CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 2002, p. 747–752 Vol. 9, No. 4
1071-412X/02/$04.00+0 DOI: 10.1128/CDLI.9.4.747–752.2002

Evidence for the Presence of Immune Dysfunction in Chronic Fatigue Syndrome

Benjamin H. Natelson,[1]* Mohammad H. Haghighi,[2] and Nicholas M. Ponzio[3]

Departments of Neurosciences[1] and Pathology,[3] University of Medicine and Dentistry—New Jersey Medical School, and Department of Psychology, Rutgers University,[2] Newark, New Jersey

Chronic fatigue syndrome 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.

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 $CD4^+$ to $CD8^+$ 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

* Corresponding author. Mailing address: Department of Neurosciences, University of Medicine & Dentistry—New Jersey Medical School, 88 Ross St., East Orange, NJ 07018. Phone: (973) 676-1000. Fax: (973) 395-7114. E-mail: bhn@njneuromed.org.

TABLE 1. Immunological variables that do not differ significantly from control values

Immunological variable	References
% CD2	3, 59, 64
% $CD4^+$/CD45RO	18, 55, 63, 66, 69
% CD5	63, 64
$CD8^+$/ICAM-1[a]	16, 66
CD8/CD45RA	55, 63
CD4/CD29	26, 63, 66
CD4/CD25	29, 55
Leu-M3-	10, 41, 57
IgG4	4, 16, 38, 47, 58
IgM	3, 38, 40, 47, 58
IFN-γ in vivo	34, 36, 63, 74
IL-1α in vivo	34, 40, 53
IL-1α in vitro	40, 45, 58, 65
% IL-2R	10, 34, 53
IL-4 in vivo	10, 53, 74
IL-4 in vitro	10, 68
IL-12 in vitro	10, 69
IL-12 in vivo	34, 53, 74
C3	40, 47
C4	40, 47

[a] ICAM-1, intercellular adhesion molecule 1.

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 $CD11b^-$: 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 $CD3^-$/$CD56^+$ 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-/$CD16^+$ cell count, three reported decreases (16, 39, 66), with the others all being normal (18, 32, 63). Five papers examined the $CD3^-$ ($CD16^+$ and $CD56^+$) 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 $CD3^-$ ($CD16^+$ and $CD56^+$) 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-1β. One group noted that the significant monthly fluctuation in interleukin 1β (IL-1β) occurring in healthy women was not seen with CFS patients (7). However, when this variable was assessed without considering mensal 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 mensal 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-α. One study reported increases (71) in alpha interferon (IFN-α), while four others found no differences between groups (5, 34, 35, 62).

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

(viii) TNF-α. Three groups reported increases in tumor necrosis factor alpha (TNF-α) (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-β. One group reported TNF-β 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-1β. One group noted IL-1β 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-γ. One group noted IFN-γ 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-γ by CD4 cells was decreased.

(vii) TNF-α. One group noted TNF-α 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 antinuclear 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 cyclicity 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. $CD3^-/CD56^+$ 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-γ 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-γ 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."

ACKNOWLEDGMENT

Preparation of this report was supported by NIH Center grant no. AI-32247.

REFERENCES

1. **Baraniuk, J. N., D. Clauw, A. MacDowell-Carneiro, J. Bellanti, S. Foong, and M. Ali.** 1998. IgE concentrations in chronic fatigue syndrome. J. Chronic Fatigue Syndr. **4:**13–21.
2. **Barker, E., S. F. Fujimura, M. B. Fadem, A. L. Landay, and J. A. Levy.** 1994. Immunologic abnormalities associated with chronic fatigue syndrome. Clin. Infect. Dis. **18**(Suppl. 1)**:**S136–S141.
3. **Bates, D. W., D. Buchwald, J. Lee, P. Kith, T. Doolittle, C. Rutherford, W. H. Churchill, P. H. Schur, M. Wener, D. Wybenga, J. Winkelman, and A. L. Komaroff.** 1995. Clinical laboratory test findings in patients with chronic fatigue syndrome. Arch. Intern. Med. **155:**97–103.
4. **Bennett, A. L., C. C. Chao, S. Hu, B. Buchwald, L. R. Fagioli, P. H. Schur, P. K. Peterson, and A. L. Komaroff.** 1997. Elevation of bioactive transforming growth factor-β in serum from patients with chronic fatigue syndrome. J. Clin. Immunol. **17:**160–166.

