers of CD8<sup>+</sup> cells and CD4/CD8 ratio; Jones (46) and colleagues (47,48), Borysiewicz (49), Gupta (50), Landay (51), Lloyd (52) and Tirelli (53) and their coworkers found normal percentages of CD4<sup>+</sup> and CD8<sup>+</sup> cells as well as a normal CD4/CD8 ratio; Lloyd and coauthors (54) found decreased numbers of both CD4<sup>+</sup> and CD8<sup>+</sup> cells; Buchwald and Komaroff (55) found reduced numbers of CD8<sup>+</sup> cells and higher-than-normal CD4/CD8 ratios; and Klimas and colleagues (56) found that most CFS subjects studied had a normal number of CD4<sup>+</sup> cells and an elevated number of CD8<sup>+</sup> cells that resulted in a decrease in the CD4/CD8 ratio

(56). Decreased CD4/CD8 ratios in 2% to 100% of patients have been demonstrated by other investigators (46-49, 57-59).

These conflicting results may be associated with the fluctuation in clinical manifestations of these patients or with other factors mentioned previously. In fact, several researchers have detected fluctuations in several immunological parameters and in the severity of symptoms in longitudinal follow-up investigations of patients with CFS. Moreover, Mawle and coworkers (60) found that although only marginal differences in cytokine responses and in cell surface markers were apparent in the total CFS population they studied, when the patients were subgrouped by type of disease onset (gradual or sudden) or by how well they were feeling on the day of testing, more pronounced differences were seen. It is also worth noting that although Peakman and coworkers did not find significant differences in the percentage levels of total CD3+, CD4+, CD8+, and activated, naive and memory T-cell subsets between CFS subjects and controls, they cryopreserved the cells before flow cytometric analysis and cryopreservation can differentially affect the representation of T-cell subsets (61).

A study by Sandman and colleagues (62) found that elevated CD4+ and CD8+ cell counts in CFS patients were related to decreases in priming of memory, speed of memory scanning and increases in errors on a memory fragility test. However the latter study did not control for depression severity, and it is not clear whether the finding is related to co-morbid depression or to CFS itself.

Klimas and co-workers (56) found a decreased proportion of CD4+CD45RA+ cells, which are associated with suppressor/cytotoxic cell induction (63) but Natelson and coworkers (64) found no significant change in the proportions of CD4+CD45RA+ and CD4+CD45RO+ cells in CFS patients. Franco and coinvestigators (65) also described a decrease in the number of CD4+CD45RA+ lymphocytes in two patients with severe, chronic, active Epstein-Barr virus (EBV) infection; one of the two patients showed a persistent diminished number of cells despite clinical improvement with interleukin-2 (IL-2) treatment. Several publications have associated alterations in the latter subset with a number of clinical entities, particularly autoimmune diseases (22, 33, 63, 66-68).

Increased numbers of T cells expressing the activation marker CD26, probably as a result of CD8+ activation, have also been reported in CFS patients (56). In this respect, an increased proportion of CD8+ cells expressing the activation marker human leukocyte antigen (HLA)-DR (51, 56, 69, 70) have been reported in CFS patients, whereas normal proportions of CD4+ T cells co-expressing the HLA-DR marker or the IL-2 receptor (CD25) were found in one study (51), normal proportions of CD8+ CD38+, CD8+CD11b-, CD8+HLA-DR+ and CD8+CD28+ were found in another study (64), and normal proportions of CD8+HLA-DR+ and CD8+CD38+ were found by Swanink and coworkers (71). In contraposition to the latter findings, Hassan and colleagues (70) found significantly decreased expression of CD28 on CD8 cells and Barker (69), Landay (51) and Swanink (71) and their coworkers found significantly decreased expression of CD11b on CD8 cells. Higher expression of CD38 on CD8 cells was found by Barker (69), Landay (51) and Peakman (72) and their coworkers.

It is worth noting that relatively higher proportions of HLA-DR+ T cells have been reported in a number of autoimmune disorders (73-77), and that Hassan and coworkers (70) found that CFS patients with increased HLA-DR expression had significantly lower Short Form-36 health questionnaire (SF-36) total scores, worse body pains, and poorer general health perception and physical functioning scores. The increased expression of class II antigens and the reduced expression of the costimulatory receptor CD28, which is a marker of terminally differentiated cells, lend further support to the concept of immunoactivation of T-lymphocytes in CFS and may be consistent with the notion of a viral etiopathogenesis in the illness.

We studied the association between CFS physical symptoms, illness burden and lymphocyte activation markers in 27 newly-recruited CFS patients (78). Elevations in T-helper/inducer cells were associated with a greater frequency and severity of tender lymph nodes, greater severity of memory and concentration difficulties and headaches. Greater numbers of activated T cells (CD2+CD3+CD26+) were associated with a greater frequency of tender lymph nodes and cognitive difficulties while more activated cytotoxic/suppressor cells (CD8+CD38+HLA-DR+) were associated with greater severity of tender lymph nodes, fatigue and sleep

problems. Conversely, lower percentages of regulatory cells such as CD3+CD8+ were associated with a greater number of cognitive difficulties, greater Sickness Impact profile(SIP)-Total, SIP Physical Impairment, and an increased frequency and severity of memory problems, increased frequency of headaches, and increased severity of fatigue. Thus, among CFS patients the degree of cellular immune activation is associated with the severity of CFS-related physical symptoms, cognitive complaints, and perceived illness burden.

## **B Lymphocytes**

Gupta (50), Klimas (56), Landay (51), Lloyd (52) and Barker (69) and their colleagues found normal levels of CD20+ resting B cells, whereas other teams reported both increased and decreased levels (49, 53, 55, 59). The proportion of CD5-bearing B cells was found to be increased in two studies (53, 56) or decreased in one study (51). B cells bearing the cell marker CD5 have been associated with autoimmunity (79).

