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Clinical Improvement in Chronic Fatigue Syndrome Is Not Associated with Lymphocyte Subsets of Function or Activation

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The relationship between markers of immune function and chronic fatigue syndrome (CFS) is controversial. To examine the relationship directly, 43 subjects with CFS entering a randomized controlled trial of a nonpharmacological treatment for CFS gave samples for immunological analysis before and after treatment. Percentage levels of total $CD3^+$ T cells, CD4 T cells, CD8 T cells, and activated subsets did not differ between CFS subjects and controls. Naive ($CD45RA^+R0^-$) and memory ($CD45RA^-R0^+$) T cells did not differ between subjects and controls. Natural killer cells ($CD16^+/CD56^+/CD3^-$) were significantly increased in CFS patients compared to controls, as was the percentage of $CD11b^+$ CD8 cells. There were no correlations between any immune variable and measures of clinical status, with the exception of a weak correlation between total CD4 T cells and fatigue. There was a positive correlation between memory CD4 and CD8 T cells and depression scores and a negative correlation between naive CD4 T cells and depression. No immune measures changed during the course of the study, and there was no link between clinical improvement as a result of the treatment program and immune status. Immune measures did not predict response or lack of response to treatment. In conclusion, we have been unable to replicate previous findings of immune activation in CFS and unable to find any important associations between clinical status, treatment response, and immunological status.

INTRODUCTION

There has been considerable interest in the possibility that immune dysfunction plays a role in the etiology of the still controversial condition known as chronic fatigue syndrome (CFS). Over 30 studies of various immunological parameters in patients with CFS have been published to date [reviewed by Strober (1)]. However, reported abnormalities have been generally modest and inconsistent. One or two trends have been noted, such as a tendency for natural killer cell activity or number to be reduced (2, 3, 4, 5) and a tendency for the population of $CD45RA^+R0^-$ "naïve" T cells to be reduced (4, 6, 7), but even these are not confirmed by all investigators. The abnormalities described have been interpreted as showing a combination of chronic immune activation associated with impaired cell-mediated immunity, as has been claimed in depression (8, 9).

There are several reasons for these inconsistent and controversial results. These include variations in sample sizes and sample selection, differing laboratory techniques, as well as the confounding effects of such variables as psychological disorder, sleep disturbance, drug therapy, and inactivity. All of these potential confounders are unable to resolve the question of whether immune dysfunction in CFS is a cause or effect. A second question is whether any observed immunological abnormalities have any clinical significance—do they relate to any measures of symptoms, duration, and/or functional impairment? There is no convincing evidence that this is the case, mainly because correlating immunological changes with changes in clinical status requires that clinical status actually changes. Regrettably, most cases seen in specialist practice do not show any meaningful improvement—hence in one of the largest series to date Tirelli and colleagues report that only 30 of the 235 cases studied showed any improvement during the period of study (6, 7).

We report a study designed to address these issues by examining possible relationships between immune abnormalities and clinical status. We have studied subjects with CFS on two occasions, before and after a nonpharmacological intervention which was successfully employed to improve functional disability, reduce fatigue, and improve psychological health.

SUBJECTS, MATERIALS, AND METHODS

All patients were referred to a Chronic Fatigue Syndrome specialist clinic at an inner London teaching hospital. One hundred forty-two cases were screened in the clinic. Sixty-three were excluded. Thirty-five (25%) did not meet the positive criteria for CFS (10) (usually failing to fulfill the functional impairment criteria or not having fatigue as the principal complaint).

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Twenty-eight (20%) satisfied criteria for diagnoses incompatible with Chronic Fatigue Syndrome. Seventy-nine (56%) cases thus fulfilled criteria for CFS. Twelve were not offered treatment as part of the trial because they lived too far away, were about to move abroad, or were too disabled for outpatient treatment. Sixty-seven were thus eligible to take part, of whom 60 consented. All fulfilled the Oxford criteria for CFS (10). Nonmelancholic depression (22%) and anxiety disorders (10%) were not exclusion criteria. There were 19 males and 41 females, with a mean age of 35 years (range 19–77).