4a. **Bennett, A. L., L. R. Fagioli, P. H. Schur, R. S. Schacterle, and A. L. Komaroff.** 1996. Immunoglobulin subclass levels in chronic fatigue syndrome. J. Clin. Immunol. **16:**315–320.

5. **Borish, L., K. Schmaling, J. D. DiClementi, J. Streib, J. Negri, and J. F. Jones.** 1998. Chronic fatigue syndrome: identification of distinct subgroups on the basis of allergy and psychologic variables. J. Allergy Clin. Immunol. **102:**222–230.
6. **Buchwald, D., M. H. Wener, T. Pearlman, and P. Kith.** 1997. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J. Rheumatol. **24:**372–376.
7. **Cannon, J. G., J. B. Angel, L. W. Abad, E. Vannier, M. D. Mileno, L. Fagioli, S. M. Wolff, and A. L. Komaroff.** 1997. Interleukin-1β, interleukin-1 receptor antagonist, and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. J. Clin. Immunol. **17:**253–261.
8. **Cannon, J. G., J. B. Angel, R. W. Ball, L. W. Abad, L. Fagioli, and A. L. Komaroff.** 1999. Acute phase responses and cytokine secretion in chronic fatigue syndrome. J. Clin. Immunol. **19:**414–421.
9. **Chao, C. C., M. Gallagher, J. Phair, and P. K. Peterson.** 1990. Serum neopterin and interleukin-6 levels in chronic fatigue syndrome. J. Infect. Dis. **162:**1412–1413.
10. **Chao, C. C., E. N. Janoff, S. Hu, K. Thomas, M. Gallagher, M. Tsang, and P. K. Peterson.** 1991. Altered cytokine release in peripheral blood mononuclear cell cultures from patients with the chronic fatigue syndrome. Cytokine **3:**292–298.
11. **Cheney, P. R., S. E. Dorman, and D. S. Bell.** 1989. Interleukin-2 and the chronic fatigue syndrome. Ann. Intern. Med. **110:**321.
12. **Conti, F., L. Magrini, R. Priori, G. Valesini, and S. Bonini.** 1996. Eosinophil cationic protein serum levels and allergy in chronic fatigue syndrome. Allergy **51:**124–127.
13. **Fukuda, K., S. E. Straus, I. Hickie, M. C. Sharpe, A. Komaroff, A. Schluederberg, J. F. Jones, A. R. Lloyd, S. Wessely, N. G. Gantz, G. P. Holmes, L. Steele, M. Reyes, S. Abbey, J. Rest, H. Jolson, D. L. Peterson, J. H. M. M. Vercoulen, U. Tirelli, B. Evengard, B. H. Natelson, and W. C. Reeves.** 1994. The chronic fatigue syndrome: a comprehensive approach to its definition and study. Ann. Intern. Med. **121:**953–959.
14. **Gupta, S., S. Aggarwal, D. See, and A. Starr.** 1997. Cytokine production by adherent and non-adherent mononuclear cells in chronic fatigue syndrome. J. Psychiatr. Res. **31:**149–156.
15. **Gupta, S., S. Aggarwal, D. See, and A. Starr.** 1999. Increased production of interleukin-6 by adherent and non-adherent mononuclear cells during 'natural fatigue' but not following 'experimental fatigue' in patients with chronic fatigue syndrome. Int. J. Mol. Med. **3:**209–213.
16. **Gupta, S., and B. Vayuvegula.** 1991. A comprehensive immunological analysis in chronic fatigue syndrome. Scand. J. Immunol. **33:**319–327.
