

# **Immunological abnormalities in Myalgic Encephalomyelitis/ICD-CFS**

From January 2007 International Conference on ME/ICD-CFS summary of immunological abnormalities:

Anthony Komaroff (Professor of Medicine, Harvard) summarised the immune abnormalities that have been demonstrated in ME/ICD-CFS. These include activated CD8 (T cells); poorly functioning Natural Killer cells; novel findings -seen only in ME/ICD-CFS -- of abnormalities of the 2-5A pathway (RNase-L ratio); cytokine abnormalities (pro-inflammatory dysregulation); increased TGF, and 27 times more circulating immune complexes than in controls.

## **Numerous additional studies cited below:**

### **Evidence for the Presence of Immune Dysfunction in Chronic Fatigue Syndrome**

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## **INTRODUCTION**

Chronic fatigue syndrome (Myalgic Encephalomyelitis) is a medically unexplained ailment characterized by new onset of fatigue accompanied by rheumatological, infectious, and neuropsychiatric symptoms. Because the ailment often begins suddenly with a flu-like presentation, early pathophysiological ideas as to cause included viral infection and immune activation. When early reports identified putative immunological abnormalities in this illness, it was given the name of chronic fatigue and immune dysfunction syndrome, or CFIDS.

The purpose of this review is to evaluate the immunological literature to determine if strong evidence to support this notion exists. We collected and reviewed 239 published papers, of which only 72 fulfilled a set of criteria for use in this review. For this review, we developed the following criteria: papers had to be published in the peer review literature; patients had to be from a group with substantial fatigue lasting at least 6 months (the vast majority fulfilled either the 1988 [21] or the 1994 [13] case definition of chronic fatigue syndrome [CFS]); papers had to compare CFS patients to healthy controls; and actual data had to be shown with evidence of testing for statistical significance. So, for example, when a paper reported no difference between patients and controls for some immunological variables but actual data were not included, we did not include it. Also, if a report compared patient data to normative values rather than to the study's own control group, we did not include it.

The numbers of immunologically active cells and immunologically active substances such as cytokines reported in the literature have mushroomed in the past decade. To keep this review manageable, we are reporting scientific papers only on those variables for which either consistent or inconsistent abnormalities were reported by more than one group. We did not review papers reporting immunological variables to be within normal limits but have listed those studies in which more than

one group found such results in a table. We have chosen not to list those variables reported abnormal in only one study because those results have not yet been replicated. When inconsistent results among laboratories were found for any immunological variable, we reviewed the methods described in those papers in an effort to identify reasons for such discrepancies.

Note: when a group published more than one paper and it was apparent that the two studies used many if not all of the patients whose data are in the second paper, we chose to include only the more recent paper or the one with the largest number of subjects. To provide several examples, Tirelli published two papers, the first with 205 subjects and the second with 265 patients (66, 67). When data from one variable appeared in both papers, we included data from only the latter. Our own group has published three papers using different statistical methods and making different comparisons when reviewing lymphocyte populations. Thus, we used the one paper that controlled for multiple comparisons (74), except in those situations where variables not included in that paper were published elsewhere.

Table 1 lists those immune variables that were found to be normal in at least two studies.

Available at: <http://cdli.asm.org/cgi/content/full/9/4/747/T1>

#### INCONSISTENT IMMUNE MARKERS

**Lymphocyte subsets studied by flow cytometry in cases of CFS developing sporadically. (i) CD2 cells** Ten papers reported data on total numbers of CD2 cells (or of total lymphocytes), with one reporting decreases (38), a second reporting decreases for women only (59), and a third reporting an increase in those CD2-labeled cells bearing the activation marker CD26 (26); the remaining seven showed no differences compared to controls (10, 16, 37, 40, 65, 66, 74).

**(ii) CD3 cells.** Concerning CD3 cells (i.e., total T cells), seven studies found no differences in the total numbers of these cells (10, 16, 18, 37, 66, 69, 74) while one noted a decrease (38) and another noted a similar decrease but for women only (59). When data for this marker were expressed as percentages of total lymphocyte count, one reported a decrease (64) and the remaining nine studies done showed no differences (16, 18, 29, 39, 41, 55, 59, 63, 74).

**(iii) CD4 cells.** Concerning CD4 cells (i.e., major histocompatibility complex class II [MHC II]-restricted T cells), two studies reported abnormalities in cell counts—both showing decreases (38), with one finding this result for women only (59); the remaining eight found no differences (10, 16, 18, 29, 37, 66, 69, 74). One study reported decreases in percentages of CD4 cells relative to total lymphocyte count (63), while ten studies found normal percentages (16, 18, 26, 29, 39, 41, 55, 59, 64, 74).

**(iv) CD4/CD8 ratios.** Six studies have reported ratios of CD4<sup>+</sup> to CD8<sup>+</sup> cells. Three of them noted this ratio to be reduced for CFS patients relative to controls (26, 32, 63), while the others found these ratios to be similar to those of controls (28, 29, 59).

**(v) CD4 subsets.** A number of studies have evaluated MHC II-restricted T-cell subsets of CFS patients and controls. Of those examining cells expressing CD45RA (i.e., naïve T cells), three reported decreases in the percentage of total T cells bearing this marker (26, 63, 66) and a fourth paper reported an increase (69), while two papers reported no differences (55, 74). Although no single study found differences in CD45RO (Table 1), one report did note increased levels of adhesion markers on these memory T cells (63). Three papers evaluated CD4 cells for the CD54 marker (intercellular adhesion

molecule 1), with two papers finding the percentages of these cells to be increased ([16](#), [66](#)) and one finding them to be at normal levels ([63](#)). Of those papers reporting data on the percentage of CD4 cells bearing the HLA-DR activation marker, one reported an increase ([38](#)) and five found no difference from controls ([18](#), [29](#), [37](#), [55](#), [63](#)).

**(vi) CD8 cells.** Eleven papers evaluated absolute numbers of CD8 cells (i.e., MHC-I-restricted T cells), and 10 evaluated these cells as percentages of total T-cell counts. Of the former group of studies, two reported finding decreases ([38](#), [60](#)), with the rest finding normal results ([10](#), [16](#), [18](#), [29](#), [37](#), [59](#), [66](#), [69](#), [74](#)). Of the latter set of studies, one reported an increase ([26](#)) while the rest found no differences ([16](#), [18](#), [29](#), [39](#), [55](#), [59](#), [63](#), [64](#), [74](#)).

**(vii) CD8 subsets.** A number of studies also assessed CD8 cells for the percentage expressing the CD45RA and CD45RO markers. The National Institutes of Health group found normal values for the former (Table [1](#)) but increased values for the latter marker ([63](#)), results that two other groups did not find ([18](#), [55](#)). One activation marker on CD8 cells which has been studied is the CD28 marker; one study reported the percentage of CD8 cells bearing this marker to be reduced ([18](#)), and two others found it to be at normal levels ([65](#), [74](#)). A landmark paper in the *Lancet* indicated abnormal numbers of three activation markers on these CD8 cells ([29](#)). A series of papers followed this report. Of those papers reporting data on the percentage of CD8 cells bearing the HLA-DR activation marker (either percentage of CD8 cells or some measure of expression of the subset of cells), three reported increases ([26](#), [29](#), [38](#)), with six finding no differences from controls ([18](#), [37](#), [55](#), [63](#), [65](#), [74](#)). Of those additional papers reporting the percentage of all MHC I-restricted T cells bearing the CD38 marker, one reported an increase ([29](#)) while five found the percentages to be normal ([18](#), [55](#), [62](#), [65](#), [74](#)). Finally, five papers did a similar determination for CD11b<sup>+</sup>: two reported decreases ([29](#), [65](#)) and one reported increase ([55](#)), with the remaining finding no differences ([63](#), [74](#)).

**(viii) B cells.** Of 11 studies done quantifying the CD19, CD20, or CD21 markers for B cells, two showed increases ([26](#), [66](#)), with the rest showing no differences from controls ([10](#), [16](#), [18](#), [29](#), [39](#), [59](#), [63](#), [69](#), [74](#)).

**(ix) NK cells.** A number of different cell surface markers have been counted to evaluate NK cell number. For the CD3<sup>+</sup>/CD56<sup>+</sup> cells, two studies reported decreases ([16](#), [39](#)) and one reported increases ([26](#)), with the remaining three showing normal results ([2](#), [49](#), [63](#)). For the six reports of CD3<sup>-</sup>/CD16<sup>+</sup> cell count, three reported decreases ([16](#), [39](#), [66](#)), with the others all being normal ([18](#), [32](#), [63](#)). Five papers examined the CD3<sup>-</sup> (CD16<sup>+</sup> and CD56<sup>+</sup>) cell population: one noted increases ([55](#)), and the remaining found no difference from controls ([29](#), [63](#), [69](#), [74](#)).

**(x) Monocytes.** One group reported the percentage of these cells in whole blood to be increased ([3](#)), while four other groups found no difference between patients and controls ([28](#), [40](#), [59](#), [69](#)). Two groups have evaluated the presence of HLA-DR on monocytes: one group noted it to be decreased ([57](#)), while another group found it to be at control levels ([10](#)).

**Lymphocyte subsets studied by flow cytometry in cases of CFS developing in a quasiepidemic pattern.** In one study comparing Gulf veterans with CFS to healthy Gulf veterans ([74](#)), both numbers and percentages of CD3 and MHC-II T cells were elevated in the patient group and patients had a lower percentage of CD3<sup>-</sup> (CD16<sup>+</sup> and CD56<sup>+</sup>) NK cells than controls. In contrast, Levine et al. found no differences in these cells in a small cluster of patients that occurred in a women's residential facility ([31](#)).

**White blood cell function studied in cases of CFS developing sporadically. (i) T-cell function.**

Three studies evaluated T-cell function via skin testing. While one group reported decreases in delayed hypersensitivity skin reaction to injection of common antigens (38), two others did not (40, 61). Of those studies evaluating in vitro peripheral blood mononuclear cell (indicated below by an asterisk) or T-cell function in response to various lymphocyte stimulants, five showed decreases (10, 26, 38, 57, 63), one showed increases (24), and seven showed no differences (2, 10,\* 16,\* 18, 40, 41, 59). The reader should note that one group reported increases in some variables, decreases in others, and no change in yet others (10); moreover, one group reported no significant differences between patients and controls when phytohemagglutinin, concanavalin A, or poke weed antigen were the stimulants but did find significantly lower activity in CFS patients when soluble antigens such as tetanus toxoid were used (16).

**(ii) NK cell activity.** This was the first variable for which the majority of studies showed significant decreases, seven studies having this result (2, 26, 32, 39, 49, 50, 60) and one finding no differences (40).

**(iii) Phagocytic activity of monocytes.** One group reported a decrease in the phagocytosis index of CFS patients (57), while another group found phagocytized opsonized activity to be normal (2).

**Immunoglobulins and other substances with immunological activity studied in cases of CFS developing sporadically. (i) IgG.**

One study reported that total immunoglobulin G (IgG) was increased (3), while one study reported it to be decreased (72); in contrast, six studies reported normal levels (16, 38, 40, 41, 47, 58). In studies of IgG1, two reports noted decreases (38, 47) while three reports noted levels to be normal (4a, 16, 58). For IgG2, one study noted levels to be reduced (72) while five found levels to be at control levels (4, 16, 38, 47, 58). For IgG3, two reports noted decreases (47, 72) while four noted levels to be normal (4, 16, 38, 58); however, the Australian group did find that CFS patients had elevated IgG3 levels significantly more often than controls (38).

**(ii) IgE.** IgE levels were measured in four studies, with one reporting reductions (58) and all the others finding normal results (1, 40, 47). One study reported a significantly higher rate of IgE-directed radioallergosorbent test positivity in patients than in controls (12).

**(iii) IgA.** One study reported decreases in IgA (58) but attributed this to abnormally high control values consistent with their being no difference in IgA. Four other studies found this variable to be normal (3, 38, 40, 47).

**(iv) Immune complexes.** One study reported CFS patients to have increased amounts of immune complexes in the blood (3), whereas a second one did not (47). A third study found no difference in the percentage of patients showing these abnormalities relative to controls (40)

**(v) Antinuclear antibodies.** A number of papers have reported that CFS patients have a higher rate of autoantibody positivity than controls (3, 27, 48). However, a follow-up report using data from a number of CFS centers did not find similar differences between patients and controls (M. Sugiura, D. Daniels, D. Buchwald, A. Komaroff, M. Hossein, M. Peakman, S. Wessely, B. Natelson, I. Hay, P. Levine, and E. M. Tan, Abstr. Fifth Int. Conf. Am. Assoc. Chronic Fatigue Syndr., abstr. 36, p. 46, 2001). One other group found no evidence for increased rates of autoantibody positivity, but the size of the sample studied was very small (58).

(vi) **Neopterin.** One study reported levels to be elevated relative to controls (9), but four others reported levels to be no different from control levels (6, 34, 36, 53).

(vii) **Beta microglobulin.** Two studies reported increases (8, 52), while two reported levels to be normal (6, 9).

## INCONSISTENT PERIPHERAL BLOOD LEVELS OF CYTOKINES OR THEIR RECEPTORS

**Peripheral blood levels of cytokines or their receptors studied in cases of CFS developing sporadically.** (i) **IL-1 $\beta$ .** One group noted that the significant monthly fluctuation in interleukin 1 $\beta$  (IL-1 $\beta$ ) occurring in healthy women was not seen with CFS patients (7). However, when this variable was assessed without considering menstrual variability, no significant differences were found (5, 10, 34, 35, 53, 62).

(ii) **IL-1 Ra.** One group reported receptor antagonist IL-1 Ra to be increased in women during the follicular but not in the luteal phase of their menstrual cycle (7), while three other studies that did not consider menstrual variability found no increases (51, 58, 65).

(iii) **IL-2.** One study found IL-2 to be at higher levels than controls (11), but four others did not (10, 53, 62, 74).

(iv) **IL-6.** One group reported increases in IL-6 between CFS and controls (14) in 33% of a CFS group but not in controls (9). However, the latter group in a later report (10) plus seven others (6, 8, 34, 40, 53, 56, 74) found levels to be normal.

(v) **IL-10.** One group reported decreases in IL-10 (5), but one found levels to be normal (74).

(vi) **IFN- $\alpha$ .** One study reported increases (71) in alpha interferon (IFN- $\alpha$ ), while four others found no differences between groups (5, 34, 35, 62).

(vii) **Transforming growth factor  $\beta$ .** One study reported increases in transforming growth factor  $\beta$  (4), while two reported levels to be normal (40, 65).

(viii) **TNF- $\alpha$ .** Three groups reported increases in tumor necrosis factor alpha (TNF- $\alpha$ ) (5, 46, 51), while a fourth group noted increases in monocytes but not lymphocytes in cell culture (14). In contrast, four groups reported levels to be normal (10, 35, 62, 74).

(ix) **TNF- $\beta$ .** One group reported TNF- $\beta$  to be increased relative to controls (53), while a second group found levels of this cytokine to be at control levels (58).

## INCONSISTENT LEVELS OF SOLUBLE MEDIATORS, CYTOKINES, OR THEIR RECEPTORS FROM BLOOD FOLLOWING IN VITRO STIMULATION

### **Soluble mediators and cytokines or their receptors from blood studied following in vitro stimulation in cases of CFS developing sporadically.**

(i) One group reported decreases in sCD8, a soluble marker of lymphocyte activation (40), while two others reported it to be normal compared to controls (34, 53).

(ii) **IL-1 $\beta$** . One group noted IL-1 $\beta$  to be increased (10), while a second group noted this variable to be decreased (65), and a third found it to be at control levels (40). A fourth group reported total IL-1 levels to be at control levels (45).

(iii) **IL-2**. One group reported increases in IL-2 cytokine (58), while three groups found amounts of this cytokine to be at control levels (10, 40, 70).

(iv) **IL-6**. Two groups reported IL-6 to be increased compared to controls (8, 10), while two groups found this cytokine to be at normal levels (14, 58). However, Gupta et al. did a follow-up study in which they reported this variable to be higher in CFS patients when fatigued than when rested (15).

(v) **IL-10**. Visser and coworkers reported IL-10 to be increased (69) and normal (70), while another group reported it to be decreased (14).

(vi) **IFN- $\gamma$** . One group noted IFN- $\gamma$  to be higher in patients following activation (58), while one group reported it to be decreased (26) and three groups found this cytokine to be at control levels (42, 44, 68); however, while Visser et al. did not find differences in overall production, they did note that the production of IFN- $\gamma$  by CD4 cells was decreased.

(vii) **TNF- $\alpha$** . One group noted TNF- $\alpha$  to be increased in stimulated cells of CFS patients (10), and one group reported it to be decreased (65). Five groups found that levels of this cytokine did not differ significantly from controls (14, 24, 40, 58, 69).