As well as simply fulfilling the criteria, the sample was very typical of those seen in specialist referral centers, the setting of all investigations of immune function and CFS to date. The sample was characterized by an excess of professional and higher social classes (36% belonged to Registrar General Social Class 1, the highest of the five categories) and of women (68%). Duration of illness was long (mean duration of fatigue was 4 years), and disability was profound (70% were unable to work due to ill health, 60% were taking long-term disability payments). Most of the patients taking part in this study also took part in a multicenter study looking at symptoms and diagnostic profiles in CFS (11). The results confirmed our clinical impression that our sample of CFS patients was typical of those seen in specialist settings in both the United Kingdom and other countries. The sample is not typical of patients presenting with symptoms of CFS seen in primary care (12).

In addition, 20 healthy individuals with no history of chronic fatigue or psychiatric morbidity were recruited as control subjects from laboratory and hospital workers at the referral center. There were 8 males and 12 females and their mean age was 30 years (range 19–50).

All of the patients in this report were taking part in a randomized controlled trial comparing cognitive behavior therapy (CBT) with relaxation therapy for the management of CFS. The design and results of the study are reported elsewhere (13). CBT is a nonpharmacological intervention that involves a combination of a graded behavioral activation program with elements of cognitive therapy designed to help cognitive distortions. An uncontrolled pilot study reported this approach to be promising in the management of CFS (14). In consequence we undertook a randomized controlled trial comparing CBT with relaxation therapy, the latter chosen as a control for nonspecific effects of therapist interest and contact. A second trial of CBT was also undertaken simultaneously by colleagues in Oxford, United Kingdom (15). Both our study and the Oxford study have reported that CBT is a safe and effective way of improving outcome in CFS.

Following informed consent and randomization, but before commencing treatment, subjects were invited to give a blood sample. Treatment then took place according to the randomization code. Both treatment conditions involved 12 individual treatment sessions and lasted for between 4 and 6 months. At the end of active treatment, and before follow up commenced, all those who had agreed to take part in this study were then requested to give a second sample.

No new medication was used during the study. One patient took antidepressants together with amphetamines outside the study. This subject has been excluded.

Outcome Measures

Measurements were made of a variety of relevant variables before, during, and at the end of treatment. Further measures were made 3 and 6 months after the end of treatment. For the purposes of this study the important instruments were as follows:

1. *Fatigue Questionnaire (FQ)* (16): a self-report measure developed for the study of CFS and validated in primary care (16). It consists of 11 items covering the physical and mental aspects of fatigue. Additional questions concern the duration of fatigue, the percentage of time during the day the respondent felt tired, and two questions on muscle pain at rest and after exercise.
2. *General Health Questionnaire (GHQ-12)* (17): a well-validated questionnaire measure of psychological disorder. We used Likert scoring, which follows a normal distribution in large samples.
3. *Beck Depression Inventory (BDI)* (18): a well-known self-administered scale for measuring depression.
4. *Medical Outcomes Study Health Survey (MOS-Short Form)* (19): a 20-item questionnaire measuring functional impairment, scored on a scale of 0–100, the higher score indicating better health, recommended for studies of CFS (20, 21).
5. *Somatic Symptom Check List:* a check list containing 32 somatic symptoms, modified from the Somatic Discomfort Questionnaire (22) and previously used in hospital-based studies of CFS (14, 23).

Flow Cytometric Analysis of Lymphocyte Subsets

Lymphocyte subsets were identified by two- and three-color immunofluorescence staining of peripheral blood mononuclear cells (PBMC) and analyzed by flow cytometry. Briefly, PBMC were separated by density gradient centrifugation and cryopreserved as previously described (24). Once the collection of both samples from the subjects was complete, immunofluorescence studies were performed in one batch over a period of 2 months by an individual blinded to the clinical

TABLE 1

Monoclonal Antibodies Used and the Cell Populations Identified

Lymphocyte population	FITC-conjugated	PE-conjugated	PerCP-conjugated
Activated T cells	CD3	CD25	—
Activated T cells	CD3	HLA-DR	—
Activated helper/inducer T cells	HLA-DR	CD25	CD4
Activated cytotoxic/suppressor T cells	HLA-DR	CD25	CD8
Activated cytotoxic/suppressor T cells	CD8	CD38	—
Naïve and memory CD4 cells	CD45RO*	CD45RA†	CD4
Naïve and memory CD8 cells	CD45RO	CD45RA	CD8
Natural killer cells	CD3	CD16; CD56	—
$CD11b^+$ CD8 cells	CD8	CD11b	—

Note. FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin–chlorophyll a–protein. All monoclonal antibodies were obtained from Becton–Dickinson apart from *Dako (High Wycombe, UK) and †Coulter (Luton, UK).