17. **Hanson, S. J., W. C. Gause, and B. H. Natelson.** 2001. Detection of immunologically significant factors for chronic fatigue syndrome using neural network classifiers. Clin. Diagn. Lab. Immunol. **8:**658–662.
18. **Hassan, I. S., B. A. Bannister, A. Akbar, W. Weir, and M. Bofill.** 1998. A study of the immunology of the chronic fatigue syndrome: correlation of immunologic parameters to health dysfunction. Clin. Immunol. Immunopathol. **87:**60–67.
19. **Herbert, T. B., and S. Cohen.** 1993. Stress and immunity in humans: a meta-analytic review. Psychosom. Med. **55:**364–379.
20. **Hoffman-Goetz, L., and B. K. Pedersen.** 1994. Exercise and the immune system: a model of the stress response. Immunol. Today **15:**382–387.
21. **Holmes, G. P., J. E. Kaplan, N. M. Gantz, A. L. Komaroff, L. B. Schonberger, S. E. Straus, et al.** 1988. Chronic fatigue syndrome: a working case definition. Ann. Intern. Med. **108:**387–389.
22. **Irwin, M., U. Lacher, and C. Caldwell.** 1992. Depression and reduced natural killer cytotoxicity: a longitudinal study of depressed patients and control subjects. Psychol. Med. **22:**1045–1050.
23. **Irwin, M., J. McClintick, C. Costlow, M. Fortner, J. White, and J. C. Gillin.** 1996. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. FASEB J. **10:**643–653.
24. **Kavelaars, A., W. Kuis, L. Knook, G. Sinnema, and C. J. Heijnen.** 2000. Disturbed neuroendocrine-immune interactions in chronic fatigue syndrome. J. Clin. Endocrinol. Metab. **85:**692–696.
25. **Klimas, N.** 1995. Immunology workshop summary. J. Chronic Fatigue Syndr. **1:**203–206.
26. **Klimas, N. G., F. R. Salvato, R. Morgan, and M. A. Fletcher.** 1990. Immunologic abnormalities in chronic fatigue syndrome. J. Clin. Microbiol. **28:** 1403–1410.
27. **Konstantinov, K., A. Von Mikecz, D. Buchwald, J. Jones, L. Gerace, and E. M. Tan.** 1996. Autoantibodies to nuclear envelope antigens in chronic fatigue syndrome. J. Clin. Investig. **98:**1888–1896.
28. **LaManca, J. J., S. Sisto, J. E. Ottenweller, S. Cook, A. Peckerman, Q. Zhang, T. N. Denny, W. C. Gause, and B. H. Natelson.** 1999. Immunological response in chronic fatigue syndrome following a graded exercise test to exhaustion. J. Clin. Immunol. **19:**135–142.
29. **Landay, A. L., C. Jessop, E. T. Lennette, and J. A. Levy.** 1991. Chronic fatigue syndrome: clinical condition associated with immune activation. Lancet **338:**707–712.
30. **Levi, F. A., C. Canon, Y. Touitou, A. Reinberg, and G. Mathe.** 1988. Seasonal modulation of the circadian time structure of circulating T and natural killer lymphocytes subsets from healthy subjects. J. Clin. Investig. **81:**407–413.
31. **Levine, P. H., J. K. Dale, E. Benson-Grigg, S. Fritz, S. Grufferman, and S. E. Straus.** 1996. A cluster of cases of chronic fatigue and chronic fatigue syndrome: clinical and immunologic studies. Clin. Infect. Dis. **23:**408–409.
32. **Levine, P. H., T. L. Whiteside, D. Friberg, J. Bryant, G. Colclough, and R. B.**