## **Natural Killer Cells**

Klimas (56), Morrison (80), Peakman (72) and Tirelli (53) and their associates found increased numbers of NK cells, whereas Barker (69), Landay (51), Lloyd (52) and Natelson (64) and their coworkers found normal numbers and Masuda (81) and Gupta (50) and their coworkers found decreased numbers of NK cells. Despite the discrepancy in total numbers of NK cells measured by different groups, Caligiuri (82) and Morrison (80) and their coworkers found an increased proportion of CD56+CD3+ T cells, which may account for the decreased natural killer (NK) cell cytotoxic activity seen in several studies of CFS patients. Morrison and coworkers (80) also found a decreased percentage of CD56+Fcγ receptor+ NK cells, which suggests a reduced capacity for antibody-dependent cellular toxicity.

## **Neutrophils**

Previously described relationships in healthy women between basal circulating neutrophil numbers and plasma progesterone concentrations and between exercise-induced neutrophilia and urinary cortisol and plasma creatine kinase concentrations were not observed in CFS women, observations which suggest that normal endocrine influences on the circulating neutrophil pool may be disrupted in CFS patients (83).

## **IMMUNE CELL FUNCTION**

### **T and B Lymphocytes**

Depressed responses to phytohemagglutinin (PHA) and pokeweed mitogen (PWM), an indication of dysfunction in cellular immunity, were found in the CFS patients studied by most teams (45-49, 52, 54, 56, 57, 70, 84, 85) while Mawle and coworkers (60) found no change. Gupta and coworkers (50) found that the lymphocyte DNA synthesis in response to PHA, PWM and concanavalin A was normal in CFS patients, but the response to soluble antigens (mumps, *E. coli*) was significantly reduced. Roberts and colleagues (42) found that PWM lymphoproliferative response is associated with Rh status among healthy controls but not among CFS patients and recommended to control future studies for Rh status. In terms of the functional implications of decreased lymphoproliferative activities in CFS, Hassan and coworkers (70) reported that PHA proliferative responses were lower in patients with poor emotional and mental health scores, and the anti-CD3/anti-CD28 response was low in those with low general health perception scores. T-cell dysfunction in CFS patients has been suggested to result from decreased surface expression of CD3, an important component of the T-cell receptor complex (86) and Barker and coworkers (69) found no significant increase in the mean proliferation of peripheral blood cells when stimulated with anti-CD3 antibody.

In terms of B-cell function, spontaneous and mitogen-induced immunoglobulin synthesis is also affected as discussed later. Despite these deficits in B-cell function, stimulation with allergens provides differential lymphocyte responsiveness. Greater *in vitro* lymphocyte responses to specific allergens, greater baseline levels of lymphocyte incorporation of tritiated thymidine, and an increased number of immunoglobulin E-bearing B and T lymphocytes have been reported (87,88). Elevation in levels of certain cytokines, such as IL-4, IL-5 and

IL-6 may underlie the latter effects as discussed later.

In a sample of 65 CFS patients, we observed that decreased lymphoproliferative responses to PHA and PWM were associated with increased cognitive difficulties and greater SIP physical illness burden (89).

Another area of research in CFS is that of apoptosis, the process of programmed cell death, which is regulated by several genes including Bax and Bcl-2. The Bcl-2 protein forms a heterodimer with Bax that inhibits apoptosis, whereas the Bax-Bax homodimer promotes it. A report by Hassan and coworkers (70) on surface and intracellular immunologic and apoptotic markers and functional lymphocyte assays after stimulation with anti-CD3/anti-CD28 antibodies or PHA in 44 CFS patients revealed increased expression of the apoptosis repressor ratio of bcl-2/bax in both CD4 and CD8. However, recent evidence indicates that induction of apoptosis might be mediated in a dysregulated immune system, such as that present in CFS, by the upregulation of growth inhibitory cytokines. In this respect, Vojdani and colleagues (90) found an increased apoptotic cell population in CFS individuals as compared to healthy controls. The increased apoptotic subpopulation in CFS individuals was accompanied by an abnormal cell arrest in the S phase and the G2/M boundary of the cell cycle as compared to the control group. In addition, CFS individuals exhibited enhanced mRNA and protein levels of the IFN-induced protein kinase RNA (PKR) product as compared to healthy controls. In 50% of the CFS samples treated with 2-aminopurine (a potent inhibitor of PKR) the apoptotic population was reduced by more than 50%. PKR-mediated apoptosis may thus contribute to the pathogenesis and the fatigue symptomatology associated with CFS. See and colleagues (91) found that addition of a glyconutrient compound (dietary supplement that supplies the crucial eight monosaccharides required for synthesis of glycoproteins) to peripheral blood cells of CFS patients *in vitro* significantly decreased the percentage of apoptotic cells (all three parameters were deficient at baseline).

In contrast to the studies described above, Swanink and coworkers (71) found no obvious difference in apoptosis in leukocyte cultures from CFS patients.

### **Natural Killer Cells**

Several studies revealed impaired NK cell function in CFS patients as assessed by cytotoxic activity against K562 cells (45, 56, 58, 69, 82, 92-95) and a decreased number of CD56+CD3- lymphocytes (80, 82). A study by Levine and coworkers (96) on NK cell activity in a family with members who had developed CFS as adults, as compared to those who had not, documented low NK cell activity in 6/8 cases and in 4/12 unaffected family members. Two of the offspring of the CFS cases had pediatric malignancies. Based on these observations, the authors suggested that the low NK cell activity in this family may be a result of a genetically determined immunologic abnormality predisposing to CFS and cancer. Gold and colleagues (97) were the only group to find elevated NK cell activity among the CFS patients they studied while Mawle and colleagues (60) found no change in NK cell function.

The changes in NK cell cytotoxic activity found by most groups could be related to several findings: [1] CD56+CD3- cells are the lymphoid subset with highest NK cell activity; and a decrease in their representation is expected to lower the value for the NK cell activity per effector cells; [2] The reduction in CD4+CD45+ T cells described previously may also result in decreased induction of suppressor/cytotoxic T cells; and [3] Reduced NK cell activity may be associated with deficiencies in the production of IL-2 and interferon(IFN)-gamma by T cells or in the ability of NK cells to respond to these lymphokines. In the terms of the latter possibility, Buchwald and Komaroff (55) found that stimulation with IL-2 failed to result in improvement of cytolytic activity in many patients with CFS.