## DISCUSSION

The overriding result of this review is the remarkable inconsistency of results for each of the immunological parameters that were reported by the various laboratories. There was no single marker in which more than one laboratory reported consistent abnormalities. Moreover, of all the variables studied, we found only three for which the majority of studies reported abnormalities relative to controls. These were anti-nuclear antibody positivity, NK cell function, and the percentage of lymphocytes bearing the CD4 and CD45RA cell surface markers. The first of these is interesting in that at least 15% of CFS patients exhibit antinuclear antibody titers, whereas these are much less commonly found in healthy controls (3). Although a recent abstract from the La Jolla group and other participating centers (Sugiura et al., Abstr. Fifth Int. Conf. Am. Assoc. Chronic Fatigue Syndr., 2001) indicated that such results were not consistently found when patients and controls from the same geographical area were compared, the results still suggest that a small percentage of CFS patients may have a form of

mild autoimmune disease. Exactly which autoantibodies to test for to identify this subgroup of patients remains an important research question.

Considering the NK cell activity results, the critical question is whether the reported abnormalities reflect an effect of some underlying pathophysiological process involved in CFS (i.e., actual immune dysfunction) or represent epiphenomena specific to CFS. A number of variables in the characteristics of patients are known to reduce NK cell activity. These include age (33), cigarette smoking (43), stress (19), lesser fitness levels of patients relative to those of controls (20), presence of depression (22), and disrupted sleep (23). Unfortunately, none of the seven studies reporting decreased NK cell function assessed or controlled for these variables. Another possible reason for discrepancies among studies could be the time elapsed between sampling and the time of NK cell function testing. If one waits over 18 h to test fresh cells, NK cell activity can decrease by as much as 20% (73).

A number of major reasons could possibly contribute to the variability of results of the other immune variables studied. These fall within the following categories: methodological issues, issues related to the populations studied, and statistical issues.

Methodological issues are broad. First, circadian rhythms are known to exist for lymphocyte subsets, including numbers of NK cells (30). If samples were collected from patients at one time of day and from controls at another time of day, as could happen, such a systematic difference could explain results suggesting significant differences between groups. Since two of the studies reporting diminished NK cell activity sampled patients and controls within a narrow time window (39, 50), circadian factors cannot totally explain the decreases reported in NK cell function; however, using restricted sampling to reduce variability is obviously important. In addition, Cannon et al. (7) have shown that certain immune variables are sensitive to the menstrual cycle. No other group controlled for menstrual cyclicality in its study.

Next, methodological differences from an immunological or assay-related perspective might exist. Klimas notes that NK cell activity can be artificially lower if separated mononuclear cell fractions, rather than whole blood, are assessed for activity (25). However, since this group used whole blood and did find reductions in NK cell activity, this concern is probably not important. Similarly, results are thought to be most reliable when patient and control samples are tested for NK cell activity in parallel with multiple (e.g., four) effector-to-target ratios. Indeed, four of the groups reporting diminished NK cell activity did this (2, 26, 32, 50). Another possible source of variability in NK cell numbers could be whether cells were counted when fresh or after a period of cryopreservation (for an example, see reference 63). Some cell surface markers, including those expressed on NK cells, are cryosensitive (54). However, these methodological differences cannot be critical in explaining the discrepancies, because one study in which differences between CFS patients and controls in NK cell number were found counted fresh cells within a few hours of collection (66). One surprise is that it is rare for a published paper to note that samples were counted or assayed with the laboratory staff blinded to the identity of the samples and groups.

Another possible contribution to the observed variability involved the cell populations studied. First, NK activity is a function of which NK cell populations are in the circulation. CD3<sup>-</sup>/CD56<sup>+</sup> cells are those with the highest NK cell activity. While NK cell numbers were not consistently low for CFS patients, Masuda et al. did report decreases in NK cells expressing this specific phenotype (39), and such a decrease would explain their report of decreased NK cell activity. Another immunological variable which could reduce NK cell activity is low levels of IFN- $\gamma$  in the blood, as was reported by

Klimas et al. to occur in CFS (26). However, similar reductions in specific NK cell populations or in IFN- $\gamma$  levels have not been consistently reported by other research groups.

There are also issues related to the patient and comparison control subjects that could lead to discrepancies in immunological results across laboratories. Concerning patients, there are three important variables that are often used to stratify the entire CFS sample into subgroups: presence or absence of Axis I psychopathology, illness severity, and whether illness onset was sudden or gradual. Mawle et al. examined illness onset and concluded that it had no influence on NK cell activity (40). However, in other work, Mawle and our group (74) did find small differences in a few other immune parameters between CFS patients stratified based on illness onset. When illness severity was evaluated, one group found that NK cell activity decreased as illness severity increased (50), and another group reported greater IL-6 production when patients were symptomatic than when they were not (15). Besides these few reports, our group appears to be the only one to have evaluated the other stratifying variables, and no further differences from controls were found after evaluating any of these subgroups.

Another critical difference is if the patients are drawn from the civilian population or from Gulf veterans. We found some immunological differences in veterans but not in civilians; the apparent reason for this was significantly less variability in the results obtained from veterans relative to the civilians (74). This could possibly be due to the similarity of the veteran population with respect to age, education, social background, history of immunizations, etc. Concerning the control population, one critical factor would be to try to match controls to patients based on either fitness or activity. To our knowledge, our group was the only one to do this (74). Finally, decreases in NK cell function are not rare in healthy people: over 14% have consistently reduced NK cell function, and these reductions are seen mostly in young people reporting significant stress in their lives (33).

Finally, choice of statistical analyses is critical in determining the significance of a set of immunological results. If a group compares 20 different immunological parameters between patients and controls, at least one could turn out to be statistically significant merely by chance. The possibility of investigators finding a difference which is not really there has attracted little attention in the clinical immunological literature related to CFS. In fact, attention to the problem of multiple comparisons for statistically significant differences was the exception and not the rule. Another option that is now available is the use of a "neural nets" approach. This methodology provides data on a pattern of immunological parameters that differentiates the target population from the comparison population; we have done a preliminary study using this methodology in our work with CFS (17).

In summary, any further studies seeking to identify immunological abnormalities in CFS patients require careful attention to methodological issues. First, efforts should be made to reduce the heterogeneity of the patient sample; alternatively, large sample sizes are required in order to evaluate the importance of subgroups within the overall CFS population. Similarly, efforts must be made to reduce any potential major differences between patients and controls in areas such as the level of fitness and the presence of psychiatric disorders. Next, samples should be coded and, perhaps, provided as split samples to evaluate within-assay variability in a laboratory. Finally, appropriate statistical methods are required if differences between patients and controls are done on more than one immunological parameter. Our conclusion is that the available evidence does not support chronic fatigue syndrome as being due to any consistent immunological dysfunction. Until that evidence is better documented, we believe that the term "chronic fatigue syndrome" is preferable to the older "chronic fatigue and immune dysfunction syndrome."

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**Immune System Research** (source: A Hummingbirds Guide to M. E.--  
<http://www.ahummingbirdsguide.com/>)

Interleukin-1 beta, interleukin-1 receptor antagonist, and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. Cannon JG, Angel JB, Abad LW, Vannier E, Mileno MD, Fagioli L, Wolff SM, Komaroff AL. Department of Medicine, Tufts University-New England Medical Center, Boston, Massachusetts 02111, USA.

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. The signs and symptoms, which include fatigue, myalgia, and low-grade fever, are similar to those experienced by patients infused with cytokines such as interleukin-1. The present study was carried out to test the hypotheses that (1) cellular secretion of interleukin-1 beta (IL-1 beta), interleukin-1 receptor antagonist (IL-1Ra), and soluble interleukin-1 receptor type II (IL-1sRII) is abnormal in female CFS patients compared to age- and activity-matched controls; (2) that these abnormalities may be evident only at certain times in the menstrual cycle; and (3) that physical exertion (stepping up and down on a platform for 15 min) may accentuate differences between these groups. 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 ( $R^2 = 0.65$ ,  $P < 0.01$ ). IL-1Ra secretion for CFS patients was twofold higher than controls during the follicular phase ( $P = 0.023$ ), 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 ( $P = 0.002$ ). 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 ( $P = 0.020$ ). 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.

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Chronic fatigue Syndrome: evaluation of a 30 criteria-score and correlation with immune activation. Hilgers A, Frank J. *Journal of Chronic Fatigue Syndrome* 1996; 2(4): 35-47.

Abstract: OBJECTIVE. The development of a score for severity of Chronic Fatigue Syndrome (CFS), the correlation of CFS with parameters of immune activation and the association with pathogens. METHODS. Five hundred five patients with suspicion of Chronic Fatigue Syndrome and no other definitive diagnosis were checked by a 45-criteria-score, basic laboratory programs and immunological profiles. In most of the patients further tests concerning complement system, immune activation markers, hormones and serology of herpesviruses, Chlamydia and Borrelia could be evaluated. Comparison of the symptoms of CFS patients with healthy controls lead to a 30-criteria-score and this score was correlated with laboratory parameters (Spearman rank-correlation-coefficient  $r(s)$ , ties corrected). RESULTS. Three hundred eighty-five patients fulfilling stronger criteria according to the Centers for Disease Control (CDC) definition showed significant differences to 53 healthy controls in 40 of the 45 criteria ( $p < 0.001$ , twitches and food allergies  $p < 0.05$ ). Thirteen symptoms corresponding to CDC criteria were all significant ( $p < 0.001$ ), 17 further significant criteria of descending precision were added: respiratory infections, palpitations, dizziness, dyspepsia, dryness of mouth/eyes, allergies,

nausea, paresthesia, loss of hair, skin alterations, dyscoordination, chest pain, personality changes, eczema, general infections, twitches, urogenital infections. A correlation between the 30-criteria-score and immunological parameters could be evaluated in 472 of the 505 patients. Significant positive correlation with the 30-criteria-score was found in numbers of CD8+ T-lymphocytes, HLA-DR+ T-lymphocytes, gamma globulins, IgM, IgG, and for the number of types of autoantibodies (mainly ANA, ACA, antithyroid and antiparietal cell antibodies). Significant negative correlation was found in albumin-globulin-ratio, eosinophils and IgE. Most of these parameters also correlated with one another. On the other hand, in subgroups of the 505 patients the frequency of positivity in serological tests for HHV-6 (49.9%), EBV (35.4%), HSV (29.2%), CMV (12.5%) and Chlamydia (35.0%) was striking. Borrelia Western blots showed 3 or more specific IgG-bands in 54 of 131 patients (41.2%). In some cases infection with EBV, HHV-6 and CMV, respectively, was confirmed by DNA-PCR-test and antigen detection. SUMMARY. In increasingly larger groups of patients with CFS and related constellations we often see clinical signs and longer anamnesis of other symptoms besides the classical criteria of CFS, especially a high prevalence of local and general susceptibility to infections and hints to prolonged inflammation processes. Together with other results, the data confirm the hypothesis that a reduced or unstable immune control or delayed immune reaction to persisting viruses or bacterial intracellular pathogens, possibly triggered by common infections or other environmental factors, can lead to a chronic neuroimmune activation state and auto-immune disorders. Hypersensitivity symptoms of the patients might not be mediated by classical allergies alone but also result from a type-IV-hypersensitivity.

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Immunologic Status Correlates with Severity of Physical Symptoms and Perceived Illness Burden in Chronic Fatigue Syndrome Patients Stacy E. Cruess, PhD; Nancy Klimas, MD; Michael H. Antoni, PhD; Lynn Helder, PhD; Kevin Maher, PhD; Robert Keller, MD; Mary Ann Fletcher, PhD *Journal of Chronic Fatigue Syndrome*, Vol. 7(1) 2000 pp. 39-52 Affiliations: Stacy E. Cruess and Michael H. Antoni are affiliated with the Department of Psychology, University of Miami. Michael H. Antoni is also with the Department of Psychiatry and Behavioral Sciences, University of Miami. Nancy Klimas, Kevin Maher and Mary Ann Fletcher are affiliated with the Department of Medicine, University of Miami. Lynn Helder and Robert Keller are affiliated with Biororan Medical Center, Ft. Lauderdale, FL. Address correspondence to: Nancy Klimas, MD, 200 BMRC - Section 6D, c/o VA Medical Center, 1201 NW 16th Street, Miami, FL 33125.

ABSTRACT. The purpose of the present study was to investigate the relationship between immunologic status and physical symptoms in Chronic Fatigue Syndrome (CFS) patients. Twenty-seven patients diagnosed with CFS were included. Participants completed a questionnaire including selected subscales of the Sickness Impact Profile, the Cognitive Difficulties Scale, and frequency and severity of CFS-related physical symptoms. Cellular immune markers measured included number and percent of T-helper/inducer cells (CD3+CD4+), T-cytotoxic/ suppressor cells (CD3+CD8+), activated T-lymphocytes (CD26+CD2+ CD3+), activated T cytotoxic/suppressor cells (CD38÷HLA-DR+CD8+), and CD4/CD8 ratio. Spearman's correlation coefficients revealed significant associations between a number of immunologic measures and severity of illness suggesting that the degree of cellular immune activation was associated with the severity of CFS-related physical symptoms, cognitive complaints, and perceived impairment secondary to CFS. Specifically, elevations in T-

helper/inducer cells, activated T-cells, activated cytotoxic/suppressor T-cells, and CD4/CD8 ratio were associated with greater severity of several symptoms. Furthermore, reductions in T-suppressor/cytotoxic cells also appeared related to greater severity of some CFS-related physical symptoms and illness burden. Multiple regression analyses demonstrated that decreased percentage of CD3+CD8+ cells and increased number of CD38+HLA-DR+CD8+ cells were the strongest predictors of total illness burden and fatigue severity, accounting for almost 30% of the variance in these measures.

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Chronic fatigue syndrome: intracellular immune deregulations as a possible etiology for abnormal exercise response Jo Nijs(a,b,\*), Kenny De Meirleir(a,c), Mira Meeus(a), Neil R. McGregor(d,e), Patrick Englebienne(f,g) a Department of Human Physiology, Faculty of Physical Education and Physical Therapy Science, Vrije Universiteit Brussel (VUB), Brussel 1090, Belgium b Institute for Occupational and Physical Therapy, Department of Health Sciences, Hogeschool Antwerpen, Belgium c Chronic Fatigue Clinic, Vrije Universiteit Brussel (VUB), Belgium d Bio21, Institute of Biomedical Research, University of Melbourne, Parkville, Victoria 3000, Australia e Dental Clinical School, Westmead Hospital, Westmead, Australia f Department of Nuclear Medicine, Universit e Libre de Bruxelles (ULB), Belgium g RED Laboratories N. V., Zellik, Belgium \* Corresponding author. Present address: Vakgroep MFYS/Sportgeneeskunde, AZ-VUB KRO gebouw - 1, Laarbeeklaan 101, 1090 Brussel, Belgium. Tel.: +32-2-477-4604; fax: +32-2-477-4607. E-mail address: jo.nijs@vub.ac.be Received 1 October 2003; accepted 9 November 2003 Source: Medical Hypotheses Vol 62, #5, pp 759-765 Date: April 2004 URL: <http://www.sciencedirect.com/science/journal/03069877>

**Summary** The exacerbation of symptoms after exercise differentiates Chronic fatigue syndrome (CFS) from several other fatigue-associated disorders. Research data point to an abnormal response to exercise in patients with CFS compared to healthy sedentary controls, and to an increasing amount of evidence pointing to severe intracellular immune deregulations in CFS patients. This manuscript explores the hypothetical interactions between these two separately reported observations. First, it is explained that the deregulation of the 2-5A synthetase/RNase L pathway may be related to a channelopathy, capable of initiating both intracellular hypomagnesaemia in skeletal muscles and transient hypoglycemia. This might explain muscle weakness and the reduction of maximal oxygen uptake, as typically seen in CFS patients. Second, the activation of the protein kinase R enzyme, a characteristic feature in atleast subsets of CFS patients, might account for the observed excessive nitric oxide (NO) production in patients with CFS. Elevated NO is known to induce vasidilation, which may limit CFS patients to increase blood flow during exercise, and may even cause and enhanced postexercise hypotension. Finally, it is explored how several types of infections, frequently identified in CFS patients, fit into these hypothetical pathophysiological interactions.

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Mitochondrial abnormalities in the postviral fatigue syndrome. Behan WM, More IA, Behan PO  
Department of Pathology, University of Glasgow, Scotland. *Acta Neuropathol* 1991;83(1):61-5

We have examined the muscle biopsies of 50 patients who had postviral fatigue syndrome (PFS) for from 1 to 17 years. We found mild to severe atrophy of type II fibres in 39 biopsies, with a mild to moderate excess of lipid. On ultrastructural examination, 35 of these specimens showed branching and fusion of mitochondrial cristae. Mitochondrial degeneration was obvious in 40 of the biopsies with swelling, vacuolation, myelin figures and secondary lysosomes. These abnormalities were in obvious contrast to control biopsies, where even mild changes were rarely detected. The findings described here provide the first evidence that PFS may be due to a mitochondrial disorder precipitated by a virus infection.