status of the subjects. Paired samples from six patients and two samples from control subjects were analyzed in each batch. Cytofluorometric analysis of cells simultaneously coexpressing differentiation and activation and markers was carried out as described (25). Monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or peridinin–chlorophyll a–protein (PerCP) were used in direct immunofluorescence. The monoclonal antibody combinations used are shown in Table 1. Samples were analyzed using a bench-top flow cytometer (FACScan; Becton–Dickinson, Oxford, UK). At least 5000 cells were counted, and nonspecific binding of monoclonal antibodies assessed using isotype- and fluorochrome-matched monoclonal antibodies directed against an irrelevant target (antikeyhole limpet hemocyanin, Becton–Dickinson) was subtracted from all results. Inter- and intraassay coefficients of variation established using 10 consecutive determinations from a control sample on a single day and on successive days were <5 and $<10\%$, respectively. Data acquisition was performed in list mode using Lysis II software (Becton–Dickinson), amplified logarithmically for all fluorescence channels and acquired over five decades. Analysis of the double-stained cells was performed in Lysis II and the tripled stained cells using Paint-A-Gate software (Becton–Dickinson). To identify cells stained with any one of the fluorochromes, markers were set on each fluorescence channel (FL1, FITC; FL2, PE; FL3, PerCP) around a population of unstained cells. Cells lying beyond these markers were considered positive. Cells lying simultaneously beyond the markers in two or three channels could thus be identified as double or triple stained.

Values of total $CD3^+$ T cells and the $CD4^+$ and $CD8^+$ subsets are expressed as a percentage of total lymphocytes. Activated ($CD25^+$, $HLA\text{-}DR^+$, $CD38^+$), naïve ($CD45RA^+R0^-$), and "memory" ($CD45RA^-R0^+$) subsets are expressed as a percentage of the relevant population ($CD3^+$, $CD4^+$, $CD8^+$) stained in the same tube. Natural killer cells ($CD16^+CD56^+$, $CD3^-$) are expressed as a percentage of total lymphocytes, and $CD11b^+CD8^+$ lymphocytes are expressed as a percentage of the CD8 subset.

Statistical Analysis

Most of the immunological variables satisfied criteria for the normal distribution, with the exception of values for activated (HLA^-DR^+) $CD8^+$ T cells, which required log transformation. Before and after comparisons were made using paired *t* tests. Outcome measures (GHQ, Beck Depression, and measures of functional impairment) were not normally distributed, so rank correlation coefficients are quoted for correlations. All statistical calculations were performed using the Statistical Packages for the Social Sciences. Significance testing was two tailed unless otherwise stated. Conventional statistical significance of $P < 0.05$ was used, but exact *P* values are quoted.

We report three sets of analyses. In the first we compare immunological profiles between the subjects with CFS assessed at the start of the study and the control samples. Associations between these variables and clinical status are then sought. In the third part of the analysis, we compare the immunological profiles of the CFS subjects before and after treatment.

RESULTS

Forty-three of the 60 subjects (72%) gave both samples and are reported in this study. CFS patients not giving samples did not differ by sex, treatment group, age, duration of illness, initial fatigue score, initial psychological distress, initial depression, and initial physical functioning. Of those giving an initial sample, 51% were subsequently rated as improved, compared to 12% of those who did not give a sample ($\chi^2 = 7.87$, $df = 1$, $P = 0.005$).

TABLE 2

Major T Cell Populations and Their Activated Subsets in Patients with CFS and Control Subjects

Variable	Initial sample in CFS patients [mean % (SD)]	Control subjects [mean % (SD)]	*t* test (initial vs controls; *P* value)	Second sample in CFS patients	Paired *t* test (vs initial sample; *P* value)
Total $CD3^+$ T cells	70.5 (10.1)	74.6 (8.8)	0.13	72.4 (11.5)	0.33
Activated ($CD25^+$) $CD3^+$ T cells	19.6 (6.6)	19.4 (6.6)	0.89	19.8 (8.6)	0.80
Activated (HLA^-DR^+) $CD3^+$ T cells	12.8 (7.1)	11.6 (7.8)	0.54	12.5 (6.3)	0.62
Total $CD4^+$ T cells	44.3 (8.4)	46.5 (6.9)	0.30	44.7 (8.2)	0.93
Activated ($CD25^+$) $CD4^+$ T cells	30.5 (10.0)	30.0 (7.9)	0.80	30.8 (3.3)	0.80
Activated (HLA^-DR^+) $CD4^+$ T cells	6.0 (2.3)	5.2 (2.0)	0.16	6.5 (3.3)	0.33
Total $CD8^+$ T cells	30.4 (8.6)	30.1 (5.6)	0.86	28.6 (9.2)	0.04
Activated ($CD25^+$) $CD8^+$ T cells	4.1 (2.2)	5.0 (4.8)	0.34	4.6 (2.7)	0.23
Activated (HLA^-DR^+) $CD8^+$ T cells	11.2 (7.3)	14.2 (1.7)	0.23	11.0 (7.0)	0.87
Activated ($CD38^+$) CD8 T cells	64.2 (12.5)	58.5 (10.7)	0.09	62.4 (14.6)	0.14