Herberman. 1998. Dysfunction of natural killer activity in a family with chronic fatigue syndrome. Clin. Immunol. Immunopathol. **88:**96–104.
33. **Levy, S. M., R. B. Herberman, A. Simons, T. L. Whiteside, J. Lee, R. McDonald, and M. Beadle.** 1989. Persistently low natural killer cell activity in normal adults: immunological, hormonal and mood correlates. Nat. Immun. Cell Growth Regul. **8:**173–186.
34. **Linde, A., B. Andersson, S. B. Svenson, H. Ahrne, M. Carlsson, P. Forsberg, H. Hugo, A. Karstorp, R. Lenkei, A. Lindwall, A. Loftenius, C. Säll, and J. Andersson.** 1992. Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr virus infection and in patients with chronic fatigue syndrome. J. Infect. Dis. **165:**994–1000.
35. **Lloyd, A., S. Gandevia, A. Brockman, J. Hales, and D. Wakefield.** 1994. Cytokine production and fatigue in patients with chronic fatigue syndrome and healthy control subjects in response to exercise. Clin. Infect. Dis. **18**(Suppl. 1)**:**S142–S146.
36. **Lloyd, A., I. Hickie, A. Brockman, J. Dwyer, and D. Wakefield.** 1991. Cytokine levels in serum and cerebrospinal fluid in patients with chronic fatigue syndrome and control subjects. J. Infect. Dis. **164:**1023–1024.
37. **Lloyd, A., I. Hickie, C. Hickie, J. Dwyer, and D. Wakefield.** 1992. Cell-mediated immunity in patients with chronic fatigue syndrome, healthy control subjects and patients with major depression. Clin. Exp. Immunol. **87:** 76–79.
38. **Lloyd, A. R., D. Wakefield, C. R. Boughton, and J. M. Dwyer.** 1989. Immunological abnormalities in the chronic fatigue syndrome. Med. J. Austral. **151:**122–124.
39. **Masuda, A., S.-I. Nozoe, T. Matsuyama, and H. Tanaka.** 1994. Psychobehavioral and immunological characteristics of adult people with chronic fatigue and patients with chronic fatigue syndrome. Psychosom. Med. **56:** 512–518.
40. **Mawle, A. C., R. Nisenbaum, J. G. Dobbins, H. E. Gary, Jr., J. A. Stewart, M. Reyes, L. Steele, D. S. Schmid, and W. C. Reeves.** 1997. Immune responses associated with chronic fatigue syndrome: a case-control study. J. Infect. Dis. **175:**136–141.
41. **Milton, J. D., G. B. Clements, and R. H. T. Edwards.** 1991. Immune responsiveness in chronic fatigue syndrome. Postgrad. Med. J. **67:**532–537.
42. **Milton, J. D., A. G. Morris, S. E. Christmas, and R. H. T. Edwards.** 1991. Interferon production by mononuclear cells from patients with chronic fatigue syndrome. Med. Sci. Res. **19:**205–206.
43. **Morimoto, K., T. Takeshita, C. Inoue-Sakurai, and S. Maruyama.** 2001. Lifestyles and mental health status are associated with natural killer cell and lymphokine-activated killer cell activities. Sci. Total Environ. **270:**3–11.
44. **Morte, S., A. Castilla, M. Civeira, M. Serrano, and J. Prieto.** 1988. Gamma-interferon and chronic fatigue syndrome. Lancet **ii:**623–624.
45. **Morte, S., A. Castilla, M. P. Civeira, M. Serrano, and J. Prieto.** 1989. Production of interleukin-1 by peripheral blood mononuclear cells in patients with chronic fatigue syndrome. J. Infect. Dis. **159:**362.
46. **Moss, R. B., A. Mercandetti, and A. Vojdani.** 1999. TNF-α and chronic fatigue syndrome. J. Clin. Immunol. **19:**314–316.
47. **Natelson, B. H., J. J. LaManca, T. Denny, A. C. Vladutiu, J. Oleske, M. Hill, M. T. Bergen, L. Korn, and J. Hay.** 1998. Immunological parameters in chronic fatigue syndrome, major depression, and multiple sclerosis. Am. J. Med. **105:**43S-49S.