Poor NK cell function may also be related to the finding of an impaired ability of lymphocytes from CFS patients to produce IFN-gamma in response to mitogenic stimuli (56, 92). Although one study reported elevated IFN-gamma production (98) and another demonstrated normal production (99), the inability of lymphocytes from CFS patients to produce IFN-gamma found by Klimas (56), Kibler (92) and Visser (100) and their associates might represent a cellular exhaustion as a consequence of persistent viral stimulus. The latter postulate is supported by Morag (101) and Straus (45) and their colleagues' finding of elevated levels of

leukocyte 2'5'-oligoadenylate synthetase, an IFN-inducible enzyme, in lymphocytes of CFS patients. Furthermore, the lack of IFN-gamma production in CFS patients may be responsible for the impaired activation of immunoregulatory circuits, which in turn facilitates the reactivation and progression of viral infections. In this respect, Lusso and associates (102) described the prevention of intercellular spread of EBV mediated by the IFN released as a consequence of cellular response, and Borysiewicz and co-workers (49) described normal NK cell activity but reduced EBV-specific cytotoxic T-cell activity in their CFS patients. Reactivation/replication of a latent virus (such as Epstein Barr virus) secondary to decreased NK cell activity has also been proposed to modulate the immune system to induce CFS (103).

More recent research has provided alternative explanations for the decreased NK cell activity observed in CFS. A study by Ogawa and coworkers (104) revealed a possible dysfunction in the nitric oxide (NO)-mediated NK cell activation in CFS patients based on the observations that 24 hours treatment of NK cells with L-Arginine (L-Arg), one of the essential amino acids, enhanced NK cell activity in controls but not CFS patients. Although the expression of inducible NO synthase (iNOS) (the enzyme involved in the synthesis of NO from L-Arg) transcripts in peripheral blood mononuclear cells was not significantly different between healthy control subjects and CFS patients, and incubation with S-nitroso-N-acetyl-penicillamine, an NO donor, stimulated NK cell activity in healthy control subjects but not in CFS patients. See and coworkers (1998) reported that addition *in vitro* of a glyconutrient compound (dietary supplement that supplies the crucial eight monosaccharides required for synthesis of glycoproteins) to peripheral blood cells from CFS patients significantly enhanced natural killer cell activity, increased the expression of the glycoproteins CD5, CD8 and CD11a, and decreased the percentage of apoptotic cells, parameters which were all deficient at baseline. The latter observation would be consistent with a defect in glycoprotein synthesis.

See and Tilles (105) treated 30 CFS patients IFN-alpha 2a or placebo in a double-blind crossover study. Outcome was evaluated by NK cell function, lymphocyte proliferation to mitogens and soluble antigens, CD4/CD8 counts and a 10 item Quality of Life (QOL) survey. Although mean NK function rose with 12 weeks of IFN therapy, there was no significant change in the other immunologic parameters or QOL scores. When the 26 patients who completed the study were stratified according to their baseline NK cell function and lymphocyte proliferation, 4 groups were identified: 3 patients had normal NK cell function and lymphocyte proliferation when compared to normal, healthy controls, 9 had isolated deficiency in lymphocyte proliferation, 7 had diminished NK function only, and 7 had abnormalities for both parameters. QOL scores were not significantly different for the four groups at baseline. After 12 weeks of interferon therapy, QOL score significantly improved in each of the seven patients with isolated NK cell dysfunction compared to baseline. In these patients the mean NK cell function increased. Significant improvement was not recorded for QOL in the other three groups. Thus, therapy with IFN-alpha has a significant effect on the QOL of that subgroup of patients with CFS manifesting an isolated decrease in NK cell function.

Pursuing the hypothesis that the low-grade fever and fatigue in low NK syndrome (LNKS) - a condition resembling CFS in many but not all ways (57, 106)- might be abrogated by interventions that normalize NK functioning, one group has tested the effects of immunopotentiators with patients diagnosed with LNKS. They found in single-blind trials (contents of medication were not revealed to patients) that while the administration of antipyretics, non-steroidal anti-inflammatory drugs or antibiotics had no detectable effects on fever, lentinan, a glucon extracted from Japanese mushrooms improved clinical symptoms and increased NKCC and antibody-dependent cellular cytotoxicity (ADCC) in patients with LNKS (107). Although preliminary, this is one of the only studies to document parallel improvement in CFS-like clinical symptoms and NKCC following an experimental manipulation. However, this study did not focus specifically on CDC-diagnosed CFS patients.

## **Monocytes**

Prieto and coauthors (108) found significant monocyte dysfunction in patients with CFS, such as reduced display of vimentin, phagocytosis index, and surface expression of HLA-DR. These deficits responded to naloxone treatment, which suggests that increased interaction of endogenous opioids with monocyte receptors might account for the monocyte dysfunction. Gupta and coworkers (109) found that monocytes from CFS patients display an increased density of ICAM-1 and LFA-1, but showed decreased enhancing response to recombinant IFN-gamma *in vitro*. In contrast to the latter studies, Barker and coworkers (69) did not find

abnormalities in superoxide anion production and phagocytosis in CFS patients. Moreover, lack of a consistent elevation of neopterin, a macrophage activation marker (see later discussion), suggests that monocytes do not appear to account for the imbalances in IL-1 described below.

### **Eosinophils**

Conti and colleagues (110) provided evidence for eosinophil activation in CFS by demonstrating elevated serum levels of eosinophil cationic protein (ECP). In the CFS population they studied, the prevalence of RAST positivity to one or more allergens was 77%, while no control showed positive RAST. Twelve of the 14 CFS patients with increased ECP serum levels were RAST-positive. However, CFS RAST-positive patients had no significantly higher ECP serum levels than CFS RAST-negative patients. It remains to be determined whether eosinophil activation has a pathogenetic role in CFS or whether a common immunologic background may exist for both atopy and CFS.