Lymphocyte Subsets in CFS Patients at Entry into the Study and in Control Subjects

Percentage levels of total ($CD3^+$) T cells, CD4 T cells, CD8 T cells, and the activated subsets ($CD25^+$, $HLA\text{-}DR^+$) of these populations were similar in CFS patients and control subjects when tested at entry into the study (Table 2). There was a trend for percentage levels of activated ($CD38^+$) CD8 T cells to be elevated in patients with CFS compared with control subjects, (64.2% ± 12.5 versus 58.5% ± 10.7, $P = 0.09$; Table 2).

Percentage levels of naïve ($CD45RA^+R0^-$) and memory ($CD45RA^-R0^+$) CD4 and CD8 T cells were similar in CFS patients and control subjects (Table 3). In addition, the percentage of CD4 and CD8 T cells in transition between the naïve and memory states ($CD45RA^+R0^+$) was similar in patients and control subjects.

Percentage levels of natural killer cells ($CD16^+$ $CD56^+$, $CD3^-$) were significantly higher in patients with CFS (mean ± SD, 20.7 ± 10.4%) compared with control subjects (14.6 ± 5.8%, $P = 0.017$; Table 4). Similarly, the percentage of CD8 T cells coexpressing CD11b was significantly higher in patients with CFS (61.1 ± 13.2%) compared with control subjects (49.6 ± 8.3%, $P < 0.001$; Table 4).

Immunological Variables and Clinical Status

We looked for associations between immune variables and clinical status at the start of the study. Percentage levels of total CD4 T cells were positively correlated with fatigue scores ($r = 0.34$, $P = 0.02$), while there was a reciprocal relationship between these and CD8 ($r = -0.33$, $P = 0.02$). Memory CD4 T cells increased, and naïve CD4 T cells declined as Beck Depression Index (BDI) scores increased ($r = 0.39$, $P = 0.02$ and $r = -0.35$, $P = 0.038$, respectively). Percentage levels of memory CD8 T cells increased with BDI ($r = 0.35$, $P = 0.03$). Percentage levels of $CD11b^+$ CD8 T cells decreased as fatigue increased ($r = -0.28$, $P = 0.04$). None of the other immune parameters showed significant correlations with fatigue, psychological distress, functional impairment, or duration of illness (all rank correlation coefficients between −0.016 and 0.25, all *P* values >0.05), with the sole exception of a weak

TABLE 3

Naïve and Memory CD4 and CD8 T Cell Subsets in Patients with CFS and Control Subjects

Variable	Initial sample in CFS patients [mean % (SD)]	Control subjects [mean % (SD)]	*t* test (initial vs control; *P* value)	Second sample in CFS patients [mean % (SD)]	Paired *t* test (vs initial sample; *P* value)
Naïve ($CD45RA^+R0^-$) $CD4^+$ T cells	43.8 (14.3)	44.3 (14.2)	0.91	44.2 (15.3)	0.90
Memory ($CD45RA^-R0^+$) $CD4^+$ T cells	44.0 (13.1)	43.9 (12.8)	0.97	43.7 (13.6)	0.75
$CD45RA^+R0^+$ $CD4^+$ T cells	10.6 (5.9)	11.3 (7.9)	0.70	10.5 (6.2)	0.65
Naïve ($CD45RA^+R0^-$) $CD8^+$ T cells	62.1 (15.5)	59.2 (16.2)	0.51	63.9 (12.8)	0.20
Memory ($CD45RA^-R0^+$) $CD8^+$ T cells	18.0 (10.6)	22.8 (13.7)	0.16	16.7 (7.2)	0.22
$CD45RA^+R0^+$ $CD8^+$ T cells	17.0 (9.0)	17.9 (7.4)	0.74	17.2 (9.8)	0.91