48. **Nishikai, M., S. Tomomatsu, R. W. Hankins, S. Takagi, K. Miyachi, S. Kosaka, and K. Akiya.** 2001. Autoantibodies to a 68/48 kDa protein in chronic fatigue syndrome and primary fibromyalgia: a possible marker for hypersomnia and cognitive disorders. Rheumatology **40:**806–810.
49. **Ogawa, M., T. Nishiura, M. Yoshimura, Y. Horikawa, H. Yoshida, Y. Okajima, I. Matsumura, Y. Ishikawa, H. Nakao, Y. Tomiyama, Y. Kanayama, Y. Kanakura, and Y. Matsuzawa.** 1998. Decreased nitric oxide-mediated natural killer cell activation in chronic fatigue syndrome. Eur. J. Clin. Investig. **28:**937–943.
50. **Ojo-Amaize, E. A., E. J. Conley, and J. B. Peter.** 1994. Decreased natural killer cell activity is associated with severity of chronic fatigue immune dysfunction syndrome. Clin. Infect. Dis. **18**(Suppl. 1)**:**S157–S159.
51. **Patarca, R., N. Klimas, D. Sandler, M. N. Garcia, and M. A. Fletcher.** 1996. Interindividual immune status variation patterns in patients with chronic fatigue syndrome: association with gender and the tumor necrosis factor. J. Chronic Fatigue Syndr. **2:**13–37.
52. **Patarca, R., N. G. Klimas, M. N. Garcia, M. J. Walters, D. Dombroski, H. Pons, and M. A. Fletcher.** 1995. Dysregulation expression of soluble immune mediator receptors in a subset of patients with chronic fatigue syndrome: cross-sectional categorization of patients by immune status. J. Chronic Fatigue Syndr. **1:**81–96.
53. **Patarca, R., N. G. Klimas, S. Lugtendorf, M. Antoni, and M. A. Fletcher.** 1994. Dysregulated expression of tumor necrosis factor in chronic fatigue syndrome: interrelations with cellular sources and patterns of soluble immune mediator expression. Clin. Infect. Dis. **18**(Suppl. 1)**:**S147–S153.
54. **Patarca, R., K. Maher, K. Goodkin, and M. A. Fletcher.** 1997. Cryopreservation of peripheral blood mononuclear cells, p. 281–286. *In* N. R. Rose, E. Conway de Macario, J. D. Folds, H. C. Lane, and R. M. Nakamura (ed.), Manual of clinical laboratory immunology. American Society for Microbiology, Washington, D.C.
55. **Peakman, M., A. Deale, R. Field, M. Mahalingam, and S. Wessely.** 1997. Clinical improvement in chronic fatigue syndrome is not associated with lymphocyte subsets of function or activation. Clin. Immunol. Immunopathol. **82:**83–91.
56. **Peterson, P. K., S. A. Sirr, F. C. Grammith, C. H. Schenck, A. M. Pheley, S. Hu, and C. C. Chao.** 1994. Effects of mild exercise on cytokines and cerebral blood flow in chronic fatigue syndrome patients. Clin. Diagn. Lab. Immunol. **1:**222–226.
57. **Prieto, J., M. L. Subira, A. Castilla, and M. Serrano.** 1989. Naloxone-reversible monocyte dysfunction in patients with chronic fatigue syndrome. Scand. J. Immunol. **30:**13–20.
58. **Rasmussen, Å. K., H. Nielsen, V. Andersen, T. Barington, K. Bendtzen, M. B. Hansen, L. Nielsen, B. K. Pedersen, and A. Wiik.** 1994. Chronic fatigue syndrome—a controlled cross sectional study. J. Rheumatol. **21:**1527–1531.
59. **Roberts, T. K., N. R. McGregor, R. H. Dunstan, M. Donohoe, R. N. Murdoch, D. Hope, S. Zhang, H. L. Butt, and W. G. Taylor.** 1998. Immunological and haematological parameters in patients with chronic fatigue syndrome. J. Chronic Fatigue Syndr. **4:**51–65.
60. **See, D. M., P. Cimoch, S. Chou, J. Chang, and J. Tilles.** 1998. The *in vitro* immunomodulatory effects of glyconutrients on peripheral blood mononuclear cells of patients with chronic fatigue syndrome. Integr. Physiol. Behav. Sci. **33:**280–287.