Although a higher prevalence of allergy (111) and delayed type hypersensitivity (52,54) can be detected in CFS patients, a trial with antihistamine treatment did not provide significant improvement (112) and other authors such as Mawle and coworkers (60) found no significant difference in the incidence of delayed type hypersensitivity and allergic responses among CFS patients. Baraniuk and coworkers (113,114) found that 30% of CFS patients had positive skin tests suggesting the potential for allergic rhinitis complaints, and 46% had non-allergic rhinitis and suggested that while atopy may coexist in some CFS subjects, it is unlikely that it plays a causal role in CFS pathogenesis. Borish and coworkers (115) proposed that in at least a large subgroup of subjects with CFS with allergies, the concomitant influences of immune activation brought on by allergic inflammation in an individual with the appropriate psychologic profile may interact to produce the symptoms of CFS, and Borok (116) suggested that food intolerance, in a genetically predisposed group of people, causes symptoms akin to both the major and minor criteria of CFS and it should be screened for to avoid confusion. Although the controversy of atopy and CFS continues, it may be possible that these two conditions share some common denominators that are worth pursuing particularly in light of the proposed Th2 cytokine predominant pattern.

### **CYTOKINES AND OTHER SOLUBLE IMMUNE MEDIATORS**

Stimulated lymphoid cells either express or induce the expression in other cells of a heterogeneous group of soluble mediators that exhibit either effector or regulatory functions. These soluble mediators include cytokines, hormones, and neurotransmitters, which in turn affect immune function and may underlie many of the pathological manifestations seen in CFS (41). The studies of cytokines in CFS have been done in the peripheral blood compartment and a recent review by Vollmer-Conna and coworkers (117) on the immunopathogenesis of CFS concludes that neuropsychiatric symptoms in CFS patients may be more closely related to disordered cytokine production by glial cells within the CNS than to circulating cytokines. The hypothesis that expression of proinflammatory cytokines within the CNS plays a role in the pathogenesis of immunologically-mediated fatigue is underscored by the study by Sheng and coworkers (118) who, using two strains of mice with differential patterns of cytokine expression in response to an injection challenge with *Corynebacterium parvum*, demonstrated that elevated IL-1 and TNF cytokine mRNA expression in the central nervous system corresponded to development of fatigue. Injection of antibodies specific to either IL-1 or TNF did not alter immunologically induced fatigue, suggesting a lack of involvement of these cytokines produced outside of the CNS. We will nonetheless describe the potential implications of the cytokine imbalances detected in peripheral blood to physiological and psychological functions.

### **Cytokines**

The decreased NK cell cytotoxic and lymphoproliferative activities and increased allergic and autoimmune manifestations in CFS would be compatible with the hypothesis that the immune system of affected individuals is biased towards a Th2 type, or humoral immunity-oriented cytokine pattern (119). The factors that could lead to a Th2 shift and to mood changes associated with immunoendocrine changes among CFS patients are unknown. Vaccines and stressful stimuli have been shown to lead to long-term, non-specific shifts in cytokine

balance. Therapeutic regimens that induce a systemic Th1 bias are being tested including repeated stimulation with bacterial antigens or poly (I)-poly (C12U) (120) and *ex vivo* activation of lymph node cells (121).

#### *Interleukin-1 (IL-1) and soluble IL-1 receptors*

IL-1 is the term for two distinct cytokines - IL-1a and IL-1b - that share the same cell-surface receptors and biological activities (122,123). One study of CFS patients (124) found elevated levels of serum IL-1 alpha but not of plasma IL-1 beta in 17% of patients studied. When the cohort was examined as to severity of symptoms, it was noted that the top quartile in terms of disability had the highest level of IL-1. Curiously, use of reverse transcriptase-coupled polymerase chain reaction (RT-PCR) revealed IL-1b but not IL-1a messenger RNA (mRNA) in peripheral blood mononuclear cells (PBMCs) of several CFS patients with highly elevated levels of IL-1a. RT-PCR of fractionated cell populations showed that lymphocytes accounted for the IL-1b mRNA detected in PBMCs. No IL-1 mRNA was apparent in control subjects. That IL-1a mRNA was not detectable by RT-PCR in either PBMCs or granulocytes suggests that serum IL-1a in CFS patients is probably derived from a source other than peripheral blood cells. Other potential sources are tissue macrophages, endothelial cells, lymph node cells, fibroblasts, central nervous system microglia, astrocytes, and dermal dendritic cells (122).

Linde and coworkers (125) found significantly higher levels of IL-1 alpha in CFS and mononucleosis patients but Lloyd (52), Peakman (72) and Rasmussen (126) and their coworkers found no difference. Five studies, in addition to one described above by Patarca and colleagues (124) found no difference in the levels of IL-1 beta in CFS patients (72, 125-128).

The signs and symptoms of CFS, which include fatigue, myalgia, and low-grade fever, are similar to those experienced by patients infused with cytokines such as interleukin-1. Elevated serum levels of IL-1a found in a significant number of CFS patients could underlie several of the clinical symptoms. IL-1 can gain access to the brain through the preoptic nucleus of the hypothalamus, where it induces fever and the release of adrenocorticotropin hormone (ACTH)-releasing factor (129-132), which in turn would lead to release of ACTH and cortisol. The observation that cortisol levels tend to be low in CFS patients regardless of IL-1a levels suggests a role of a defective hypothalamic feedback loop in the pathogenesis of CFS. The presence of such a defect has been documented in Lewis rats, which are particularly susceptible to the induction of a variety of inflammatory and autoimmune diseases and exhibit reduced levels of ACTH-releasing hormone, ACTH and cortisol in response to IL-1.