TABLE 4

Natural Killer Cells, $CD11b^+$, and $CD38^+$ CD8 T Cells in Patients with CFS and Control Subjects

Variable	Initial sample in CFS patients [mean % (SD)]	Control subjects [mean (SD)]	*t* test (initial vs control sample; *P* value)	Second sample in CFS patients [mean % (SD)]	Paired t test (vs initial sample; *P* value)
Natural killer cells	20.7 (10.4)	14.6 (5.8)	0.017	20.5 (10.3)	0.90
$CD11b^+$ CD8 T cells	61.1 (13.2)	49.6 (8.3)	0.001	60.9 (13.3)	0.94

correlation between age and activated ($CD25^+$) CD4 cells ($r = 0.41$, $P = 0.04$).

Changes in Lymphocyte Subsets in CFS Patients during the Study

Paired sample *t* tests were used to test for changes between the immune variables at the start and end of the study in the treatment groups (i.e., CBT and placebo combined). The mean percentages of CD3 and CD4 T cells and their activated ($CD25^+$, HLA^-DR^+) subsets did not change significantly during the study (Table 2). Mean levels of activated ($CD25^+$, HLA^-DR^+, $CD38^+$) CD8 T cells did not change during the study. However, there was a trend for percentage levels of total CD8 cells to decline in the study group (Table 2).

Similarly, mean percentages of naïve and memory CD4 and CD8 T cells, NK cells, and $CD11b^+$ CD8 T cells did not change significantly during the study (Tables 3 and 4). Treatment thus had no detectable impact on any immune variable.

Changes in Lymphocyte Subsets in Treated Patients in Relation to Outcome

The sample was divided into those who finished the study much improved versus the rest. This was defined a priori as an increase of 50 or more and/or an end score of 83 or more, representing the ability to carry out moderate activities without limitations, on the Physical Functioning Scale of the Medical Outcomes Survey (MOS)-Short Form (13). There were no group differences for the initial percentage levels of activated ($CD25^+$, HLA^-DR^+) CD4 T cells, activated ($CD25^+$, $CD38^+$) CD8 T cells (Table 5), nor for levels of naïve and memory CD4 and CD8 T cells, NK cells, and $CD11b^+$ CD8 T cells (Tables 6 and 7), with the exception of a significant elevation of memory ($CD45RA^-RO^+$) $CD4^+$ T cells in those who did not improve (Table 6). Although unimproved patients were significantly older than improved patients (mean age 29.4 versus 38.8, $P = 0.003$), age itself was only weakly associated with activated $CD25^+$ CD4 cells ($P = 0.36$, $P = 0.02$). Adjusting for age revealed no hidden effect of immune function on outcome.

Percentage levels of activated (HLA^-DR^+) CD8 T cells at onset were significantly higher in those who did not improve versus those who did (13.6% versus 8.2%, $t = 2.52$, $P = 0.014$; Table 5). However, this was due to a chance of randomization, since levels of HLA^-DR^+ CD8 T cells were significantly higher at onset in those who were randomized to relaxation compared to those who received CBT. Mean percentage of HLA^-DR^+ CD8 T cells at the start of study for those who would go on to receive CBT was 8.6%, compared to 14.2% for those receiving relaxation ($t = 2.48$, $P = 0.02$). As the main result of the trial was a significant effect of CBT over relaxation, the apparent association of elevated HLA^-DR^+ CD8 T cells with poor outcome is explained by the chance imbalance of this variable between the two treatment conditions. Once treatment condition was entered as a covariate, this effect disappeared ($f = 1.898$, $P = 0.18$). Using logistic regression to predict outcome gave the same result, confirming independent effects of age ($P = 0.03$) and treatment condition ($P = 0.03$), but not HLA^-DR^+ CD8 levels ($P = 0.44$). An alternative method of adjustment was to use multiple regression. Using physical functioning as the end of the treatment (the principal predeclared outcome measure of the clinical trial) as a continuous dependent variable, there was a significant effect for activated CD8 T cell levels at the start of treatment, but this was accounted for by entering treatment condition. Treatment condition (CBT) had a strong influence on physical functioning ($B = 23.81$, SE = 6.76. $\beta = 0.4$, $r = 0.001$) but not activated CD8 T cell levels ($B = -0.18$, SE = 0.39, $\beta = -0.06$, $r = 0.63$). In conclusion, there is no evidence that activated CD8 T cell levels at the start of treatment had any influence on outcome once adjusted for treatment condition.