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Is chronic fatigue syndrome an autoimmune disorder of endogenous neuropeptides, exogenous infection and molecular mimicry? Staines DR. Gold Coast Public Health Unit, 10-12 Young Street, Southport 4215, Qld, Australia. don\_staines@health.qld.gov.au

Chronic fatigue syndrome is a disorder characterised by prolonged fatigue and debility and is mostly associated with post-infection sequelae although ongoing infection is unproven. Immunological aberration is likely and this may prove to be associated with an expanding group of vasoactive neuropeptides in the context of molecular mimicry and inappropriate immunological memory. Vasoactive neuropeptides including vasoactive intestinal peptide (VIP) and pituitary adenylate activating polypeptide (PACAP) belong to the secretin/glucagon superfamily and act as hormones, neurotransmitters, immune modulators and neurotrophes. They are readily catalysed to smaller peptide fragments by antibody hydrolysis. They and their binding sites are immunogenic and are known to be associated with a range of autoimmune conditions. Vasoactive neuropeptides are widely distributed in the body particularly in the central, autonomic and peripheral nervous systems and have been identified in the gut, adrenal gland, reproductive organs, vasculature, blood cells and other tissues. They have a vital role in maintaining vascular flow in organs, and in thermoregulation, memory and concentration. They are co-transmitters for acetylcholine, nitric oxide, endogenous opioids and insulin, are potent immune regulators with primarily anti-inflammatory activity, and have a significant role in protection of the nervous system to toxic assault, promotion of neural development and the maintenance of homeostasis. This paper describes a biologically plausible mechanism for the development of CFS based on loss of immunological tolerance to the vasoactive neuropeptides following infection, significant physical exercise or de novo. It is proposed that release of these substances is accompanied by a loss of tolerance either to them or their receptor binding sites in CFS. Such an occurrence would have predictably serious consequences resulting from compromised function of the key roles these substances perform. All documented symptoms of CFS are explained by vasoactive neuropeptide compromise, namely fatigue and nervous system dysfunction through impaired acetylcholine activity, myalgia through nitric oxide and endogenous opioid dysfunction, chemical sensitivity through peroxynitrite and adenosine dysfunction, and immunological disturbance through changes in immune modulation. Perverse immunological memory established against these substances or their receptors may be the reason for the protracted nature of this condition. The novel status of these substances together with their extremely small concentrations in blood and tissues means that clinical research into them is still in its infancy. A biologically plausible theory of CFS

causation associated with vasoactive neuropeptide dysfunction would promote a coherent and systematic approach to research into this and other possibly associated disabling conditions.

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Clinical and Immunologic Effects of Autologous Lymph Node Cell Transplant in Chronic Fatigue Syndrome Nancy G. Klimas, MD; Roberto Patarca-Montero, MD, PhD; Kevin Maher, PhD; Mack Smith, RN, ARNP; Oliver Bathe, MD; Mary Ann Fletcher, PhD *Journal of Chronic Fatigue Syndrome*, Vol. 8(1) 2001, pp. 39-55 Nancy G. Klimas, Roberto Patarca-Montero, Kevin Maher, and Mary Ann Fletcher are Directors, E. M. Papper Laboratory of Clinical Immunology and Researchers at the Center for Behavioral Medicine Research, Miami Veterans Administration Medical Center, University of Miami School of Medicine, PO. Box 016960 (R-42), Miami, FL 33101. Mack Smith is Research Nurse at the Center for Behavioral Medicine Research. Oliver Bathe is affiliated with the Department of Surgery, Tom Baker Cancer Centre, University of Calgary, 1331/29th Street North West, Calgary, AB, T2N 4N2, Canada. Address correspondence to: Nancy G. Klimas at the above address (E-mail: Nancy.Klimas@med.va.gov ). This work was supported, in part, by a grant from the CFIDS Association of America, by NIH Center Grant 1UD1-AI 45940-02, and by funds from Neoprobe Corp. and Ciratech Corp.

**ABSTRACT:** An open labeled, phase 1, safety and feasibility study using lymph node extraction, ex vivo lymph node cell expansion, followed by autologous cell reinfusion was evaluated as a potential immunomodulatory treatment strategy in patients with chronic fatigue syndrome (CFS). The experimental therapy utilized the cells of the lymph node, activated and grown in culture with defined media, interleukin-2 (IL-2) and anti-CD3 to activate and enhance cellular immunological functions. This procedure was designed to change the cytokine pattern of the lymph node lymphocytes to favor expression of T-helper (Th)1-type over Th2-type cytokines. The mixed population of ex vivo immune-enhanced cells were reinfused into the donor, who was carefully monitored for adverse events and possible clinical benefit. There were no adverse events. There were significant improvements in clinical status in association with a significant decrease in Th2-type cytokine production.

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Disturbed neuroendocrine-immune interactions in chronic fatigue syndrome. Kavelaars A, Kuis W, Knook L, Sinnema G, Heijnen CJ *J Clin Endocrinol Metab* 2000 Feb;85(2):692-6 Department of Pediatric Immunology, Wilhelmina Children's Hospital of the University Medical Center Utrecht, The Netherlands. a.kavelaars@wkgz.azu.nl PMID: 10690878, UI: 20152737

The present study was designed to investigate the interaction between neuroendocrine mediators and the immune system in chronic fatigue syndrome (CFS). We examined the sensitivity of the immune system to the glucocorticoid agonist dexamethasone and the beta2-adrenergic agonist terbutaline in 15 adolescent girls with CFS and 14 age- and sex-matched controls. Dexamethasone inhibits T-cell proliferation in healthy controls and in CFS patients.

However, the maximal effect of dexamethasone on T-cell proliferation is significantly reduced in CFS patients as compared with controls. The beta2-adrenergic receptor agonist terbutaline inhibits tumor necrosis factor-alpha production and enhances interleukin-10 production by monocytes. Our data demonstrate that the capacity of a beta2-adrenergic agonist to regulate the production of these two cytokines is also reduced in CFS patients. We did not observe differences in baseline or CRH-induced cortisol and ACTH between CFS patients and controls. Baseline noradrenaline was similar in CFS and controls, whereas baseline adrenaline levels were significantly higher in CFS patients. We conclude that CFS is accompanied by a relative resistance of the immune system to regulation by the neuroendocrine system. Based on these data, we suggest CFS should be viewed as a disease of deficient neuroendocrine-immune communication.

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Comparative Analysis of Lymphocytes in Lymph Nodes and Peripheral Blood of Patients with Chronic Fatigue Syndrome Mary Ann Fletcher, PhD; Kevin Maher, PhD; Roberto Patarca-Montero, MD, PhD; Nancy Klimas, MD *Journal of Chronic Fatigue Syndrome*, Vol. 7(3) 2000, pp. 65-75 Department of Medicine, University of Miami School of Medicine and the Miami VA Medical Center. Address correspondence to: Mary Ann Fletcher, E.M. Papper Laboratory of Clinical Immunology - R-42, R.M.S.B. Room 8168, 1600 NW 10th Avenue, Miami, FL 33136 (E-mail: mfletche@med.miami.edu).

This work was supported, in part, by a grant from the CFIDS Association of America, by NIH Center Grant 1UDI-AI 45940-02, and by funds from Neoprobe Corporation and Ciratech Corporation.

#### ABSTRACT.

Blood and lymph node samples were obtained from patients with chronic fatigue syndrome (CFS) who had volunteered to undergo a lymph node biopsy while participating in a phase 1 clinical trial of a novel immunomodulatory therapy. The surface marker phenotypes of the peripheral blood and lymph node samples were examined using four-color flow cytometry and compared to published proportions of cells in peripheral blood and lymph nodes from control individuals. While a greater proportion of T lymphocytes from both lymph nodes and peripheral blood of control subjects are immunologically "naive" (CD45RA+), the proportions of lymphocytes with a "memory" phenotype predominate in lymph nodes and peripheral blood of CFS patients. CFS has been proposed to be a disease of autoimmune etiology and in this respect it is interesting to note that decreased proportions of CD45RA+ T ("naive") cells are also seen in the peripheral blood of patients with autoimmune diseases.

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Detection of Immunologically Significant Factors for Chronic Fatigue Syndrome Using Neural-Network Classifiers. Hanson SJ, Gause W, Natelson B. *Clin Diagn Lab Immunol* 2001 May;8(3):658-662 Rutgers University, Newark, New Jersey. PMID: 11329477

Neural-network classifiers were used to detect immunological differences in groups of chronic fatigue syndrome (CFS) patients that heretofore had not shown significant differences from controls. In the

past linear methods were unable to detect differences between CFS groups and non-CFS control groups in the nonveteran population.

An examination of the cluster structure for 29 immunological factors revealed a complex, nonlinear decision surface. Multilayer neural networks showed an over 16% improvement in an n-fold resampling generalization test on unseen data. A sensitivity analysis of the network found differences between groups that are consistent with the hypothesis that CFS symptoms are a consequence of immune system dysregulation. Corresponding decreases in the CD19(+) B-cell compartment and the CD34(+) hematopoietic progenitor subpopulation were also detected by the neural network, consistent with the T-cell expansion.

Of significant interest was the fact that, of all the cytokines evaluated, the only one to be in the final model was interleukin-4 (IL-4). Seeing an increase in IL-4 suggests a shift to a type 2 cytokine pattern. Such a shift has been hypothesized, but until now convincing evidence to support that hypothesis has been lacking.

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Directions in Immunotherapy Roberto Patarca-Montero, MD, PhD The CFS Research Review, Winter 2001 University of Miami School of Medicine

In a subset of chronic fatigue syndrome (CFS) patients, the immune system is always activated. Although it is unknown why this happens, one hypothesis is that it is caused by a lingering infection or an infection that leaves an autoimmune sequelae.

Although the immune systems of some CFS patients are chronically activated, parts function poorly, particularly the T cells (the "generals" of the immune system army) and natural killer cells (destroyers of infected or cancerous cells). CFS patients' T cells have a decreased capacity to divide and generate new T cells, and their natural killer cells have significantly decreased cytotoxic activity.

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Elevated Peroxynitrite as the cause of chronic fatigue syndrome: Other Inducers and Mechanisms of Symptom Generation Martin L Pall School of Molecular Biosciences, Washington State University, Pullman, WA. Source: J Chronic Fatigue Syndrome 2000; 7(4):45-58.

Abstract: In an earlier paper, I proposed that chronic fatigue syndrome (CFS) is caused by a response to infection, involving the induction of inflammatory cytokines which induce, in turn, the inducible nitric oxide synthase, producing elevated nitric oxide. Nitric oxide reacts with superoxide to form the potent oxidant, peroxynitrite. Six positive feedback loops were proposed by which peroxynitrite may stay elevated, acting to increase levels of both nitric oxide and superoxide, which react to form more peroxynitrite. This vicious cycle based on known biochemistry is proposed to be the central cause of CFS. The current paper discusses additional inducers which may act by increasing nitric oxide

(physical or psychological trauma) or increasing superoxide (hypoxia) and the role of orthostatic intolerance, Ehlers-Danlos syndrome, excessive exercise, exercise intolerance and carbon monoxide in inducing hypoxia and consequently superoxide and peroxynitrite. The major symptoms of CFS can all be interpreted as relatively direct consequences of the pathophysiology predicted by the elevated peroxynitrite theory of CFS. Attractive mechanisms are proposed by which elevated peroxynitrite, nitric oxide and/or related physiological changes may induce CFS symptoms including fatigue, immune dysfunction, learning and memory dysfunction, multi-organ pain, exercise intolerance/postexertional malaise and orthostatic intolerance. Roles are discussed for six factors likely to influence the frequency of CFS induction in response to infection or other inducing events.

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Prevalence of abnormal cardiac wall motion in the cardiomyopathy associated with incomplete multiplication of Epstein-barr Virus and/or cytomegalovirus in patients with chronic fatigue syndrome. Lerner AM, Dworkin HJ, Sayyed T, Chang CH, Fitzgerald JT, Beqaj S, Deeter RG, Goldstein J, Gottipolu P, O'Neill W. Department of Medicine, William Beaumont Hospital, Royal Oak, Michigan, USA. lerner@cdimed.com

We reported unique incomplete herpesvirus (Epstein-Barr Virus (EBV) and/or nonstructural (HCMV) cytomegalovirus) multiplication in 2 distinct subsets of CFS patients. The CFS subsets were identified by: a) presence of IgM serum antibodies to HCMV nonstructural gene products p52 and CM2 (UL44 and UL57), and/or b) IgM serum antibodies to Epstein-Barr virus viral capsid antigen (EBV, VCA IgM). Diagnostic IgM serum antibodies were found in two independent blinded studies involving 49 CFS patients, but the same antibodies were absent in 170 control patients ( $p < 0.05$ ). Abnormal 24 Hr-electrocardiographic monitoring, tachycardias at rest and, in severe chronic cases, abnormal cardiac wall motion (ACWM) were seen in these same CFS patients. We now report a prospective consecutive case control study from 1987--1999 of cardiac dynamics as measured by radionuclide ventriculography in 98 CFS patients from 1987--1999. Controls were patients with various malignancies who were evaluated in protocols requiring radionuclide ventriculography before initiation of cardiotoxic chemotherapeutic agents. The prevalence of abnormal cardiac wall motion (ACWM) at rest in CFS patients was 10 out of 87 patients (11.5%). With stress exercise, 21 patients (24.1%) demonstrated ACWM. Cardiac biopsies in 3 of these CFS patients with ACWM showed a cardiomyopathy. Among the controls, ACWM at rest was present in 4 out of 191 patients (2%) ( $p = 0.0018$ ). A progressive cardiomyopathy caused by incomplete virus multiplication of EBV and/or HCMV in CFS patients is present.

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Relationships of cognitive difficulties to immune measures, depression and illness burden in CFS. Lutgendorf S, Klimas NG, Antoni M, Brickman A, Fletcher MA University of Miami School of Medicine, Miami, Florida, USA. Journal of Chronic Fatigue Syndrome 1995; 1(2): 23-41.

Abstract: OBJECTIVE. We related the subjective assessment of cognitive difficulties with lymphocyte phenotypes, cell-mediated immunity (CMI), cytokine and neopterin levels in patients with chronic fatigue syndrome (CFS), in order to determine if CFS patients complaining of greater cognitive difficulties would show greater impairments in cell-mediated immunity and a greater degree of immune system dysregulation, and to determine if these cognitive difficulties would correlate with the other non-affective measures of CFS-associated illness burden. We also assessed whether these relationships would hold independent of depression in two ways, by statistically covarying depression severity scores and by comparing subsets of CFS patients with and without a concurrent diagnosis of major depressive disorder. DESIGN. A case series of CFS patients. SETTING. Outpatient tertiary referral clinic at the University of Miami School of Medicine, Miami, FL. PATIENTS. Consecutive sample of 65 patients who were referred as CFS to the University of Miami Diagnostic Immunology Clinic, who met the Centers for Disease Control and Prevention (CDC) criteria for diagnosis of CFS and consented to participate. MAIN MEASURES. Self-assessment of cognitive difficulties, depression and illness burden, clinician-assessed depression and CFS symptoms, lymphocyte phenotype, proliferative response to mitogens, serum levels of cytokines and neopterin. RESULTS. Among CFS patients, high Cognitive Difficulty Scale (CDS) scores were significantly related to lower lymphocyte proliferative responses to mitogens, higher neopterin levels, and higher CD4 and lower CD8 lymphocyte counts. These relationships, with the exception of T cell subset percentages, were maintained when depression severity was used as a co-variate. High CDS scores were also significantly related to lower Karnofsky scores, and greater illness burden as measured by the Sickness Impact Profile. Evidence is presented that CFS patients with higher cognitive difficulty scores have more immune and clinical dysfunction than those with less cognitive difficulty, and that these relationships are independent of depression. These observations provide support for the concept that although both cognitive difficulties and immunologic abnormalities, such as immune activation and impaired cell-mediated immunity, may represent secondary sequelae to the same event(s), they are not likely to be secondary sequelae to depression.