61. **Steinberg, P., A. Pheley, and P. K. Peterson.** 1996. Influence of immediate hypersensitivity skin reactions on delayed reactions in patients with chronic fatigue syndrome. J. Allergy Clin. Immunol. **98:**1126–1128.
62. **Straus, S. E., J. K. Dale, J. B. Peter, and C. A. Dinarello.** 1989. Circulating lymphokine levels in the chronic fatigue syndrome. J. Infect. Dis. **160:**1085–1086.
63. **Straus, S. E., S. Fritz, J. K. Dale, B. Gould, and W. Strober.** 1993. Lymphocyte phenotype and function in the chronic fatigue syndrome. J. Clin. Immunol. **13:**30–40.
64. **Subira, M. L., A. Castilla, M.-P. Civeira, and J. Prieto.** 1989. Deficient display of CD3 on lymphocytes of patients with chronic fatigue syndrome. J. Infect. Dis. **160:**165–166.
65. **Swanink, C. M. A., J. H. M. M. Vercoulen, J. M. D. Galama, M. T. L. Roos, L. Meyaard, J. Van der Ven-Jongekrijg, R. De Nijs, G. Bleijenberg, J. F. M. Fennis, F. Miedema, and J. W. M. Van der Meer.** 1996. Lymphocyte subsets, apoptosis, and cytokines in patients with chronic fatigue syndrome. J. Infect. Dis. **173:**460–463.
66. **Tirelli, U., G. Marotta, S. Improta, and A. Pinto.** 1994. Immunological abnormalities in patients with chronic fatigue syndrome. Scand. J. Immunol. **40:**601–608.
67. **Tirelli, U., A. Pinto, G. Marotta, M. Crovato, M. Quaia, P. De Paoli, E. Galligioni, and G. Santini.** 1993. Clinical and immunologic study of 205 patients with chronic fatigue syndrome: a case series from Italy. Arch. Intern. Med. **153:**116–117.
68. **Visser, J., B. Blauw, B. Hinloopen, E. Brommer, E. R. De Kloet, C. Kluft, and L. Nagelkerken.** 1998. CD4 T lymphocytes from patients with chronic fatigue syndrome have decreased interferon-γ production and increased sensitivity to dexamethasone. J. Infect. Dis. **177:**451–454.
69. **Visser, J., W. Graffelman, B. Blauw, I. Haspels, E. Lentjes, R. De Kloet, and L. Nagelkerken.** 2001. LPS-induced IL-10 production in whole blood cultures from chronic fatigue syndrome patients is increased but supersensitive to inhibition by dexamethasone. J. Neuroimmunol. **119:**343–349.
70. **Visser, J., E. Lentjes, I. Haspels, W. Graffelman, B. Blauw, R. De Kloet, and L. Nagelkerken.** 2001. Increased sensitivity to glucocorticoids in peripheral blood mononuclear cells of chronic fatigue syndrome patients without evidence for altered density or affinity of glucocorticoid receptors. J. Investig. Med. **49:**195–204.
71. **Vojdani, A., M. Ghoneum, P. C. Choppa, L. Magtoto, and C. W. Lapp.** 1997. Elevated apoptotic cell population in patients with chronic fatigue syndrome: the pivotal role of protein kinase RNA. J. Intern. Med. **242:**465–478.
72. **Wakefield, D., A. Lloyd, and A. Brockman.** 1990. Immunoglobulin subclass abnormalities in patients with chronic fatigue syndrome. Pediatr. Infect. Dis. J. **9**(Suppl.)**:**S50–S53.
73. **Whiteside, T. L.** 1991. Natural killer activity in the diagnosis of immune dysfunction. Clin. Immunol. Newsl. **11:**27–31.
74. **Zhang, Q., X. Zhou, T. Denny, J. Ottenweller, G. Lange, J. J. LaManca, M. H. Lavietes, C. Pollet, W. C. Gause, and B. H. Natelson.** 1999. Changes in immune parameters in Gulf War veterans but not in civilians with chronic fatigue syndrome. Clin. Diagn. Lab. Immunol. **6:**6–13.