Besides its effects on the HPA axis, IL-1 has other effects on the pituitary; it has been shown to augment release of prolactin and growth hormone and to inhibit release of thyrotropin and luteinizing hormone (133,134). The growth hormone deficiency state associated with CFS may also be a reflection of the defect in hypothalamic feedback loop which renders it inadequately responsive to IL-1.

IL-1 and tumor necrosis factor (TNF) provoke slow-wave sleep when placed in the lateral ventricles of experimental animals (135). The inordinate fatigue, lassitude, and excessive sleepiness associated with CFS (136,137) could well be a consequence of the direct action of these cytokines on neurons. Neurotoxic effects due to chronic overexpression of IL-1a and/or b of S100 - a small (10KDa), soluble calcium-binding protein that is synthesized and released by astroglia (138) - have been proposed to underlie progressive neurological degeneration in Alzheimer's disease (139).

IL-1 induces prostaglandin (PGE<sub>2</sub>, PGI<sub>2</sub>) synthesis by endothelial and smooth muscle cells (140). These substances are potent vasodilators, and IL-1 administration in animals and humans produces significant hypotension. IL-1 has a natriuretic effect (141) and may affect plasma volume.

Gulick and colleagues (142) showed that IL-1 and TNF inhibit b-adrenergic agonist-mediated cardiac myocyte contractility in cultures and intracellular accumulation of cyclic adenosine monophosphate. Cytokine imbalances may, therefore, also underlie the cardiovascular manifestations of CFS.

Chronic fatigue syndrome is a condition that affects women in disproportionate numbers, and that is often exacerbated in the premenstrual period and following physical exertion. Cannon and coworkers (143) found that isolated peripheral blood mononuclear cells from healthy women, but not CFS patients, exhibited significant menstrual cycle-related differences in IL-1 beta secretion that were related to estradiol and progesterone levels. IL-1Ra secretion for CFS patients was twofold higher than controls during the follicular phase, but luteal-phase levels were similar between groups. In both phases of the menstrual cycle, IL-1sRII release was significantly higher for CFS patients compared to controls. The only changes that might be attributable to exertion occurred in the control subjects during the follicular phase, who exhibited an increase in IL-1 beta secretion 48 hr after the stress. These results suggest that an abnormality exists in IL-1 beta secretion in CFS patients that may be related to altered sensitivity to estradiol and progesterone. Furthermore, the increased release of IL-1Ra and sIL-1RII by cells from CFS patients is consistent with the hypothesis that CFS is associated with chronic, low-level activation of the immune system.

In contrast to the studies described above, Swanink and coworkers (71) found no obvious difference in the levels of circulating cytokines, and *ex vivo* production of IL-1 alpha and IL-1 receptor antagonist. Although endotoxin-stimulated *ex vivo* production of tumor necrosis factor-alpha and IL-beta was significantly lower in CFS, none of the immunologic test results correlated with fatigue severity or psychologic well-being scores. Swanink and coworkers (71) concluded that these immunologic tests cannot be used as diagnostic tools in individual CFS patients.

#### *Tumor necrosis factors (TNFs) and soluble TNF-receptors*

TNF-alpha and TNF-beta are cytokines produced on lymphoid cell activation (144). Twenty-eight percent of CFS patients studied by Patarca and colleagues (124) had elevations in serum levels of TNF-alpha and TNF-beta usually with elevation in serum levels of IL-1 or sIL-2R. TNF-alpha expression in CFS patients is also evident at the mRNA level, which suggests *de novo* synthesis rather than release of a preformed inducible surface TNF-alpha protein upon activation of monocytes and CD4+ T cells (145). The levels of spontaneously (unstimulated) produced TNF-alpha by non-adherent lymphocytes were also significantly increased as compared to simultaneously studied matched controls by Gupta and colleagues (109). TNF-alpha may be associated with CNS pathology because it has been associated with demyelination and may also lead to loss of appetite (144, 146). A study by Dreisbach and coworkers (147) suggests that TNF-alpha may be involved in the pathogenesis of post-dialysis fatigue. In contrast to the studies discussed above, Lloyd et al. (52) found no difference in the levels of TNF-alpha or -beta in CFS patients and Rasmussen et al. (126) and Peakman et al. (72) found no differences in the levels of TNF-alpha and -beta, respectively. The latter discrepancies are likely due to the fact that TNF levels decrease precipitously if the serum or plasma is not frozen within 30 minutes from collection (61).

TNF-alpha's proinflammatory effects may be mediated by induction of gene expression for neutrophil activating protein-1 and macrophage inflammatory proteins resulting in neutrophil migration and degranulation (148). Thus, it is reasonable that TNF elevations may also be associated with markers of macrophage activation such as serum neopterin (see below). Among patients studied in our laboratory, we found that illness burden scores were significantly positively correlated with elevated TNF-alpha serum levels.

CFS patients have higher levels of sTNF-RI or sCD120a and sTNF-RII or sCD120b (24, 25). Levels of sTNF-Rs are negatively correlated with NK cell cytotoxic and lymphoproliferative activities in CFS, an observation that is consistent with the activities of these soluble mediators.

#### *Interleukin-2 (IL-2) and soluble IL-2 receptor*

IL-2, formerly termed "T-cell growth factor," is a glycosylated protein produced by T lymphocytes after mitogenic or antigenic stimulation (149). IL-2 acts as a growth factor (150) and promoted proliferation of T cell (151) and, under particular conditions, of B cells and macrophages (152, 153).

Although serum IL-2 levels were found to be elevated in CFS patients compared with control individuals in

one study (154), decreased levels were reported in two other studies (92, 97) and no difference was reported in three studies (124, 125, 128). Rasmussen and coworkers (126) reported a higher production of IL-2 by stimulated peripheral blood cells from CFS patients as compared to controls. Cheney and coworkers (154) found no obvious relation between IL-2 serum levels and severity or duration of illness in CFS.