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Elevated apoptotic cell population in patients with chronic fatigue syndrome: the pivotal role of protein kinase RNA Vojdani A; Ghoneum M; Choppa PC; Magtoto L; Lapp CW Immunosciences Laboratory Inc., Beverly Hills, California, USA. J Intern Med 1997 Dec;242(6):465-78 (ISSN: 0954-6820)

OBJECTIVES: A prominent feature of chronic fatigue syndrome (CFS) is a disordered immune system. Recent evidence indicates that induction of apoptosis might be mediated in a dysregulated immune system by the upregulation of growth inhibitory cytokines. Therefore, the purpose of this study was to evaluate the apoptotic cell population, interferon-alpha (IFN-alpha) and the IFN-induced protein kinase RNA (PKR) gene transcripts in peripheral blood lymphocytes (PBL) of CFS individuals, as compared to healthy controls. SUBJECTS AND METHODS: PBL were isolated from CFS (n = 29) and healthy control individuals (n = 15) and subjected to quantitative analysis of apoptotic cell population and cell cycle progression by flow cytometry. Quantitative competitive polymerase chain reaction (Q/C PCR) and Western blot analysis were used to assess the levels of PKR mRNA and protein in control and CFS individuals. In addition, circulating IFN-alpha was measured by ELISA assay. RESULTS: Increased apoptotic cell population was observed in CFS individuals, as compared to healthy controls (26.6 +/- 12.9% and 9.9 +/- 4.2%, respectively). 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 (8.6 +/- 1.2 to 22.8 +/- 2.4 and 3.6 +/- 0.82 to 24.3 +/- 3.4, respectively). In addition, CFS individuals exhibited enhanced PKR mRNA and protein levels (mean basal level 3538 +/- 1050 and 2.7 +/- 0.26, respectively) as compared to healthy controls (mean basal level 562 +/- 162 and 0.89 +/- 0.18, respectively). In 50% of the CFS samples (n = 29) treated with 2-aminopurine (2-AP) (a potent inhibitor of PKR) the apoptotic population was reduced by more than 50%. CONCLUSIONS: PKR-mediated apoptosis in CFS individuals may contribute to the pathogenesis and the fatigue symptomatology associated with CFS.

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Role of cysteine and glutathione in HIV infection and other diseases associated with muscle wasting and immunological dysfunction W Droge and E Holm Division of Immunochemistry, Deutsches Krebsforschungszentrum, Heidelberg, Germany. The FASEB Journal, 1997 Vol 11, 1077-1089  
REVIEW

The combination of abnormally low plasma cystine and glutamine levels, low natural killer (NK) cell activity, skeletal muscle wasting or muscle fatigue, and increased rates of urea production defines a complex of abnormalities that is tentatively called "low CG syndrome." These symptoms are found in patients with HIV infection, cancer, major injuries, sepsis, Crohn's disease, ulcerative colitis, chronic fatigue syndrome, and to some extent in overtrained athletes. The coincidence of these symptoms in diseases of different etiological origin suggests a causal relationship. The low NK cell activity in most cases is not life-threatening, but may be disastrous in HIV infection because it may compromise the initially stable balance between the immune system and virus, and trigger disease progression. This hypothesis is supported by the coincidence observed between the decrease of CD4+ T cells and a decrease in the plasma cystine level. In addition, recent studies revealed important clues about the role of cysteine and glutathione in the development of skeletal muscle wasting. Evidence suggests that 1) the cystine level is regulated primarily by the normal postabsorptive skeletal muscle protein catabolism, 2) the cystine level itself is a physiological regulator of nitrogen balance and body cell mass, 3) the cyst(e)ine-mediated regulatory circuit is compromised in various catabolic conditions, including old age, and 4) cysteine supplementation may be a useful therapy if combined with disease-specific treatments such as antiviral therapy in HIV infection.

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Clinical Improvement in Chronic Fatigue Syndrome Is Associated with Enhanced Natural Killer Cell-Mediated Cytotoxicity: The Results of a Pilot Study with Isoprinosine® Francisco Diaz-Mitoma MD, PhD, FRCP(C), Chief and Professor, Children's Hospital of Eastern Ontario, Ottawa, ON, K1H 8L1, Canada, K1H 8L1 Eva Turgonyi MD, Newport Pharmaceuticals Ltd., Franz Maas House Swords Business Park, Swords, Co., Dublin, Ireland Ashok Kumar PhD, Children's Hospital of Eastern Ontario, Ottawa, ON, K1H 8L1, Canada Wilfred Lim, Division of Virology, Children's Hospital of Eastern Ontario, Ottawa, ON, K1H 8L1, Canada Louise Larocque, Division of Virology, Children's

Hospital of Eastern Ontario, 401 Smyth Road, Ottawa, ON, K1H 8L1, Canada Byron M. Hyde MD,  
The Nightingale Research Foundation, 121 Iona Street. Ottawa, Canada K1Y 3M1

Abstract: Chronic fatigue syndrome is associated with systemic and cognitive symptoms and with several immune abnormalities. The clinical impact of Isoprinosine® was evaluated in sixteen CFS patients, followed for 28 weeks in a single-blind, placebo controlled trial. Patients were also monitored for various immune parameters. Improvement based on clinical staging was observed in six of ten treated patients (60%). Clinically improved patients showed significantly enhanced natural killer (NK) cell activity, which correlated with the duration of Isoprinosine treatment ( $p < 0.03$ ). Treatment with Isoprinosine resulted in significantly increased numbers of CD4+ T helper cells ( $p < 0.03$ ). Treatment with Isoprinosine for 12 weeks did not appreciably influence the in vitro production of IFN- $\gamma$ , IL-1 $\alpha$ , IL-10 or IL-12. However, IL-12 was significantly increased at week 28 ( $p < 0.02$ ) in patients who improved after treatment with Isoprinosine. These results suggest that taking Isoprinosine may benefit a subgroup of patients with CFS, and this clinical improvement is associated with enhanced NK cell function and IL-12 levels. Further trials to evaluate the use of Isoprinosine in the treatment of CFS patients are warranted

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Monitoring a Hypothetical Channelopathy in Chronic Fatigue Syndrome: Preliminary Observations  
Journal of Chronic Fatigue Syndrome: Multidisciplinary Innovations in Research, Theory, and Clinical Practice  
Jo Nijs MSc, Department of Human Physiology, Faculty of Physica Christian Demanet MD, PhD, Division of Hematology and Immunology, Academic Ho Neil R. McGregor BDS, MSc, PhD, Collaborative Pain Research Unit, Department of Bio Pascale De Becker, Department of Human Physiology, Faculty of Physica Michel Verhas MD, PhD Patrick Englebienne PhD Kenny De Meirleir MD PhD, Department of Human Physiology  
Volume: 11 Issue: 1 ISSN: 1057-3321 Pub Date: 1/23/2003

Abstract: This study was aimed at monitoring of a previously suggested channelopathy in Chronic Fatigue Syndrome, and at searching for possible explanations by means of immune system characteristics. Twenty-seven CFS patients and 20 age and sex matched healthy volunteers were recruited. RNase L-ratio, percent of the norm of whole body potassium content, serum electrolytes (sodium, calcium and potassium), immune cells, blood cell count and erythrocyte sedimentation rate were determined. More than fifty percent of our patients presented with abnormal whole body potassium content. Eight patients had increased, while six had depleted potassium content. Discriminant function analysis revealed that the CFS patients and control subjects could be differentiated on immunophenotyping with the predominant cell differences being the increase in CD19+ CD5+ (mature B-) cells and the decrease in CD3CD16+ CD56+ (NK) cells in both the percentage and count distributions. The fall in NK-cells was very strongly associated with increases in the RNase L-ratio and falls in serum calcium levels. In addition, four patients with low serum calcium levels showed lower whole body potassium levels. In conclusion, these observations suggest a channelopathy in a subset of CFS patients, probably induced by the deregulated 2-5A RNase L antiviral pathway

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Neuroimmune mechanisms in health and disease: Part 2. Anisman H, Baines MG, Berczi I, Bernstein CN, Blennerhassett MG, Gorczynski RM. Canadian Medical Association Journal 1996; 155(8): 1075-82.

Abstract: In the second part of their article on the emerging field of neuroimmunology, the authors present an overview of the role of neuroimmune mechanisms in defence against infectious diseases and in immune disorders. During acute febrile illness, immune-derived cytokines initiate an acute phase response, which is characterized by fever, inactivity, fatigue, anorexia and catabolism. Profound neuroendocrine and metabolic changes take place: acute phase proteins are produced in the liver, bone marrow function and the metabolic activity of leukocytes are greatly increased, and specific immune reactivity is suppressed. Defects in regulatory processes, which are fundamental to immune disorders and inflammatory diseases, may lie in the immune system, the neuro endocrine system or both. Defects in the hypothalamus-pituitary-adrenal axis have been observed in autoimmune and rheumatic diseases, chronic inflammatory disease, chronic fatigue syndrome and fibromyalgia. Prolactin levels are often elevated in patients with systemic lupus erythematosus and other autoimmune diseases, whereas the bioactivity of prolactin is decreased in patients with rheumatoid arthritis. Levels of sex hormones and thyroid hormone are decreased during severe inflammatory disease. Defective neural regulation of inflammation likely plays a pathogenic role in allergy and asthma, in the symmetrical form of rheumatoid arthritis and in gastrointestinal inflammatory disease. A better understanding of neuroimmunoregulation holds the promise of new approaches to the treatment of immune and inflammatory diseases with the use of hormones, neurotransmitters, neuropeptides and drugs that modulate these newly recognized immune regulators.

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Low NK syndrome and its relationship to chronic fatigue syndrome. Aoki T, Miyakoshi H, Usuda Y, Herberman RB. Clinical Immunology and Immunopathology 1993; 69(3): 253-65.

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Immunologic abnormalities associated with chronic fatigue syndrome. Barker E, Fujimura SF, Fadem MB, Landay AL, Levy JA. Clinical Infectious Diseases 1994; 18(Supp 1): S136-41.

Abstract: Several aspects of cellular immunity in patients with clinically defined chronic fatigue syndrome (CFS) were evaluated and compared with those in healthy individuals. Flow cytometric analyses revealed normal expression of total T (CD3+), B (CD19+), and NK (natural killer) (CD16+, CD56+) markers on the surface of peripheral blood mononuclear cells (PMC) from patients with CFS. However, compared with those of healthy individuals, patients' CD8+ T cells expressed reduced levels of CD11b and expressed the activation markers CD38 and HLA-DR at elevated levels. In many of the individuals in whom expression of CD11b was reduced the expression of CD28 was increased. These

findings indicate expansion of a population of activated CD8+ cytotoxic T lymphocytes. A marked decrease in NK cell activity was found in almost all patients with CFS, as compared with that in healthy individuals. No substantial abnormalities in monocyte activity or T cell proliferation were observed. The results of this study suggest that immune cell phenotype changes and NK cell dysfunction are common manifestations of CFS.

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The postviral fatigue syndrome - an analysis of the findings in 50 cases. Behan PO, et al. *Journal of Infection* 1985; 10: 211-22.

**Abstract:** The clinical, pathological, electrophysiological, immunological and virological abnormalities in 50 patients with the postviral fatigue syndrome are recorded. These findings confirm the organic nature of the disease. A metabolic disorder, caused by persistent virus infection and associated with defective immunoregulation, is suggested as the pathogenetic mechanism.

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A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active human herpesvirus type 6 infection. Buchwald D, Cheney PR, Peterson DL, Henry B, Wormsley SB, Geiger A, Ablashi DV, Salahuddin SZ, Saxinger C, Biddle R, et al. *Annals of Internal Medicine* 1992; 116(2): 103-13.

**Abstract:** **OBJECTIVE:** To conduct neurologic, immunologic, and virologic studies in patients with a chronic debilitating illness of acute onset. **DESIGN:** Cohort study with comparison to matched, healthy control subjects. **PATIENTS:** We studied 259 patients who sought care in one medical practice; 29% of the patients were regularly bedridden or shut-in. **MAIN OUTCOME MEASURES:** Detailed medical history, physical examination, conventional hematologic and chemistry testing, magnetic resonance imaging (MRI) studies, lymphocyte phenotyping studies, and assays for active infection of patients' lymphocytes with human herpesvirus type 6 (HHV-6). **MAIN RESULTS:** Patients had a higher mean ( $\pm$  SD) CD4/CD8 T-cell ratio than matched healthy controls ( $3.16 \pm 1.5$  compared with  $2.3 \pm 1.0$ , respectively;  $P$  less than 0.003). Magnetic resonance scans of the brain showed punctate, subcortical areas of high signal intensity consistent with edema or demyelination in 78% of patients (95% CI, 72% to 86%) and in 21% of controls (CI, 11% to 36%) ( $P$  less than  $10^{-9}$ ). Primary cell culture of lymphocytes showed active replication of HHV-6 in 79 of 113 patients (70%; CI, 61% to 78%) and in 8 of 40 controls (20%; CI, 9% to 36%) ( $P$  less than  $10^{-8}$ ), a finding confirmed by assays using monoclonal antibodies specific for HHV-6 proteins and by polymerase chain reaction assays specific for HHV-6 DNA. **CONCLUSIONS:** Neurologic symptoms, MRI findings, and lymphocyte phenotyping studies suggest that the patients may have been experiencing a chronic, immunologically mediated inflammatory process of the central nervous system. The active replication of HHV-6 most likely represents reactivation of latent infection, perhaps due to immunologic dysfunction. Our study did not directly address whether HHV-6, a lymphotropic and gliotropic virus, plays a role in producing the symptoms or the immunologic and neurologic dysfunction seen in this illness. Whether the

findings in our patients, who came from a relatively small geographic area, will be generalizable to other patients with a similar syndrome remains to be seen.

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A Chronic "postinfectious" fatigue syndrome associated with benign lymphoproliferation, B-cell proliferation, and active replication of human herpesvirus-6. Buchwald D, Freedman AS, Ablashi DV, Sullivan JL, Caligiuri M, Weinberg DS, Hall CG, Ashley RL, Saxinger C, Balachandran N, Ritz J, Nadler LM, Komaroff AL. *Journal of Clinical Immunology* 1990; 10(6): 335-343.

Abstract: A 17-year-old, previously healthy woman developed an acute "mononucleosis-like" illness with an associated "atypical" pneumonitis, followed by years of debilitating chronic fatigue, fevers, a 10-kg weight loss, night sweats, and neurocognitive symptoms. Thereafter, her sister developed a similar but less severe illness. The patient developed marked, chronic lymphadenopathy and splenomegaly, with associated persistent relative lymphocytosis and atypical lymphocytosis and with thrombocytopenia. After 3 years of illness, a splenectomy was performed, which resulted in some symptomatic improvement, prompt weight gain, and resolution of all hematologic abnormalities. Serial immunologic studies revealed a strikingly elevated number of activated B lymphocytes and a T lymphopenia, which improved but did not return to normal postsplenectomy. No causal association was found with any of several infectious agents that could produce such a lymphoproliferative illness. However, both the patient and her sister had evidence of active infection with the recently discovered human herpesvirus-6. Seven years after the onset of the illness, the patient and her sister remain chronically ill.

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Review of laboratory findings for patients with chronic fatigue syndrome. Buchwald D, Komaroff AL. *Reviews of Infectious Diseases* 1991; 13(Suppl 1): S12-S28.

Abstract: Various abnormalities revealed by laboratory studies have been reported in adults with chronic fatigue syndrome. Those most consistently reported include depressed natural killer cell function and reduced numbers of natural killer cells; low levels of circulating immune complexes; low levels of several autoantibodies, particularly antinuclear antibodies and antithyroid antibodies; altered levels of immunoglobulins; abnormalities in number and function of lymphocytes; and modestly elevated levels of two Epstein-Barr virus-related antibodies, immunoglobulin G to viral capsid antigen and to early antigen.

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Eosinophil cationic protein serum levels and allergy in chronic fatigue syndrome. Conti F, Magrini L, Priori R, Valesini G, Bonini S. *Allergy* 1996; 51: 124-7.

Abstract: Chronic fatigue syndrome (CFS) is a syndrome of uncertain etiopathogenesis characterized by disabling fatigue associated with a variable number of somatic and/or neuropsychologic symptoms. In patients with CFS, several immunologic abnormalities can be detected, including a higher prevalence of allergy. The aim of this study was to determine whether CFS patients, well studied for their allergy profile, show signs of eosinophil activation, as detectable by the measurement in serum of eosinophil cationic protein (ECP) levels. In 35 consecutive CFS outpatients (diagnosis based on the Centers for Disease Control case definition), ECP was measured in serum by a competitive enzyme immunoassay (ECP-FEIA kit, Kabi Pharmacia Diagnostics, Uppsala, Sweden). Fourteen disease-free subjects with no history of CFS or allergy were selected as controls. ECP serum levels were significantly higher in CFS patients than in controls ( $18.0 \pm 11.3$  micrograms/l vs  $7.3 \pm 2.1$  micrograms/l;  $P < 0.01$ ). In the CFS population, 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 ( $19.3 \pm 12.4$  micrograms/l vs  $13.6 \pm 3.7$  micrograms/l;  $P=0.4$ )." This is the first report of increased serum levels of ECP in CFS. On the basis of the available data, it is discussed whether eosinophil activation has a pathogenetic role in CFS or is linked to the frequently associated allergic condition, or, finally, whether a common immunologic background may exist for both atopy and CFS.

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Chronic fatigue syndrome - immunological findings vary between populations [Letter]. Abbot NC, Spence VA, Lowe JG, Potts RC, Hassan AH, Belch JJ, Beck JS. *British Medical Journal* 1994; 308: 1299.

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Low serum b2 - microglobulin levels in CFS patients De Becker P, De Meirleir K, Demanet C, Wets L, Joos E, Smits J. *Journal of Chronic Fatigue Syndrome* 1996; 2(2/3): 106.

Abstract: OBJECTIVE: Elevations of serum b2-Microglobulin (b2m) have been described in patients with a variety of illnesses, including malignancies, acquired immune deficiency syndrome and rheumatic disease. A low molecular weight polypeptide synthesized by all nucleated cells of the body, b2m forms part of the HLA class I antigens on the cell membrane. Because abnormalities of both humoral and cellular immunity have been demonstrated in a substantial proportion of patients with CFS, we measured serum b2m and compared it to those of matched controls. METHODS. From our outpatient clinic we recruited 18 patients (mean age  $\pm$  SD;  $34.8 \pm 6.49$ ) who met the CDC criteria for CFS. All patients had been investigated to exclude alternative medical diagnosis. Controls were 14 age- and sex-matched healthy individuals (mean age  $\pm$  SD;  $34.7 \pm 7.25$ ); all were free of major medical or psychiatric diseases. Data were analyzed by using Student's t-test; a p value  $< 0.01$  was considered

statistically significant. RESULTS. Mean b2 microglobulin levels of CFS-patients  $\pm$  SD="0.981" UG/L  $\pm$  0.123; mean b2 microglobulin levels of matched controls  $\pm$  SD="1.179"  $\pm$  0.246 (p="0.0012)."  
CONCLUSION. Our data show that CFS patients have lower serum b2 microglobulin levels compared to controls. Although this is not a diagnostic marker, it adds to the numerous disturbances observed in the immunity of CFS patients.

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A comprehensive immunological analysis in chronic fatigue syndrome. Gupta S, Vayuvegula B. Scandinavian Journal of Immunology 1991; 33: 319-327.

Abstract: A detailed analysis of cell-mediated and antibody-mediated immunity was performed in 20 CDC-defined patients with chronic fatigue syndrome (CFS) and 20 age- and sex-matched healthy controls. CD3+, CD4+, CD8+, and CD20+ lymphocytes were comparable in two groups. Natural killer cells as defined by CD16, CD56 and CD57 antigens were significantly reduced in CFS. A significant increase in the proportions of CD4+ ICAM 1+ T cells was observed in CFS. Monocytes from CFS displayed increased density (as determined by mean fluorescence channel numbers) of intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function associated antigen 1 (LFA-1), but showed decreased enhancing response to recombinant interferon-gamma in vitro. The lymphocyte DNA synthesis in response to phytohaemagglutinin (PHA), Concanavalin A (Con A) and pokeweed mitogen (PWM) was normal but the response to soluble antigens was significantly reduced. Serum IgM, IgG, IgA, and IgG subclasses were normal. In vivo specific antibody response to pneumococcus vaccine was depressed in CFS. Forty percent of patients showed titres of anti-human herpes virus 6 (anti-HHV-6) antibody higher than that in the controls (greater than or equal to 1/80). These data suggest immunological dysfunction in patients with chronic fatigue syndrome. The significance of these observations is discussed.

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Immunological abnormalities in patients with chronic fatigue syndrome. Tirelli U, Marotta G, Improta S, Pinto A. Scandinavian Journal of Immunology 1994; 40(6): 601-8.

Abstract: Between January 1991 and January 1993, 265 patients who fulfilled the CDC criteria of the working case definition of Chronic Fatigue Syndrome (CFS) have been observed at our Institution and submitted for clinical and laboratory evaluation. One hundred and sixty-three patients were females and 102 males, the median age was 35 years (range 4-55 years); all patients reported profound and prolonged fatigue, lasting for a median of 3 years (range 6 months-10 years), preceded or accompanied at appearance by fever in 185 cases, and neuropsychologic problems including inability to concentrate, difficulty in thinking, confusion, irritability, forgetfulness, and depression. The fatigue was so severe that it required 102 patients to stop their working activities for a period of time ranging from 3 months to 2 years (range 7 months). In 40 consecutive patients a comprehensive immunologic testing by single

and two-colour flow cytometry was performed and results compared with a group of 35 healthy, age- and sex-matched controls. Whilst no significant differences were found in the absolute numbers of circulating total T cells (CD3+) and of total helper/inducer (CD4+) or suppressor/cytotoxic (CD8+) T cells, an evident reduction in CD3-/CD16+ and CD57+/CD56+ NK lymphocytes along with an expansion of the CD8+/CD56+ and CD16-/CD56+ NK subsets, were found in the CFS group. In addition, CD56+ NK cells from CFS subjects were found to express an increased amount of cell adhesion molecules (CD11b, CD11c, CD54) and activation antigens (CD38). Both the percentage and absolute numbers of CD4+ T cells bearing the CD45RA antigen appeared significantly reduced in CFS patients, and CD4+ T lymphocytes from CFS subjects displayed an increased expression of the intercellular adhesion molecule-1 (ICAM-1/CD54). Finally, the total numbers of circulating (CD19+) B lymphocytes, were significantly higher in CFS cases than in controls, and in 11 out of 30 CFS patients the increase in circulating B cells was sustained by the expansion of the CD5+/CD19+ subset of B lymphocytes. We conclude that CFS is a syndrome not previously described in Italy, with already known clinical characteristics and appears to be associated with several immunologic abnormalities, including those reported previously in cohort of patients from different countries. We also show for the first time that CD56- NK cell subsets from CFS patients display an abnormally increased expression of cell adhesion molecules and activation markers.

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Characteristic T cell dysfunction in patients with chronic active Epstein-Barr virus infection (chronic infectious mononucleosis). Tosato G et al. *Journal of Immunology* 1985; 134: 3082-8.

**Abstract:** We evaluated immune functions in 16 patients with chronic active Epstein-Barr virus (EBV) infection (chronic infectious mononucleosis). Chronic infectious mononucleosis is an illness characterized primarily by chronic and occasionally disabling fatigue and other constitutional complaints, only sometimes beginning with an episode of acute infectious mononucleosis, and associated with an abnormal pattern of serum antibodies to EBV. In these patients, the frequency of circulating EBV-infected B cells that manifested spontaneous outgrowth in vitro was comparable to that found in EBV-seropositive normals, and the levels of EBV-specific suppressor activity were also normal. Upon stimulation with polyclonal activators, unseparated cells from these patients produced a relatively normal number of immunoglobulin-secreting cells. However, when purified T cells from these patients were mixed with normal mononuclear cells in co-culture, immunoglobulin production was strikingly suppressed. The degree of this T cell suppression correlated directly with the abnormally elevated titer of antibody to the early antigens of EBV. Interestingly, during normal convalescence from acute EBV-induced infectious mononucleosis a period is also seen during which T cells suppress the response of allogeneic but not autologous cells. Thus, from an immunologic viewpoint, patients with chronic active EBV infection appear "frozen" in a state typically found only briefly during the convalescence from acute EBV infection.

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Consequences of live poliovirus vaccine administration in chronic fatigue syndrome. Vedhara K, Llewelyn MB, Fox JD, Jones M, Jones R, Clements GB, Wang EC, Smith AP, Borysiewicz LK. *Journal of Neuroimmunology* 1997; 75(1-2): 183-195.

Abstract: The effect of live oral polio virus vaccination on chronic fatigue syndrome (CFS) patients was examined in a double-blind study. CFS patients were allocated randomly to placebo (N = 7) or vaccine (N = 7) conditions. All controls subjects received the vaccine (9). Vaccine administration was not associated with clinical exacerbation of CFS. However, objective responses to the vaccine revealed differences between patients and controls: increased poliovirus isolation, earlier peak proliferative responses, lower T-cell subsets on certain days post vaccination and a trend for reduced gamma-interferon in the CFS-vaccine group. Polio vaccination was not found to be clinically contraindicated in CFS patients, however, there was evidence of altered immune reactivity and virus clearance.

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Gamma globulin therapy for chronic mononucleosis syndrome. Dubois RE. *AIDS Research* 1986; 2(1): 191-5.

Abstract: Antibodies against Epstein-Barr virus, associated with antibody dependent cytotoxic cell activity, were found to be present in diminished titer in 20 of 22 patients tested with chronic mononucleosis syndrome (CMS). Gamma globulin was shown to improve symptoms in 53% of the patients treated, compared with 32% of placebo injections. 89.5% of 57 patients treated with a gamma globulin treatment program remained in the treatment program because of relief of symptoms, and only four patients dropped out because there was no relief of symptoms or side effects. Four patients experienced complete relief of symptoms following a variable length treatment program. It would appear that intramuscular gamma globulin treatment is efficacious in the treatment of CMS and that the average interval between treatments is three weeks.

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A double-blind, placebo-controlled trial of intravenous immunoglobulin therapy in patients with chronic fatigue syndrome. Lloyd A, Hickie I, Wakefield D, Boughton CR. *American Journal of Medicine* 1990; 89: 561-68.

Abstract: **PURPOSE:** The chronic fatigue syndrome (CFS) is characterized by profound fatigue, neuropsychiatric dysfunction, and frequent abnormalities in cell-mediated immunity. No effective therapy is known. **PATIENTS AND METHODS:** Forty-nine patients (40 with abnormal cell-mediated immunity) participated in a randomized, double-blind, placebo-controlled trial to determine the effectiveness of high-dose intravenously administered immunoglobulin G. The patients received three intravenous infusions of a placebo solution or immunoglobulin at a dose of 2 g/kg/month. Assessment of the severity of symptoms and associated disability, both before and after treatment, was completed at detailed interviews by a physician and psychiatrist, who were unaware of the treatment status. In addition, any change in physical symptoms and functional capacity was recorded using visual analogue

scales, while changes in psychologic morbidity were assessed using patient-rated indices of depression. Cell-mediated immunity was evaluated by T-cell subset analysis, delayed-type hypersensitivity skin testing, and lymphocyte transformation with phytohemagglutinin. RESULTS: At the interview conducted by the physician 3 months after the final infusion, 10 of 23 (43%) immunoglobulin recipients and three of the 26 (12%) placebo recipients were assessed as having responded with a substantial reduction in their symptoms and recommencement of work, leisure, and social activities. The patients designated as having responded had improvement in physical, psychologic, and immunologic measures ( $p$  less than 0.01 for each). CONCLUSION: Immunomodulatory treatment with immunoglobulin is effective in a significant number of patients with CFS, a finding that supports the concept that an immunologic disturbance may be important in the pathogenesis of this disorder.

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Low NK syndrome and its relationship to chronic fatigue syndrome. Aoki T, Miyakoshi H, Usuda Y, Herberman RB. *Clinical Immunology and Immunopathology* 1993; 69(3): 253-65.

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Immunologic abnormalities associated with chronic fatigue syndrome. Barker E, Fujimura SF, Fadem MB, Landay AL, Levy JA. *Clinical Infectious Diseases* 1994; 18(Supp 1): S136-41.

Abstract: Several aspects of cellular immunity in patients with clinically defined chronic fatigue syndrome (CFS) were evaluated and compared with those in healthy individuals. Flow cytometric analyses revealed normal expression of total T (CD3+), B (CD19+), and NK (natural killer) (CD16+, CD56+) markers on the surface of peripheral blood mononuclear cells (PMC) from patients with CFS. However, compared with those of healthy individuals, patients' CD8+ T cells expressed reduced levels of CD11b and expressed the activation markers CD38 and HLA-DR at elevated levels. In many of the individuals in whom expression of CD11b was reduced the expression of CD28 was increased. These findings indicate expansion of a population of activated CD8+ cytotoxic T lymphocytes. A marked decrease in NK cell activity was found in almost all patients with CFS, as compared with that in healthy individuals. No substantial abnormalities in monocyte activity or T cell proliferation were observed. The results of this study suggest that immune cell phenotype changes and NK cell dysfunction are common manifestations of CFS.

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Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. Caligiuri M, Murray C, Buchwald D, Levine H, Cheney P, Peterson D, Komaroff AL, Ritz J. *Journal of Immunology* 1987; 139: 3306-13.

Abstract: Natural killer (NK)3 cells are large granular lymphocytes that appear to play a significant role in the host's defense against viral infection. We performed an extensive phenotypic and functional characterization of NK cells on 41 patients with the chronic fatigue syndrome (CFS), or "chronic active Epstein-Barr virus infection" syndrome, and on 23 age- and sex-matched asymptomatic control subjects in an attempt to further characterize this illness. These studies demonstrated that a majority of patients with CFS have low numbers of NKH1+T3- lymphocytes, a population that represents the great majority of NK cells in normal individuals. CFS patients had normal numbers of NKH1+T3+ lymphocytes, a population that represents a relatively small fraction of NK cells in normal individuals. When tested for cytotoxicity against a variety of different target cells, patients with CFS consistently demonstrated low levels of killing. After activation of cytolytic activity with recombinant interleukin 2, patients were able to display increased killing against K562 but most patients remained unable to lyse Epstein-Barr virus-infected B cell targets. Additional cytotoxicity experiments were carried out utilizing anti-T3 monoclonal antibody to block killing by NKH1+T3+ cells. These experiments indicated that the NK cell that appears to be responsible for much of the functional activity remaining in patients with CFS belongs to the NKH1+T3+ subset, which under normal circumstances represents only approximately 20% of the NK cell population.

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Immune function in chronic active Epstein-Barr virus infection. Kibler R, Lucas RO, Hicks MJ, et al. *Journal of Clinical Immunology* 1985; 5: 46-54.

Abstract: The spectrum of illness attributed to Epstein-Barr virus (EBV) includes patients with symptoms persisting for more than 1 year without any other obvious underlying disease. High titers of antibodies to EBV, either IgG antiviral capsid antigen or anti-early antigen, can be demonstrated. In this study, 13 patients diagnosed as having chronic active EBV infection were examined to determine aspects of their immunologic status. Morphological examination and fluorescent antibody analysis revealed no abnormalities in the phenotypes of peripheral blood white cells present in these patients. Compared to those from healthy control individuals, mononuclear cells from the patients showed a markedly depressed ability to produce both interleukin-2 and interferon after stimulation with mitogen and a phorbol ester. Studies of natural killer (NK) cell activity revealed that unfractionated mononuclear cells from patients with chronic active EBV infection were significantly lower in killing activity compared to the control group. Fractionation procedures to enrich for large granular lymphocytes resulted in an increase in NK activity for all individuals, but killing activity still remained slightly lower in the patients than in the control group. The dysfunctions which were found in patients with chronic active EBV infection may reflect a primary defect in natural immune functions of the patients predisposing them to a chronic or intermittent clinical disease rather than a self-limiting illness. Alternatively, the abnormalities detected in these experiments may be a result of the viral infection itself.

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Immune responses associated with CFS: a case-control study. Mawle AC, Nisenbaum R, Dobbins JG, Gary HE Jr, Stewart JA, Reyes M, Steele L. *Journal of Infectious Diseases* 1997; 175(1): 136-41.

Abstract: An exploratory case-control study was conducted to assess whether the many reported differences in the immune function of chronic fatigue syndrome (CFS) patients are detectable in rigorously defined cases of CFS. Although many studies have reported differences between cases and controls in various measures of immune function, none of these differences were found in all studies. In this study, no differences were found in white blood cell numbers; immune complex, complement, or serum immunoglobulin levels; delayed type hypersensitivity and allergic responses; NK cell function; and proliferative responses to mitogens and antigens. Marginal differences were detected in cytokine responses and in cell surface markers in the total CFS population. However, 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.

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Changes in natural killer cell phenotype in patients with post-viral fatigue syndrome. Morrison LJA, Behan WHM, Behan PO. *Clinical and Experimental Immunology* 1991; 83: 441-6.

Abstract: We analysed peripheral blood CD56+ natural killer (NK) cell subsets in 23 carefully characterized patients with post-viral fatigue syndrome (PFS), compared with 19 healthy controls, using fluorochrome-conjugated, specific monoclonal antibodies and the FACScan. We found significantly increased percentages of CD56+, and especially CD56bright+ NK cells in PFS patients. We also found significantly increased percentages of CD56+ high affinity interleukin-2 (IL-2) receptor (CD25)+ and CD56+ transferrin receptor (CD71+) subsets of cells, most of which also stained brightly for CD56. Also, we found an increased percentage of CD56+ CD3+ cells, many of which stained brightly for CD56, although there was no increase in the percentage of CD56- CD3+ T cells in these patients. These observations, in conjunction with very low percentage of CD56- CD25+ cells, suggest that there is a preferential involvement of this minor subset of CD56+ CD3+ T cells in PFS. Finally, a decreased percentage of CD56+ Fc gamma receptor (CD16)+ NK cells was identified, which suggests a reduced capacity of antibody-dependent cellular cytotoxicity in PFS patients. Subsets of CD56+ NK cells co-expressing CD2, CD4 or CD8 did not show any significant difference between PFS patients and healthy controls. These phenotypic changes provide laboratory evidence of immunological abnormalities in this syndrome, and, we suggest, may be consistent with persistent viral infection.