Elevated levels of sIL-2R, a marker of lymphoid cell activation, have been found in a number of pathological conditions including viral infections, autoimmune diseases, and lymphoproliferative and hematological malignancies (155, 156). Twelve percent of CFS patients studied by Patarca and coworkers (124) had elevated levels of sIL-2R. The latter observation is consistent with the increased proportion of activated T cells and the reduced levels of IL-2 or decreased NK cell cytotoxic activity found in several studies of CFS patients discussed above. Linde and coworkers (125) found no elevation in sIL-2R levels in CFS patients.

#### *Interleukin-4 (IL-4)*

Visser and colleagues (100) reported that although CD4 T cells from CFS patients produce less interferon-gamma than cells from controls, IL-4 production and cell proliferation are comparable. With CD4 T cells from CFS patients (compared with cells from controls), a 10- to 20-fold lower dexamethasone (DEX) concentration was needed to achieve 50% inhibition of IL-4 production and proliferation, indicating an increased sensitivity to DEX in CFS patients. In contrast to IL-4, interferon-gamma production in patients and controls was equally sensitive to DEX. A differential sensitivity of cytokines or CD4 T cell subsets to glucocorticoids might explain an altered immunologic function in CFS patients.

IL-4 acts as a growth factor for various types of lymphoid cells, including B, T, and cytotoxic T cells (157), and has been shown to be involved in immunoglobulin isotype selection *in vivo* (158). Activated T cells are the major source of IL-4 production, but mast cells can also produce it, and IL-4 has been associated with allergic and autoimmune reactions (157). It is also noteworthy that many of the effects of IL-4 are antagonized by IFN-gamma, and the decreased production of the latter may underlie a predominance of IL-4 over IFN-gamma effects.

#### *Interleukin-6 (IL-6) and soluble IL-6 receptor*

The levels of spontaneously produced IL-6 by both adherent monocytes and non-adherent lymphocytes were significantly increased in CFS patients as compared to controls (109). The abnormality of IL-6 was also observed at mRNA level. In terms of circulating IL-6, Buchwald and coworkers (23) found that IL-6 was elevated among febrile CFS patients compared to those without this finding and therefore considered it an epiphenomenon possibly secondary to infection. Chao and coworkers (159, 160) also found elevated levels of IL-6 in CFS patients, but five other groups found no difference (23, 52, 72, 94, 125).

Most of the cell types that produce IL-6 so in response to stimuli such as IL-1 and TNF, among others (161). Excessive IL-6 production has been associated with polyclonal B-cell activation, resulting in hypogammaglobulinemia and auto antibody production (162). As is the case with IL-4, IL-6 may contribute to activation of CD5-bearing B cells, leading to autoimmune manifestations. IL-6 also synergizes with IL-1 in inflammatory reactions and may exacerbate many of the features described previously for IL-1.

Study of cytokine production by stimulated peripheral blood mononuclear cells from patients with a closely related syndrome to CFS, the post-Q-fever fatigue syndrome (QFS) (inappropriate fatigue, myalgia and arthralgia, night sweats, changes in mood and sleep patterns following about 20% of laboratory-proven, acute primary Q-fever cases), showed an accentuated release of IL-6 which was significantly in excess of medians for all four control groups (resolving QFS, acute primary Q-fever without subsequent QFS, healthy Q-fever vaccinees and healthy controls). Levels of induced IL-6 significantly correlated with total symptom scores and scores for other key symptoms (163).

CFS patients have higher levels of sIL-6R (24) and sIL-6R enhances the effects of IL-6.

### *Interleukin-10 (IL-10)*

A study by Gupta and coworkers (109) revealed that spontaneously produced IL-10 by both adherent monocytes and non-adherent lymphocytes and by PHA-activated non-adherent monocytes were decreased. IL-10 is part of the Th2-type response.

### *Interferons (IFNs)*

The IFNs comprise a multigenic family with pleiotropic properties and diverse cellular origin. Data from six studies indicate that circulating IFNs are present in 3% or less of patients studied (45, 48, 49, 55, 57, 164, 165).

Peripheral blood cells from children affected by postviral fatigue syndrome produced more IFN-alpha than those from controls. In line with latter observation, Vojdani and colleagues (90) found elevated IFN-alpha levels in CFS patients but Linde (125) and Straus (128) and their coworkers found no difference. Fatigue occurs in more than 70% of patients treated with IFN-alpha and it may be associated with the development of immune-mediated endocrine diseases, in particular hypothyroidism and hypothalamic-pituitary-adrenal axis-related hormonal deficiencies, in these patients (166, 167). IFN-alpha therapy-associated fatigue is often the dominant dose-limiting side effect, worsening with continued therapy, and accompanied by significant depression. Decreases in mental information processing speeds, verbal memory, and executive functions have also been reported at therapeutic doses of IFN-alpha (168). Although the direct cause of IFN-alpha-induced fatigue is unknown, it is possible that neuromuscular fatigue, similar to that observed in patients with postpolio syndrome, may also be one component of this syndrome. The induction of proinflammatory cytokines observed in patients treated with IFN-alpha is consistent with a possible mechanism of neuromuscular pathology that could manifest as fatigue. A study by Davis and colleagues (169) also revealed that IFN-alpha/beta is at least partially responsible for the early fatigue induced by polyI:C during prolonged treadmill running in mice.

IFN-gamma is an immunoregulatory substance, enhancing both cellular antigen presentation to lymphocytes (170) and NK cell cytotoxicity (171) and causing inhibition of suppressor T lymphocyte activity (172). Two groups have found impaired IFN-gamma production on mitogenic stimulation of peripheral blood mononuclear cells from CFS patients (56, 100) and one group (52) found increased production. In contrast with the findings on lymphocyte activation, four groups reported no difference in the levels of circulating IFN-gamma (72, 100, 125, 128). These results are in favor of the Th2 shift described previously, a shift that is not apparent at the level of circulating cytokines.