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Decreased natural killer cell activity is associated with severity of chronic fatigue immune dysfunction syndrome. Ojo-Amaize EA, Conley EJ, Peter JB. *Clinical Infectious Diseases* 1994; 18(Supp 1): S157-S159.

Abstract: Natural killer (NK) cell activity was measured blindly in vitro with blood specimens from 50 healthy individuals and 20 patients with clinically defined chronic fatigue immune dysfunction

syndrome (CFIDS) who met the criteria established by the Centers for Disease Control and Prevention (Atlanta). In accordance with a group scoring system of 1-10 points, with 10 being the most severe clinical status, the patient population was stratified into three clinical groups: A (> 7 points), B (5-7 points), and C (< 5 points). NK cell activity was assessed by the number of lytic units (LU), which for the 50 healthy controls varied between 20 and 250 (50%, 20-50 LU; 32%, 51-100 LU; 6%, 101-130 LU; and 12%, > 150 LU). In none of the 20 patients with CFIDS was the NK cell activity > 100 LU. For group C, the 10 patients stratified as having the least severe clinical condition, the measure was  $61.0 \pm 21.7$  LU; for group B (more severe,  $n = 7$ ), it was  $18.3 \pm 7.3$  LU; and for group A (most severe,  $n = 3$ ), it was  $8.0 \pm 5.3$  LU. These data suggest a correlation between low levels of NK cell activity and severity of CFIDS, which, if it is confirmed by additional studies of larger groups, might be useful for subgrouping patients and monitoring therapy and/or the progression of CFIDS.

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Immunological abnormalities in patients with chronic fatigue syndrome. Tirelli U, Marotta G, Improta S, Pinto A. *Scandinavian Journal of Immunology* 1994; 40(6): 601-8.

Abstract: Between January 1991 and January 1993, 265 patients who fulfilled the CDC criteria of the working case definition of Chronic Fatigue Syndrome (CFS) have been observed at our Institution and submitted for clinical and laboratory evaluation. One hundred and sixty-three patients were females and 102 males, the median age was 35 years (range 4-55 years); all patients reported profound and prolonged fatigue, lasting for a median of 3 years (range 6 months-10 years), preceded or accompanied at appearance by fever in 185 cases, and neuropsychologic problems including inability to concentrate, difficulty in thinking, confusion, irritability, forgetfulness, and depression. The fatigue was so severe that it required 102 patients to stop their working activities for a period of time ranging from 3 months to 2 years (range 7 months). In 40 consecutive patients a comprehensive immunologic testing by single and two-colour flow cytometry was performed and results compared with a group of 35 healthy, age- and sex-matched controls. Whilst no significant differences were found in the absolute numbers of circulating total T cells (CD3+) and of total helper/inducer (CD4+) or suppressor/cytotoxic (CD8+) T cells, an evident reduction in CD3-/CD16+ and CD57+/CD56+ NK lymphocytes along with an expansion of the CD8+/CD56+ and CD16-/CD56+ NK subsets, were found in the CFS group. In addition, CD56+ NK cells from CFS subjects were found to express an increased amount of cell adhesion molecules (CD11b, CD11c, CD54) and activation antigens (CD38). Both the percentage and absolute numbers of CD4+ T cells bearing the CD45RA antigen appeared significantly reduced in CFS patients, and CD4+ T lymphocytes from CFS subjects displayed an increased expression of the intercellular adhesion molecule-1 (ICAM-1/CD54). Finally, the total numbers of circulating (CD19+) B lymphocytes, were significantly higher in CFS cases than in controls, and in 11 out of 30 CFS patients the increase in circulating B cells was sustained by the expansion of the CD5+/CD19+ subset of B lymphocytes. We conclude that CFS is a syndrome not previously described in Italy, with already known clinical characteristics and appears to be associated with several immunologic abnormalities, including those reported previously in cohort of patients from different countries. We also show for the first time that CD56- NK cell subsets from CFS patients display an abnormally increased expression of cell adhesion molecules and activation markers

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Immunological and psychological dysfunction in patients receiving immunotherapy for chronic fatigue syndrome. Hickie I, Lloyd A, Wakefield D. Australian and New Zealand Journal of Psychiatry 1992; 26(2): 249-56.

Abstract: Associations between immunological and psychological dysfunction in 33 patients with Chronic Fatigue Syndrome (CFS) were examined before and in response to treatment in a double blind, placebo-controlled trial of high dose intravenous immunoglobulin. Only those patients who received active immunotherapy demonstrated a consistent pattern of correlations between improvement in depressive symptoms and markers of cell-mediated immunity (CMI). This finding lends some support to the hypothesis that depressive symptoms in patients with CFS occur secondary to, or share a common pathophysiology with, immunological dysfunction. This pattern and the lack of strong associations between depression and immunological disturbance prior to treatment are less supportive of the view that CFS is primarily a form of depressive disorder or that immunological dysfunction in patients with CFS is secondary to concurrent depression.

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Chronic fatigue Syndrome: evaluation of a 30 criteria-score and correlation with immune activation. Hilgers A, Frank J. Journal of Chronic Fatigue Syndrome 1996; 2(4): 35-47.

Abstract: OBJECTIVE. The development of a score for severity of Chronic Fatigue Syndrome (CFS), the correlation of CFS with parameters of immune activation and the association with pathogens. METHODS. Five hundred five patients with suspicion of Chronic Fatigue Syndrome and no other definitive diagnosis were checked by a 45-criteria-score, basic laboratory programs and immunological profiles. In most of the patients further tests concerning complement system, immune activation markers, hormones and serology of herpesviruses, Chlamydia and Borrelia could be evaluated. Comparison of the symptoms of CFS patients with healthy controls lead to a 30-criteria-score and this score was correlated with laboratory parameters (Spearman rank-correlation-coefficient  $r(s)$ , ties corrected). RESULTS. Three hundred eighty-five patients fulfilling stronger criteria according to the Centers for Disease Control (CDC) definition showed significant differences to 53 healthy controls in 40 of the 45 criteria ( $p < 0.001$ , twitches and food allergies  $p < 0.05$ ). Thirteen symptoms corresponding to CDC criteria were all significant ( $p < 0.001$ ), 17 further significant criteria of descending precision were added: respiratory infections, palpitations, dizziness, dyspepsia, dryness of mouth/eyes, allergies, nausea, paresthesia, loss of hair, skin alterations, dyscoordination, chest pain, personality changes, eczema, general infections, twitches, urogenital infections. A correlation between the 30-criteria-score and immunological parameters could be evaluated in 472 of the 505 patients. Significant positive correlation with the 30-criteria-score was found in numbers of CD8+ T-lymphocytes, HLA-DR+ T-lymphocytes, gamma globulins, IgM, IgG, and for the number of types of autoantibodies (mainly ANA, ACA, antithyroid and antiparietal cell antibodies). Significant negative correlation was found in albumin-globulin-ratio, eosinophils and IgE. Most of these parameters also correlated with one another. On the other hand, in subgroups of the 505 patients the frequency of positivity in serological tests for HHV-6 (49.9%), EBV (35.4%), HSV (29.2%), CMV (12.5%) and Chlamydia (35.0%) was striking. Borrelia Western blots showed 3 or more specific IgG-bands in 54 of 131 patients (41.2%). In some cases infection with EBV, HHV-6 and CMV, respectively, was confirmed by DNA-PCR-test and

antigen detection. SUMMARY. In increasingly larger groups of patients with CFS and related constellations we often see clinical signs and longer anamnesis of other symptoms besides the classical criteria of CFS, especially a high prevalence of local and general susceptibility to infections and hints to prolonged inflammation processes. Together with other results, the data confirm the hypothesis that a reduced or unstable immune control or delayed immune reaction to persisting viruses or bacterial intracellular pathogens, possibly triggered by common infections or other environmental factors, can lead to a chronic neuroimmune activation state and auto-immune disorders. Hypersensitivity symptoms of the patients might not be mediated by classical allergies alone but also result from a type-IV-hypersensitivity.

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Antibodies to Epstein-Barr virus-specific DNase and DNA polymerase in the chronic fatigue syndrome. Jones JF, Williams M, Schooley RT, Robinson C, Glaser R. Archives of Internal Medicine 1988; 148: 1957-60.

Abstract: In an attempt to examine further the association between active Epstein-Barr virus (EBV) infection and the chronic fatigue syndrome (chronic EBV syndrome, or chronic or atypical mononucleosis), antibodies acting against EBV-specific DNase and DNA polymerase, which are expressed only during virus replication, were assayed. Serum samples from 25 healthy EBV-seropositive individuals neutralized  $3.5 \pm 5.1$  U (mean  $\pm$  SD) of DNase activity and  $14.7 \pm 8.5$  U of DNA polymerase activity. From these values were selected upper limits of anti-EBV enzyme activity of 17.9 and 31.3 U neutralized in normal individuals, respectively (representing the 95% confidence limit). Serum samples from six groups of subjects representing a variety of EBV-related illnesses were then studied. Only patients with notably elevated anti-EBV antibody titers to viral capsid antigen (VCA) (greater than 10,000) had elevated levels of anti-EBV DNase (38 to 56 U neutralized) and anti-EBV DNA polymerase (72 to 106 U neutralized). Three additional patients and two geriatric controls with average anti-EBV early antigen/VCA titers had slightly elevated levels of antibody to EBV DNA polymerase. IgA anti-VCA, anti-early antigen antibodies, or both, were also detected in the same patients who had high EBV DNase and polymerase antibody levels. These antibody profiles are similar to those in patients with nasopharyngeal carcinoma. Since three of the six patients with elevated anti-EBV enzyme antibody levels developed fatal lymphomas, patients with chronic EBV and this antibody profile might be in another illness category at risk for malignant disease.

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Immunologic abnormalities in chronic fatigue syndrome. Klimas NG, Salvato FR, Morgan R, Fletcher MA. Journal of Clinical Microbiology 1990; 28: 1403-10.

Abstract: The chronic fatigue syndrome (CFS), formerly known as chronic Epstein-Barr virus syndrome, is a clinical state of some complexity and uncertain etiology. In order to characterize in a comprehensive manner the status of laboratory markers associated with cellular immune function in patients with this syndrome, 30 patients with clinically defined CFS were studied. All of the subjects

were found to have multiple abnormalities in these markers. The most consistent immunological abnormality detected among these patients, when compared with normal controls, was low natural killer (NK) cell cytotoxicity. The number of NK cells, as defined by reactivity with monoclonal antibody NKH.1 (CD56), was elevated, but the killing of K562 tumor cells per CD56 cell was significantly diminished. Lymphoproliferative responses after stimulation with phytohemagglutinin and pokeweed mitogen were decreased in most patients when compared with those in normal controls, as was the production of gamma interferon following mitogen stimulation. Lymphocyte phenotypic marker analysis of peripheral blood lymphocytes showed that there were significant differences between patients with CFS and controls. There was an increase in the percentage of suppressor-cytotoxic T lymphocytes, CD8, and a proportionally larger increase in the number of CD8 cells expressing the class II activation marker. Most patients had an elevated number of CD2 cells which expressed the activation marker CDw26. The numbers of CD4 cells and the helper subset of CD4+CD29+ cells in patients with CFS were not different from those in controls. There was, however, a significant decrease in the suppressor inducer subset of CD4+ CD45RA+ cells.

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Autoantibodies to nuclear envelope antigens in chronic fatigue syndrome. Konstantinov K, von Mikecz A, Buchwald D, Jones J, Gerace L, Tan EM. *Journal of Clinical Investigation* 1996; 98(8): 1888-96.

Abstract: We have identified and partially characterized the autoantibodies in sera of 60 patients with chronic fatigue syndrome. Approximately 52% of the sera were found to react with nuclear envelope antigens. The combination of nuclear rim staining observed in immunofluorescence microscopy and immunoblot analysis of highly purified nuclear envelope proteins provided initial characterization of these autoantibodies. Further characterization showed that some sera immunoprecipitated the in vitro transcription and translation product of a human cDNA clone encoding the nuclear envelope protein lamin B1. The autoantibodies were of the IgG isotype. The occurrence of autoantibodies to a conserved intracellular protein like lamin B1 provides new laboratory evidence for an autoimmune component in chronic fatigue syndrome.

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Chronic fatigue syndrome - clinical condition associated with immune activation. Landay AL, Jessop C, Lennette ET, Levy JA. *Lancet* 1991; 338: 707-12.

Abstract: There is much conflicting immunological and viral data about the causes of chronic fatigue syndrome (CFS); some findings support the notion that CFS may be due to one or more immune disorders that have resulted from exposure to an infectious agent. In the present study, flow cytometry and several different monoclonal antibodies recognising T, B, and natural killer (NK) cell populations as well as activation and cell adhesion antigens were used to study 147 individuals with CFS. Compared with healthy controls, a reduced CD8 suppressor cell population and increased activation markers (CD38, HLA-DR) on CD8 cells were found. The differences were significant ( $p = 0.01$ ) in patient with major symptoms of the disease. These immunological indices were not observed in 80

healthy individuals, in 22 contacts of CFS patients, or in 43 patients with other diseases. No correlation of these findings in CFS patients with any known human viruses could be detected by serology. The findings suggest that immune activation is associated with many cases of CFS.

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Immunological abnormalities in the chronic fatigue syndrome. Lloyd AR, Wakefield D, Boughton CR, Dwyer JM. *Medical Journal of Australia* 1989; 151: 122-4.

**Abstract:** The chronic fatigue syndrome is a disorder of unknown aetiology which is characterized by debilitating fatigue. Recent evidence has suggested that viruses may persist in the tissues of patients with chronic fatigue syndrome. A concurrent immunological disturbance is likely to be associated with the persistence of viral antigens. Therefore, the humoral and cellular immunity of 100 patients who were suffering from chronic fatigue syndrome and that of 100 healthy, age- and sex-matched control subjects were compared. This study documents the frequent occurrence of abnormalities within the cellular and humoral immune systems of patients with well-defined chronic fatigue syndrome. Disordered immunity may be central to the pathogenesis of chronic fatigue syndrome. In patients with chronic fatigue syndrome, a significant ( $P$  less than 0.01) reduction was found in the absolute number of peripheral blood lymphocytes in the total T-cell (CD2), the helper/inducer T-cell (CD4) and the suppressor/cytotoxic T-cell (CD8) subsets. A significant ( $P$  less than 0.001) reduction also was found in T-cell function, which was measured: in vivo by delayed-type hypersensitivity skin-testing (reduced responses were recorded in 50 [88%] of 57 patients); and in vitro by phytohaemagglutinin stimulation. Reduced immunoglobulin (Ig) levels were common (56% of patients), with the levels of serum IgG3- and IgG1-subclasses particularly ( $P$  less than 0.05) affected.

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Immunity and the pathophysiology of chronic fatigue syndrome. Lloyd AR, Wakefield D, Hickie I. *Ciba Foundation Symposium* 1993; 173: 176-87.

**Abstract:** The pathophysiology of chronic fatigue syndrome (CFS) remains unknown. The syndrome often follows a recognized or presumed infection and the disorder may therefore result from a disordered immune response to a precipitating infection or antigenic challenge. Abnormalities of both humoral and cellular immunity have been demonstrated in a substantial proportion of patients with CFS. The most consistent findings are of impaired lymphocyte responses to mitogen and reduced natural killer cell cytotoxicity. Cutaneous anergy and immunoglobulin G subclass deficiencies have also been found. Further studies are needed examining cytokine levels in serum and cerebrospinal fluid, and cytokine production in vitro in patients with CFS. Interpretation of the findings of published studies of immunity is limited by probable heterogeneity in the patient groups studied, and by the lack of standardization and reproducibility in the assays used. The pattern of abnormalities reported in immunological testing in patients with CFS is consistent with the changes seen during the resolving phases of acute viral infection. These data provide circumstantial support for the hypothesis that CFS results from a disordered immune response to an infection. Longitudinal studies of immunity in

patients developing CFS after defined infectious illnesses will provide the best means of further examining this hypothesis.

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Relationships of cognitive difficulties to immune measures, depression and illness burden in CFS. Lutgendorf S, Klimas NG, Antoni M, Brickman A, Fletcher MA. *Journal of Chronic Fatigue Syndrome* 1995; 1(2): 23-41.