### *Tumor growth factor-beta (TGF-beta)*

A study by Bennett and coworkers (173) found that patients with CFS had significantly higher levels of bioactive TGF-beta levels compared to healthy controls and to patients with various diseases known to be associated with immunologic abnormalities and/or pathologic fatigue: major depression, systemic lupus erythematosus (SLE), and multiple sclerosis (MS) of both the relapsing/remitting (R/R) and the chronic progressive (CP) types. A total of three studies supports the finding of elevated levels of TGF-beta among CFS patients.

### *Beta-2 microglobulin*

Three studies found elevated levels of beta-2 microglobulin in patients with CFS (23-25) and one study found no difference (159). Beta-2 microglobulin is a marker of immune activation.

### *Neopterin*

Neopterin is a metabolite produced during the utilization of guanosine triphosphate, and increased production of neopterin is associated with macrophage activation by IFN gamma (174, 175). Neopterin is a

presumed primate homolog of nitric oxide, which activated guanylate cyclase and is involved in neurotransmission, vasodilation, neurotoxicity, inhibition of platelet aggregation, the antiproliferative action of cytokines, and reduction of oxidative stress (176, 177). Neopterin derivatives belong to the cytotoxic arsenal of the activated human macrophage and, in high doses, enhance oxidative stress through enhancement of radical-mediated effector functions and programmed cell death by TNF-alpha, while having an opposite effect at low doses (176, 178). Buchwald (23) and Chao (159, 160) and their coworkers found elevated levels of neopterin in CFS patients, while Linde (125) and Patarca (124) and their coworkers found no difference. A report of nine CFS cases showed significantly elevated serum neopterin levels in association with high Cognitive Difficulty Scale (CDS) scores (89, 179) and neopterin levels have been shown to correlate with levels of many other mediators that have been found to be dysregulated in CFS including members of the TNF family (23, 41, 124). In terms of neurotoxicity, serum neopterin and tryptophan concentrations correlate among cancer and AIDS patients, an observation which can be accounted for by activity of indoleamine 2,3-dioxygenase, a tryptophan-degrading enzyme (180, 181). The latter enzyme also converts L-tryptophan to L-kynurenine, kynurenic acid and quinolinic acid (QUIN). QUIN is a neurotoxic metabolite that accumulates within the central nervous system following immune activation and is also a sensitive marker for the presence of immune activation within the CNS (182-184). Direct conversion of L-tryptophan into QUIN by brain tissue occurs in conditions of CNS inflammation, but not by normal brain tissue. Macrophage infiltrates, and perhaps microglia, are important sources of QUIN, an observation which is consistent with the results of inoculation of poliovirus directly into the spinal cord of rhesus macaques, resulting in increased CSF levels of both QUIN and neopterin (182, 185). Elevated serum levels of neopterin correlate with the presence of brain lesions and with neurologic and psychiatric symptoms in patients with AIDS dementia complex (179, 186). It is worth noting in this context that Buchwald and colleagues (187) found subcortical lesions consistent with edema and demyelination by magnetic resonance scans in 78% of CFS patients as compared to 20% of controls.

#### *Soluble CD8 (sCD8)*

Linde and coworkers (125) found no elevation of sCD8 in CFS patients.

#### *Soluble ICAM-1 (sICAM-1)*

Patarca and coworkers (24) found higher levels of sICAM-1 in CFS patients, an observation which is consistent with the higher expression of ICAM-1 in monocytes of CFS patients reported by Gupta and coworkers (50).

### **Immunoglobulins**

Spontaneous and mitogen-induced immunoglobulin synthesis is depressed in 10% of patients with CFS (49, 188, 189). The latter decrease may be a result of an increased T-cell suppression of immunoglobulin synthesis, because a similar effect is obtained *in vitro* when using normal allogeneic B cells (189). This inhibitory effect may also account for the reported difficulty in establishing spontaneous outgrowth of EBV-transformed B-cell lines from cells from CFS patients (45, 55, 189). The depletion of the CD4+CD45RA+ lymphocyte subset in the studies by Klimas et al. (56) and Franco and colleagues (65) may be associated with alteration in B-cell regulation.

In twelve studies, CFS patients were found to have decreased amounts of immunoglobulins of the G, A, M, or D classes (45, 48, 54, 55, 58, 126, 189-194); in five studies no difference was found (50, 52, 60, 64, 72); and in one study IgG levels were elevated while IgA levels were normal (207). IgG subclass deficiency, particularly of the opsonins IgG1 or IgG3, can be demonstrated in a substantial percentage of CFS patients (54, 56, 59, 191, 194, 195), and for a subset of these, immunoglobulin replacement therapy may be beneficial (196-199) albeit controversial (199). Bennett and coworkers (200) also failed to find immunoglobulin subclass deficiencies in CFS patients.

### **Autoantibodies**

Konstantinov and colleagues (202) found that approximately 52% of sera from CFS patients react with nuclear envelope antigens. Some sera immunoprecipitated nuclear envelope protein lamin B1, an observation which underscores an autoimmune component in CFS (203). von Mikecz and colleagues (204) found a high frequency (83%) of autoantibodies to insoluble cellular antigens (vimentin and lamin B1) in CFS, a unique feature which might help to distinguish CFS from other rheumatic autoimmune diseases. Another finding that underscores a possible autoimmune etiology is the significant association between CFS and the presence of HLA-DQ3 reported by Keller and colleagues (205).

The presence of rheumatoid factor (45-48, 85, 108, 192, 206), antinuclear antibodies (45-48, 85, 97, 108, 193, 204, 207, 208), antithyroid antibodies (84, 85, 209), anti-smooth-muscle antibodies (84), antigliadin, cold agglutinins, cryoglobulins, and false serological positivity for syphilis (45, 84) have also been reported. No circulating antimuscle and anti-CNS antibodies were found in 10 CFS patients (210) and Rasmussen and coworkers (126) found no significant differences in the number of positive tests for autoantibodies in CFS patients.