Abstract: **OBJECTIVE.** We related the subjective assessment of cognitive difficulties with lymphocyte phenotypes, cell-mediated immunity (CMI), cytokine and neopterin levels in patients with chronic fatigue syndrome (CFS), in order to determine if CFS patients complaining of greater cognitive difficulties would show greater impairments in cell-mediated immunity and a greater degree of immune system dysregulation, and to determine if these cognitive difficulties would correlate with the other non-affective measures of CFS-associated illness burden. We also assessed whether these relationships would hold independent of depression in two ways, by statistically covarying depression severity scores and by comparing subsets of CFS patients with and without a concurrent diagnosis of major depressive disorder. **DESIGN.** A case series of CFS patients. **SETTING.** Outpatient tertiary referral clinic at the University of Miami School of Medicine, Miami, FL. **PATIENTS.** Consecutive sample of 65 patients who were referred as CFS to the University of Miami Diagnostic Immunology Clinic, who met the Centers for Disease Control and Prevention (CDC) criteria for diagnosis of CFS and consented to participate. **MAIN MEASURES.** Self-assessment of cognitive difficulties, depression and illness burden, clinician-assessed depression and CFS symptoms, lymphocyte phenotype, proliferative response to mitogens, serum levels of cytokines and neopterin. **RESULTS.** Among CFS patients, high Cognitive Difficulty Scale (CDS) scores were significantly related to lower lymphocyte proliferative responses to mitogens, higher neopterin levels, and higher CD4 and lower CD8 lymphocyte counts. These relationships, with the exception of T cell subset percentages, were maintained when depression severity was used as a co-variate. High CDS scores were also significantly related to lower Karnofsky scores, and greater illness burden as measured by the Sickness Impact Profile. Evidence is presented that CFS patients with higher cognitive difficulty scores have more immune and clinical dysfunction than those with less cognitive difficulty, and that these relationships are independent of depression. These observations provide support for the concept that although both cognitive difficulties and immunologic abnormalities, such as immune activation and impaired cell-mediated immunity, may represent secondary sequelae to the same event(s), they are not likely to be secondary sequelae to depression.

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Cell-mediated immunity in patients with myalgic encephalomyelitis syndrome. N Murdoch JC. *New Zealand Medical Journal* 1988; 101: 511-2.

Abstract: Cell-mediated immunity was measured in 33 patients with myalgic encephalomyelitis syndrome and 33 age and sex matched controls, using the multitest CMI device. The multitest scores in

myalgic encephalomyelitis syndrome were significantly less than the controls suggesting a T-cell abnormality in these patients.

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Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome - interrelations with cellular sources and patterns of soluble immune mediator expression. Patarca R, Klimas NG, Lutgendorf S, Antoni M, Fletcher MA. *Clinical Infectious Diseases* 1994; 18(Supp 1): S147-53.

Abstract: Among a group of 70 individuals who met the criteria established by the Centers for Disease Control and Prevention (Atlanta) for chronic fatigue syndrome (CFS), 12%-28% had serum levels exceeding 95% of control values for tumor necrosis factor (TNF) alpha, TNF-beta, interleukin (IL) 1 alpha, IL-2, soluble IL-2 receptor (sIL-2R), or neopterin; overall, 60% of patients had elevated levels of one or more of the nine soluble immune mediators tested. Nevertheless, only the distributions for circulating levels of TNF-alpha and TNF-beta differed significantly in the two populations. In patients with CFS-but not in controls-serum levels of TNF-alpha, IL-1 alpha, IL-4, and sIL-2R correlated significantly with one another and (in the 10 cases analyzed) with relative amounts (as compared to beta-globin or beta-actin) of the only mRNAs detectable by reverse transcriptase-coupled polymerase chain reaction in peripheral-blood mononuclear cells: TNF-beta, unspliced and spliced; IL-1 beta, lymphocyte fraction; and IL-6 (in order of appearance). These findings point to polycellular activation and may be relevant to the etiology and nosology of CFS.

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Effects of mild exercise on cytokines and cerebral blood flow in chronic fatigue syndrome patients. Peterson PK, Sirr SA, Grammish FC, Schenck CH, Pheley AM, Hu S, Chao CC. *Clinical and Diagnostic Laboratory Immunology* 1994; 1(2): 222-6.

Abstract: Chronic fatigue syndrome (CFS) is an idiopathic disorder characterized by fatigue that is markedly exacerbated by physical exertion. In the present study, we tested the hypothesis that mild exercise (walking 1 mph [1 mile = 1.609 km] for 30 min) would provoke serum cytokine and cerebral blood flow abnormalities of potential pathogenic importance in CFS. Interleukin-1 beta, interleukin-6, and tumor necrosis factor alpha were nondetectable in sera of CFS patients (n = 10) and healthy control subjects (n = 10) pre- and postexercise. At rest, serum transforming growth factor beta (TGF-beta) levels were elevated in the CFS group compared with the control group ( $287 \pm 18$  versus  $115 \pm 5$  pg/ml, respectively;  $P < 0.01$ ). Serum TGF-beta and cerebral blood flow abnormalities, detected by single-photon emission-computed tomographic scanning, were accentuated postexercise in the CFS group. Although these findings were not significantly different from those in the control group, the effect of exercise on serum TGF-beta and cerebral blood flow appeared magnified in the CFS patients. Results of this study encourage future research on the interaction of physical exertion, serum cytokines, and cerebral blood flow in CFS that will adopt a more rigorous exercise program than the one used in this study.

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Naloxone-reversible monocyte dysfunction in patients with chronic fatigue syndrome. Prieto J, Subira ML, Castilla A, Serrano M. Scandinavian Journal of Immunology 1989; 30: 13-20.

Abstract: We studied monocyte function in 35 consecutive patients with chronic fatigue syndrome (CFS) and 25 healthy controls. Eighty-five per cent of the patients showed monocyte dysfunction characterized by marked reduction in the number of monocytes displaying immunoreactive cytoskeletal vimentin filaments, a low phagocytosis index, and a reduced expression of HLA-DR antigens. These values increased dramatically after incubation of the patients' monocytes with the opioid antagonist naloxone. Other immunological abnormalities also noted in the patients were low lymphocyte blastogenesis and diminished numbers of monocytes displaying receptors for Fc of IgG (FcR) and C3b (CR1). These findings suggest that an increased opioid activity acting through a classical receptor mechanism is active on monocytes from a high proportion of patients with CFS and that this represents a novel example of immunomodulation by opioid peptides in human disease. We suggest that endogenous opioids are involved in the pathogenesis of the chronic fatigue syndrome.

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Long term improvements in patients with chronic fatigue syndrome treated with Ampligen. Strayer DR, Carter W, Strauss KI, Brodsky I, Suhadolnik RJ, Ablashi D, Henry B, Mitchell WM, Bastein S, Peterson D. Journal of Chronic Fatigue Syndrome 1995; 1(1): 35-53.

Abstract: Fifteen patients who fit the CDC definition of chronic fatigue syndrome (CFS) and had evidence of severe reduction in performance levels by low Karnofsky performance scores (KPS) of 20 - 60 were treated with Ampligen. At baseline most patients showed evidence of cerebral dysfunction by neuropsychological testing, were antigen positive by cell culture assay for human herpesvirus-6 (HHV-6), and displayed reduced performance during exercise tolerance testing, as measured by oxygen consumption. These patients represented a subset of CFS patients with especially severe and sustained symptomatology. Following 12 - 48 weeks of Ampligen therapy, sustained improvements were noted in KPS ( $p < .01$ ) Cognitive function improved including IQ and memory. Oxygen uptake and treadmill duration during exercise tolerance testing was also improved after 24 weeks of treatment ( $p < 0.01$ ). Reduction in HHV-6 expression as measured by the giant cell assay was significant ( $p < 0.001$ ). Patients continued to show significant improvement late in therapy, taking 8 to 12 weeks as baseline. It was concluded that while receiving Ampligen, the severely afflicted patients studied here derived long-lasting clinical benefit from the Ampligen therapy. .

## RNase L Research

Physical performance and prediction of 2-5A synthetase/RNase L antiviral pathway activity in patients with chronic fatigue syndrome. CR Snell, JM Vanness, DR Strayer, SR Stevens. Department of Sport Sciences, University of the Pacific, Stockton, CA. *In Vivo* 2002; 16(2):107-9.

Abstract: The elevated RNase L enzyme activity observed in some Chronic Fatigue Syndrome (CFS) patients may be linked to the low exercise tolerance and functional impairment that typify this disease. The purpose of this investigation was to determine if specific indicators of physical performance can predict abnormal RNase L activity in CFS patients. Seventy-three CFS patients performed a graded exercise test to voluntary exhaustion. Forty-six patients had elevated RNase L levels. This measure was employed as the dependent variable in a discriminant function analysis, with peak V<sub>O2</sub>, exercise duration and Karnofsky Performance Scores (KPS) serving as the independent variables. All three variables entered the single significant function ( $p < 0.001$ ). The elevated RNase L group had a lower peak V<sub>O2</sub> and duration than the normal group, but a higher KPS. The results suggest that both exercise testing and the RNase L biomarker have potential to aid in the diagnosis of CFS.

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RNase L in Health and Disease -- What Did We Learn Recently? Patrick Englebienne J of Chronic Fatigue Syndrome, Vol. 11(2) 2003, pp. 97-109 Patrick Englebienne is affiliated with the Department of Nuclear Medicine, Free University of Brussels, Brugmann University Hospital, Place van Gehuchten 4, B-1020 Brussels, Belgium, and RED Laboratories N. V., Pontbeek 61, B-1731 Zellik, Belgium (E-mail: [mailto:penglebi@ulb.ac.be](mailto:mailto:penglebi@ulb.ac.be) ). RECEIVED: 09/02/02 REVISED: 09/12/02 ACCEPTED: 09/16/02

**ABSTRACT.** The 2',5'-oligoadenylate-dependent ribonuclease L (RNase L) is central to the innate cellular defense mechanism induced by type I interferons during intracellular infection. The protein, activated by 2',5'-oligoadenylates precludes the replication of the infectious agent by cleaving single-stranded RNA and, along with the double-stranded RNA-dependent protein kinase, its spreading by inducing the cell to undergo suicide (apoptosis). In absence of infection, the protein remains dormant. Recent evidence indicates, however, that the protein is activated in absence of infection and may play a role in cell differentiation, immune activation, and act as a tumor-suppressor. A deregulation in this pathway has been documented in immune cells of chronic fatigue syndrome patients which involves the presence of a catalytically active truncated RNase L. This protein escapes the normal regulation which implies the development of a cascade of unwanted cellular events. The present article reviews our current understanding of this deregulation, enlightens its relevance in the pathological process and proposes new targets for therapeutic development.

"To the best of our knowledge, the only drug so far developed and capable of regulating the 2-50AS activity is the mismatched ds-RNA Ampligen (47), which unfortunately also upregulates PKR (48), which is highly undesirable in CFS (49). Thus, other drugs are required that target more specifically and distinctly the 2-50AS and PKR. Meanwhile, however, we have other means at our disposal capable of reducing the RNase L deregulation process. Calcium antagonists, which have already shown efficacy in CFS therapy (47), reduce m-calpain activation. Elastase inhibitors are another class of drugs that show promise in regulating the dysfunction. Currently, we have reproduced the RNase L

deregulation in cell culture models which have allowed testing such drug candidates with some success. Thus, our progressive in-depth understanding of this deregulation opens the way for testing and eventually transferring new possible therapies from bench to clinic."

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Biochemical Dysregulation of the 2-5A Synthetase/RNase L Antiviral Defense Pathway in Chronic Fatigue Syndrome Robert J. Suhadolnik, PhD; Daniel L. Peterson, MD; Paul R. Cheney, MD, PhD; Susan E. Horvath, BS; Nancy L. Reichenbach, BS; Karen O'Brien, BS; Vincent Lombardi, BA; Suzanne Welsch, MS; Elizabeth G. Furr, BS; Ramamurthy Charubala, PhD; Wolfgang Pfliederer, PhD [ Journal of Chronic Fatigue Syndrome (The Haworth Medical Press, an imprint of The Haworth Press, Inc.) Vol. 5, No. 3/4, 1999 ]

SUMMARY. The aim of the current study was to examine the biochemical defects in key components of the 2',5'-oligoadenylate (2-5A) synthetase/RNase L antiviral pathway in an extended cohort of patients with chronic fatigue syndrome (CFS) from two sites. CFS patients, who met the CDC criteria for CFS, and matched controls were assessed with respect to their general health, depression, and pain. Biochemical assays were completed for three blood draws over a period of one year. Analysis of the mean values for bioactive 2-5A, RNase L activity, low molecular weight (LMW) RNase L in CFS PBMC extracts confirmed the statistically significant upregulation of the 2-5A synthetase/RNase L pathway compared to control PBMC extracts ( $p = .001$ ,  $.002$ , and  $.007$ , respectively). Clinical correlates to the biochemical findings included a negative correlation between Karnofsky Performance Score and bioactive 2-5A ( $p = .025$ ) or RNase L activity ( $p = .002$ ) and positive correlation between Metabolic Screening Questionnaire and RNase L activity ( $p = .01$ ) and between interferon- and LMW RNase L ( $p = .05$ ). The evidence presented in this study more firmly establishes the dysregulation of the 2-5A synthetase/RNase L pathway in CFS.

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Characterization of a 2-5A dependent 37-kDa RNase L. 2. Azido photoaffinity labeling and 2-5A Dependent activation. Shetzline SE, Suhadolnik RJ. J Biol Chem 2001 Apr 25; [epub ahead of print] Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140. PMID: 11323422

The preceding paper in this issue described the characterization of the molecular structure of the 37-kDa RNase L identified in peripheral blood mononuclear cell (PBMC) extracts from individuals with chronic fatigue syndrome (CFS) [Shetzline, S., et al., (2001) J. Biol. Chem. (preceding paper in this issue)].

In this study, analysis of 2-5A binding and activation of the 80-kDa and the 37-kDa forms of RNase L has been completed utilizing photolabeling/immunoprecipitation and affinity assays, respectively. Saturation of photolabeling of the 80-kDa and the 37-kDa RNase L with the 2-5A azido photoprobe,  $[^{32}\text{P}]\text{pApAp}(8\text{-azidoA})$ , was achieved.

Half-maximal photoinsertion of [32P]pApAp(8-azidoA) occurred at  $3.7 \times 10^{-8}$  M for the 80-kDa RNase L and at  $6.3 \times 10^{-8}$  M for the 37-kDa RNase L. Competition experiments using 100-fold excess unlabeled 2-5A photoaffinity probe, pApAp(8-azidoA), and authentic 2-5A (p3A3) resulted in complete protection against photolabeling, demonstrating that [32P]pApAp(8-azidoA) binds specifically to the 2-5A binding site of the 80-kDa and the 37-kDa RNase L. The rate of RNA hydrolysis by the 37-kDa RNase L was three times faster than the 80-kDa RNase L.

The data obtained from these 2-5A binding and 2-5A-dependent activation studies demonstrate the utility of [32P]pApAp(8-azidoA) for the detection of the 37-kDa RNase L in PBMC extracts.

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G-Actin Cleavage Parallels 2-5-A-Dependent RNase L Cleavage in Peripheral Blood Mononuclear Cells– Relevance to a Possible Serum-Based Screening Test for Dysregulations in the 2-5A Pathway  
Simon Roelens, C Vincent Herst, Anne D'Haese, Karen De Smet, Marc Fremont, Kenny De Meirleir, Patrick Englebienne *Innov in Chronic Fatigue Syndrome Res and Clin Practice* 2001; 8(3-4):63-82.

Summary: A dysregulation in the 2',5'-oligoadenylate (2-5A)-dependent RNase L antiviral pathway has been detected in peripheral blood mononuclear cells (PBMC) of chronic fatigue syndrome (CFS) patients, which is characterized by an unregulated RNase L activity and the presence of a low molecular weight (LMW) 2-5A-binding protein (37-kDa 2-5A-BP). This study was undertaken to test the possibility that the 37-kDa 2-5A-BP Of CFS is produced by proteolytic cleavage of the 80-kDa monomeric enzyme. Incubation of the 80-kDa human recombinant RNase L (r-hRNase L) with PBMC extracts either positive or negative for the presence of 37-kDa 2-5A-BP, respectively, demonstrates that the LMW protein is produced by the former, not the latter, and that the size of the fragment generated from the recombinant protein matches the 37-kDa size of the fragment observed in the original PBMC. Digestion of r-hRNase L with calpain generated the same 37-kDa 2-5A-BP observed in PBMC extracts and calpain immunoprecipitation from PBMC extracts reduced their proteolytic activity, an observation that suggests that calpain may be involved in the cleavage. We further examined G-actin, a known calpain substrate, for possible cleavage in PBMC. Actin fragments were observed of which the presence correlated with the presence of 37-kDa 2-5A-BP. Since G-actin is cleared by serum transport, we further screened serum samples for the presence of LMW forms. A single LMW actin fragment could be detected in serum, the presence of which correlated significantly with the presence of both G-actin and RNase L fragments in PBMC. This latter observation offers the opportunity to screen large populations of patients for dysregulations in the RNase L pathway by a serum-based assay.

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Structural and functional features of the 37-kDa 2-5A-dependent RNase L in chronic fatigue syndrome. Susan Shetzline, Camille Martinand-Mari, Nancy L Reichenbach, Zivjena Buletic, Bernard Lebleu, Wolfgang Pfliegerer, Ramamurthy Charubala, Kenney De Meirleir, Pascale De Becker, Daniel L Peterson, CVT Herst, Patrick Englebienne, Robert J Suhadolnik. Department of Biochemistry and

the Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA. *J Interferon and Cytokine Research* 2002; 22(4):443-456

Abstract: A 2',5'-oligoadenylate (2-5A)-dependent 37-kDa form of RNase L has been reported in extracts of peripheral blood mononuclear cells (PBMC) from individuals with chronic fatigue syndrome (CFS). In the current study, analytic gel permeation FPLC, azido photoaffinity labeling, two-dimensional (2-D) gel electrophoresis, and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) have been used to examine the biochemical relationship between the 80-kDa RNase L in healthy control PBMC and the 37-kDa RNase L in PBMC from individuals with CFS. Like the 80-kDa RNase L, the 37-kDa RNase L is present as a catalytically inactive heterodimer complex with the RNase L inhibitor (RLI). Formation of a 37-kDa RNase L-RLI complex indicates that the 37-kDa RNase L is structurally similar to the 80-kDa RNase L at the N-terminus, which contains the 2-5A binding domain. The enzymatically active monomer form of 37-kDa RNase L resolved by 2-D gel electrophoresis has a pI of 6.1. RT-PCR and Southern blot analyses demonstrated that the 37-kDa RNase L is not formed by alternative splicing. In-gel tryptic digestion of the 37-kDa RNase L that was excised from 2-D gels and subsequent MALDI-MS analysis identified three peptide masses that are identical to three predicted peptide masses in the 80-kDa RNase L. The electrophoretic mobility of 2-5A azido photolabeled/immunoprecipitated 37-kDa RNase L was the same under reducing and nonreducing conditions. The results presented show that the 37-kDa form of RNase L in PBMC shares structural and functional features with the native 80-kDa RNase L, in particular in the 2-5A binding and catalytic domains.

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Biochemical evidence for a novel low molecular weight 2-5A-dependent RNase L in chronic fatigue syndrome. Robert J Suhadolnik, Daniel L Peterson, K O'Brien, PR Cheney, CV Herst, Nancy L Reichenbach, N Kon, SE Horvath, KT Iacono, ME Adelson, Kenny De Meirleir, Pascale De Becker, Ramamurthy Charubala, Wolfgang Pfliederer. Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA. *J Interferon and Cytokine Research* 1997; 17(7):377-385.

Abstract: Previous studies from this laboratory have demonstrated a statistically significant dysregulation in several key components of the 2',5'-oligoadenylate (2-5A) synthetase/RNase L and PKR antiviral pathways in chronic fatigue syndrome (CFS) (Suhadolnik et al. *Clin Infect Dis* 18, S96-104, 1994; Suhadolnik et al. *In Vivo* 8, 599-604, 1994). Two methodologies have been developed to further examine the upregulated RNase L activity in CFS. First, photoaffinity labeling of extracts of peripheral blood mononuclear cells (PBMC) with the azido 2-5A photoaffinity probe, [32P]pApAp(8-azidoA), followed by immunoprecipitation with a polyclonal antibody against recombinant, human 80-kDa RNase L and analysis under denaturing conditions. A subset of individuals with CFS was identified with only one 2-5A binding protein at 37 kDa, whereas in extracts of PBMC from a second subset of CFS PBMC and from healthy controls, photolabeled/immunoreactive 2-5A binding proteins were detected at 80, 42, and 37 kDa. Second, analytic gel permeation HPLC was completed under native conditions. Extracts of healthy control PBMC revealed 2-5A binding and 2-5A-dependent RNase L enzyme activity at 80 and 42 kDa as determined by hydrolysis of poly(U)-3'-[32P]pCp. A subset of CFS PBMC contained 2-5A binding proteins with 2-5A-dependent RNase L enzyme activity at 80, 42, and 30 kDa. However, a second subset of CFS PBMC contained 2-5A binding and 2-5A-

dependent RNase L enzyme activity only at 30 kDa. Evidence is provided indicating that the RNase L enzyme dysfunction in CFS is more complex than previously reported.

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Ribonuclease L Proteolysis in Peripheral Blood Mononuclear Cells of Chronic Fatigue Syndrome Patients Edith Demettré§, Lionel Bastide§¶, Anne D'Haese§¶, Karen De Smet¶, Kenny De Meirleir, Kiet P. Tiev\*\*, Patrick Englebienne¶, and Bernard Lebleu§§ From the §UMR 5124 CNRS, Université Montpellier 2, 34293 Montpellier, France, ¶ R.E.D Laboratories, 1731 Zellik, Belgium, Department of Human Physiology and Medicine, Vrije Universiteit Brussel, 1090 Brussels, Belgium, \*\* Service de Médecine Interne, Hôpital Saint Antoine, 75571 Paris, France, and Department of Nuclear Medicine, Université Libre de Bruxelles, 1050 Brussels, Belgium *J. Biol. Chem.*, Vol. 277, Issue 38, 35746-35751, September 20, 2002

A 37-kDa binding polypeptide accumulates in peripheral blood mononuclear cell (PBMC) extracts from chronic fatigue syndrome (CFS) patients and is being considered as a potential diagnostic marker (De Meirleir, K., Bisbal, C., Campine, I., De Becker, P., Salehzada, T., Demettré, E., and Lebleu, B. (2000) *Am. J. Med.* 108, 99-105).

We establish here that this low molecular weight 2-5A-binding polypeptide is a truncated form of the native 2-5A-dependent ribonuclease L (RNase L), generated by an increased proteolytic activity in CFS PBMC extracts.

RNase L proteolysis in CFS PBMC extracts can be mimicked in a model system in which recombinant RNase L is treated with human leukocyte elastase.

RNase L proteolysis leads to the accumulation of two major fragments with molecular masses of 37 and 30 kDa. The 37-kDa fragment includes the 2-5A binding site and the N-terminal end of native RNase L. The 30-kDa fragment includes the catalytic site in the C-terminal part of RNase L.

Interestingly, RNase L remains active and 2-5A-dependent when degraded into its 30- and 37-kDa fragments by proteases of CFS PBMC extract or by purified human leukocyte elastase. The 2-5A-dependent nuclease activity of the truncated RNase L could result from the association of these digestion products, as suggested in pull down experiments.

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RNase L dysfunction disorder (R.E.D.D.) in CFS K. De Meirleir\*, LCLI, I. Campine+\*, P. De Becker, B. Van Steenberge\*, C. Bisbal\*\*, T. Salehzada\*\*, B. Lebleu\*\*, C.V. Herst\*\* Department of Human Physiology, Free University of Brussels, Brussels, Belgium \*\*Institute of Molecular Genetics, Montpellier University, Montpellier, France + I. Campine is supported by funds from the Foundation for Scientific Research, Belgium (F.W.O.).

Objective: The unknown aetiology and absence of biochemical markers in CFS are a major problem in this disorder. Activation of immune responses and infection by several viruses have been suggested in several studies. Recent work by Suhadolnik et al. has demonstrated increased levels of 2-5' A oligonucleotides, 2'-5'A Synthetase and RNase L activity in mononuclear cell pellets from CFS patients, as well as a low molecular form of RNase L in severely disabled CFS patients. This work was designed to explore the specificity and sensitivity of the presence of the LMW RNase L in CFS. Methods: Mononuclear cell pellets (PBMC) of 57 patients and 18 controls were studied. The technique used to detect the RNase L molecular weight is described by Charachon et al (Biochemistry 29:2550-2556, 1990). This technique is different from the one described by the one used by Suhadolnik et al. (Clin Inf Dis, 18 (Suppl 1), 5 96-104, 1994). An RLI binding study was also performed. Results: A low molecular weight (LMW) 2'-5'A binding polypeptide (37 kDa) was found in 50 out of 57 PBMC pellets of the CFS patients, versus 4 out of 18 healthy individuals. Both sensitivity and specificity of the LMW RNase L in relationship to CFS are high. The 37kDa 2'-5'A binding polypeptide binds RLI. Conclusion: The presence of a 37 kDa 2'-5'A binding polypeptide in the PBMC pellets of CFS patients may objectively contribute to distinguish CFS patients from healthy individuals. These observations could provide the basis for the development of a biochemical assay for the differential diagnosis of CFS and for follow up of its clinical evolution.

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A controlled trial with a specially configured RNA drug, Poly(I)∆Poly(C12U), in chronic fatigue syndrome. Strayer DR, Carter WA, Brodsky I, Cheney P, Peterson D, Salvato P, Thompson C, Loveless M, Shapiro DE, Elsasser W, Gillespie DH. Clinical Infectious Diseases 1994; 18(Supp 1): S88-95.

Abstract: Chronic fatigue syndrome (CFS) is a physically debilitating illness associated with immunologic abnormalities, viral reactivation, and impairment of cognition. In a randomized, multicenter, placebo-controlled, double-blind study of 92 patients meeting the CFS case definition of the Centers for Disease Control and Prevention, the response of several laboratory and clinical variables to an antiviral and immunomodulatory drug, poly(I)∆poly(C12U), was determined. Measures of clinical response included Karnofsky performance score, a cognition scale derived from a self-administered instrument assessing symptomatology (SCL-90-R), an activities of daily living scale, and exercise treadmill performance. After 24 weeks, patients receiving poly(I)∆poly(C12U) had higher scores for both global performance and perceived cognition than did patients receiving placebo. In particular, patients given poly(I)∆poly(C12U) had increased Karnofsky performance scores ( $P < .03$ ), exhibited a greater ability to do work during exercise treadmill testing ( $P = .01$ ), displayed an enhanced capacity to perform the activities of daily living ( $P < .04$ ), had a reduced cognitive deficit ( $P = .05$ ), and required less use of other medications ( $P < .05$ ).

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Long term improvements in patients with chronic fatigue syndrome treated with Ampligen. Strayer DR, Carter W, Strauss KI, Brodsky I, Suhadolnik RJ, Ablashi D, Henry B, Mitchell WM, Bastein S, Peterson D. *Journal of Chronic Fatigue Syndrome* 1995; 1(1): 35-53.

Abstract: Fifteen patients who fit the CDC definition of chronic fatigue syndrome (CFS) and had evidence of severe reduction in performance levels by low Karnofsky performance scores (KPS) of 20 - 60 were treated with Ampligen. At baseline most patients showed evidence of cerebral dysfunction by neuropsychological testing, were antigen positive by cell culture assay for human herpesvirus-6 (HHV-6), and displayed reduced performance during exercise tolerance testing, as measured by oxygen consumption. These patients represented a subset of CFS patients with especially severe and sustained symptomatology. Following 12 - 48 weeks of Ampligen therapy, sustained improvements were noted in KPS ( $p < .01$ ). Cognitive function improved including IQ and memory. Oxygen uptake and treadmill duration during exercise tolerance testing was also improved after 24 weeks of treatment ( $p < .01$ ). Reduction in HHV-6 expression as measured by the giant cell assay was significant ( $p < 0.001$ ). Patients continued to show significant improvement late in therapy, taking 8 to 12 weeks as baseline. It was concluded that while receiving Ampligen, the severely afflicted patients studied here derived long-lasting clinical benefit from the Ampligen therapy.

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Biochemical evidence for a novel low molecular weight 2-5A-dependent RNaseL in chronic fatigue syndrome. Suhadolnik RJ, Peterson DL, O'Brien K, Cheney PR, Herst CV, Reichenbach NL, Kon N, Horvath SE, Iacono KT, Adelson ME, De Meirleir K, De Becker P, Charubala R, Pflleiderer W. 1997; 17(7): 377-385.

Abstract: Previous studies from this laboratory have demonstrated a statistically significant dysregulation in several key components of the 2',5'-oligoadenylate (2-5A) synthetase/RNaseL and PKR antiviral pathways in chronic fatigue syndrome (CFS) (Suhadolnik et al. *Clin Infect Dis* 18, S96-104, 1994; Suhadolnik et al. *In Vivo* 8, 599-604, 1994). Two methodologies have been developed to further examine the upregulated RNaseL activity in CFS. First, photoaffinity labeling of extracts of peripheral blood mononuclear cells (PBMC) with the azido 2-5A photoaffinity probe, [32P]pApAp(8-azidoA), followed by immunoprecipitation with a polyclonal antibody against recombinant, human 80-kDa RNaseL and analysis under denaturing conditions. A subset of individuals with CFS was identified with only one 2-5A binding protein at 37 kDa, whereas in extracts of PBMC from a second subset of CFS PBMC and from healthy controls, photolabeled/immunoreactive 2-5A binding proteins were detected at 80, 42, and 37 kDa. Second, analytic gel permeation HPLC was completed under native conditions. Extracts of healthy control PBMC revealed 2-5A binding and 2-5A-dependent RNaseL enzyme activity at 80 and 42 kDa as determined by hydrolysis of poly(U)-3'-[32P]pCp. A subset of CFS PBMC contained 2-5A binding proteins with 2-5A-dependent RNaseL enzyme activity at 80, 42, and 30 kDa. However, a second subset of CFS PBMC contained 2-5A binding and 2-5A-dependent RNaseL enzyme activity only at 30 kDa. Evidence is provided indicating that the RNaseL enzyme dysfunction in CFS is more complex than previously reported.

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Changes in the 2-5A synthetase/RNaseL antiviral pathway in a controlled clinical trial with poly(I)-poly(C12U) [Ampligen] in CFS. Suhadolnik RJ, Reichenbach NL, Hitzges P, Adelson ME, Peterson DL, Cheney P, Salvato P, Thompson C, Loveless M, Muller WE, et al. *In Vivo* 1994; 8(4): 599-604.

Abstract: Latent 2', 5'-oligoadenylate (2-5A) synthetase activity, bioactive 2-5A and RNaseL activity were measured in extracts of peripheral blood mononuclear cells (PMBC) before and during a randomized, multicenter, placebo-controlled, double-blind study of poly(I)-poly(C12U) in individuals with chronic fatigue syndrome (CFS) as defined by the Centers for Disease Control and Prevention. The mean values for bioactive 2-5A and RNaseL activity were significantly elevated at baseline compared to controls ( $p < .0001$  and  $p = .001$ , respectively). In individuals that presented with elevated RNaseL activity at baseline, therapy with poly(I)-poly(C12U) resulted in a significant decrease in both bioactive 2-5A and RNaseL activity ( $p = .09$  and  $p = .005$ , respectively). Decrease in RNaseL activity in individuals treated with poly(I)-poly(C12U) correlated with cognitive improvement ( $p = .007$ ). Poly(I)-poly(C12U) therapy resulted in a significant decrease in bioactive 2-5A and RNaseL activity in agreement with clinical and neuropsychological improvements (Strayer DR, et al., *Clin. Infectious Dis.* 18:588-595, 1994). The results described show that poly(I)-poly(C12U) is a biologically active drug in CFS.

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Upregulation of the 2-5A synthetase/RNaseL antiviral pathway associated with chronic fatigue syndrome. Suhadolnik RJ, Reichenbach NL, Hitzges P, Sobol RW, Peterson DL, Henry B, Ablashi DV, Muller WE, Schroder HC, Carter WA, et al. *Clinical Infectious Diseases* 1994; 18(Supp 1): S96-S104.

Abstract: Levels of 2',5'-oligoadenylate (2-5A) synthetase, bioactive 2-5A, and RNaseL were measured in extracts of peripheral blood mononuclear cells (PBMCs) from 15 individuals with chronic fatigue syndrome (CFS) before and during therapy with the biological response modifier poly(I).poly(C12U) and were compared with levels in healthy controls. Patients differed significantly from controls in having a lower mean basal level of latent 2-5A synthetase ( $P < .0001$ ), a higher pretreatment level of bioactive 2-5A ( $P = .002$ ), and a higher level of pretherapy RNaseL activity ( $P < .0001$ ). PBMC extracts from 10 persons with CFS had a mean basal level of activated 2-5A synthetase higher than the corresponding control value ( $P = .009$ ). All seven pretherapy PBMC extracts tested were positive for the replication of human herpesvirus 6 (HHV-6). Therapy with poly(I).poly(C12U) resulted in a significant decrease in HHV-6 activity ( $P < .01$ ) and in downregulation of the 2-5A synthetase/RNaseL pathway in temporal association with clinical and neuropsychological improvement. The upregulated 2-5A pathway in CFS before therapy is consistent with an activated immune state and a role for persistent viral infection in the pathogenesis of CFS. The response to therapy suggests direct or indirect antiviral activity of poly(I).poly(C12U) in this situation.