### **Circulating immune complexes**

Elevated levels of immune complexes have been reported in four studies (45, 49, 84, 207) while the studies by Natelson (64) and Mawle (60) and their coworkers revealed no abnormality in the level of circulating immune complexes (i.e., Raji cell and C1q binding). Depressed levels of complement have also been reported in 0% to 25% of patients (45, 49, 60, 64, 84). Buchwald and coworkers (23) found elevated levels of C-reactive protein among CFS patients.

### **EXPERIMENTAL THERAPY RESULTS IN AN APPARENT SHIFT IN THE TYPE 1 TO TYPE 2 CYTOKINE PATTERN IN CFS PATIENTS**

Our group completed a safety and feasibility study using lymph node extraction, *ex vivo* cell culture, followed by autologous cell reinfusion as a treatment strategy in CFS patients (121). Lymph nodes were obtained from patients who met the current case definition for CFS and the following inclusion criteria: a history of acute onset; a Karnofsky score <80; evidence of immune dysfunction in 3 or more of the following: > 1 S.D. above controls for elevated sTNF-RI in serum, elevated sTNF-RI in PHA-stimulated blood culture or elevated IL-5 in PHA-stimulated blood culture; or lymphocyte activation (CD2+CD26+ cells > 50%); or low NK cytotoxic activity (<20%). The lymph node cells were cultured for 10 to 12 days with anti-CD3 and IL-12. These cells were then reinfused into the donor who was monitored for safety and possible clinical benefit. There were no adverse events noted in this Phase 1 clinical trial. Of 13 subjects, two had palpable lymph nodes that proved fibrotic with no viable cells. Of the remaining 11 subjects, all successfully underwent expansion and re-infusion. In some of the patients, there was an elevation in the expression of IL-2 receptor on CD4 T cells in the weeks following the reinfusion. There was a significant decrease in IL-5 production by PHA-stimulated blood cultures observed at 1 week which persisted for several weeks post-infusion. Levels of PHA-induced IFN-gamma did not change. There was a trend towards a decrease in the ratio of IFN-gamma/IL-5 starting at week 1 and persisting at least 12 weeks. Of the 11 subjects, 9 had significant cognitive improvement, other measures of severity of illness also trended towards improvement. The lack of adverse effects from this experimental approach to immunomodulation in CFS and the favorable clinical and immunologic results observed in the small number of patients studied suggest that further clinical trials are warranted.

### **STRESSORS, CYTOKINES, AND SYMPTOMS**

One of our models of CFS holds that the interaction of psychological factors (distress associated with either CFS-related symptoms or other stressful life events) and immunologic dysfunction (indicated by signs of chronic overactivation with cytokine abnormalities) contribute to: (a) CFS-related physical symptoms (e.g., fatigue, joint pain, cognitive difficulties, fever) and increases in illness burden; and (b) dysfunction in the immune system's ability to survey viruses including latent herpesviruses (indicated by impaired NKCC). As discussed above, there is a decrease in the ratio of type 1/type 2 cytokines produced by lymphocytes *in vitro*

following mitogen stimulation in CFS patients. This type of dysfunction should be expected to result in impaired immune surveillance associated with cytotoxic lymphocytes. For example, Cohen et al. (211) found an association between psychosocial stressors, immunomodulation, and the incidence and progression of rhinovirus infections in healthy normals. Here, the rates of respiratory infections and clinical colds increased in a dose-response fashion with increases in psychological stress across all five of the cold viruses studied. If viruses related to upper respiratory tract infections (URIs) are not well controlled by immune surveillance mechanisms (e.g., NKCC) in CFS patients who are exposed to stressors, then patients may suffer more frequent and protracted URIs which are accompanied by prolonged elevations in proinflammatory cytokines. Stress-associated reactivation of latent herpesviruses may also play a role in modulating the production of cytokines that underlie CFS symptom exacerbations (103, 212). Alternatively, distress increases may more directly influence cytokine dysregulation by way of neuroendocrine changes which in turn intensify physical symptoms. Importantly, for all of the possible paths, further increases in distress as a “reaction” to mounting symptoms creates a vicious cycle. Such a recursive system may act as a positive feedback loop thereby accounting for the chronic nature of CFS and its refractoriness to interventions that focus solely on symptom reduction.

Our conceptual model for CFS was supported by data from our laboratory showing that distress levels in response to the stressor Hurricane Andrew were positively correlated with: alterations in NK cells and elevated (compared to pre-storm values) circulating levels of the cytokines; exacerbation in CFS symptoms; and increases in Sickness Impact profile (SIP)-based illness burden scores among our CFS patients (178). We found that CFS patients living in a Hurricane exposure area (Dade County) had significantly greater severity of CFS symptom relapses (using clinician-rated fatigue levels and ability to engage in work-related activities) and significantly greater increases in illness burden as compared to age- and gender-matched CFS patients from the same clinical practice living in an adjacent geographical region that was not in the storm’s path, Broward/Palm beach county. We also found that pre-post hurricane NKCC changes were associated with pre-post storm symptom severity changes including cognitive symptoms, muscle weakness, and muscle pain. These data suggested that stressor-induced decrements in NKCC were associated with greater increases in the severity of cognitive difficulties, muscle weakness and pain symptoms. A final regression analysis on NKCC indicated that appraisals in greater storm impact and low social support predicted the greatest pre-post storm decrements in NKCC. Greater optimism and social support provisions were also associated with less elevations in TNF-alpha among storm victims.

## **CONCLUSIONS**

The data summarized herein indicate that CFS is associated with immune abnormalities that can potentially account for physio- and psychopathological symptomatology. Assessment of immune status reveals a heterogeneity among CFS patients that allows their categorization, thus systematizing the study of the interactions among immune, psychological, and physiological parameters in this disorder. The study of immune status at different levels also provides an integrated view of this complex syndrome and is opening doors for deciphering its cause and for developing rational treatment protocols. Future research should further elucidate the cellular basis for immune dysfunction in CFS and its implications. Other compartments such as the central nervous system have to be assessed using similar techniques to those used with peripheral blood. Nonetheless, the studies in peripheral blood have been providing insight into the physio- and psychopathologies of CFS.

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