Initial immune parameters (activated and functional T cell subsets, NK cells) also did not correlate with clinical measures taken at the end of the study. In particular, levels of activated (HLA^-DR^+) CD8 T cell levels did not correlate with physical impairment ($r = 0.06$), GHQ ($r = 0.2$), or fatigue ($r = 0.06$). Levels of neither CD4 nor CD8 memory or naïve cells correlated with any clinical variable, with the single exception of GHQ. There was a positive correlation between CD4 memory cells at the start of the study and both GHQ ($r = 0.58$, $P < 0.01$) and BDI ($r = 0.45$, $P < 0.05$) at

TABLE 5

Comparison of Initial Major T Cell Populations and Their Activated Subsets in CFS Patients Finishing the Study Improved or Unimproved

Variable	Improved [mean % (SD)]	Unimproved [mean % (SD)]	*P* value
Total $CD3^+$ T cells	71.3 (10.6)	69.7 (9.7)	0.62
Activated ($CD25^+$) $CD3^+$ T cells	17.8 (5.6)	21.5 (7.2)	0.074
Activated (HLA^-DR^+) $CD3^+$ T cells	10.7 (5.8)	14.8 (7.8)	0.062
Total $CD4^+$ T cells	44.0 (7.7)	44.5 (9.2)	0.85
Activated ($CD25^+$) $CD4^+$ T cells	27.7 (8.70)	33.4 (10.7)	0.065
Activated (HLA^-DR^+) $CD4^+$ T cells	5.5 (1.7)	6.5 (2.8)	0.18
Total $CD8^+$ T cells	30.8 (9.6)	30.1 (7.7)	0.82
Activated ($CD25^+$) $CD8^+$ T cells	3.7 (2.5)	4.5 (1.8)	0.31
Activated (HLA^-DR^+) $CD8^+$ T cells	8.2 (4.1)	13.6 (8.4)	0.014
Activated ($CD38^+$) CD8 T cells	67.3 (12.2)	61.0 (12.2)	0.10

the end. At this was noted for both GHQ and BDI (which are separate measures of general psychological distress and depression), this may be a valid finding.

DISCUSSION

In the present study of patients with CFS we aimed to examine lymphocyte phenotypes of function and activation and to correlate these with clinical status at the start of the study and also in relation to clinical improvement following the nonpharmacological intervention treatment, cognitive behavior therapy.

We do not find evidence of activation of T cells in patients with CFS; activated T cells did not relate to clinical status and did not change after successful therapy. Naïve and memory subsets of T cells were also not different in CFS patients compared with controls; memory CD4 cells were highest in depressed patients and increased with duration of illness, but did not change during the study in relation to outcome. We did find significantly elevated levels of NK cells in patients with CFS, but levels were not related to clinical status or outcome. Finally, levels of the $CD11b^+$ CD8 cell population, often referred to as "suppressor cells," were abnormally elevated in patients, but this did not relate to clinical status or outcome. In short, our study confirms the presence of abnormal distributions of lymphocyte subsets in patients with CFS, but we find no evidence to link these with the main clinical features of the disease and no evidence that these variables change when clinical status does.

There is confusion over the true meaning of any observed changes in immunological status in CFS. Some investigators suggest that these changes are those of a hyperactive immune system secondary to viral infection (26), while others speculate that they resemble those seen in more classic psychological diseases and reflect subtle endocrine changes (1). Immune dysfunction has also been reported, but to a lesser extent, in chronically fatigued adults who do not meet CFS criteria, suggesting that immune dysfunction also lies on a continuum and is affected by behavioral status (27).

In the present study, we fail to find evidence of T cell activation, using the conventional markers of the interleukin-2 receptor (CD25), the HLA^-DR molecule and CD38, on CD8 cells. Of other published studies on the same cell populations, similar negative findings have been made by Landay *et al.* (28) in relation to CD4 cells and Straus *et al.* (6) in relation to CD4 and CD8 cells. In a single study, levels of activated (HLA^-DR^+) total T cells ($CD3^+$) were elevated (7), but

TABLE 6

Comparison of Initial Naïve and Memory CD4 and CD8 T Cell Subsets in CFS Patients Finishing the Study Improved or Unimproved

Variable	Improved [mean % (SD)]	Unimproved [mean % (SD)]	*P* value
Naïve ($CD45RA^+$ $R0^-$) $CD4^+$ T cells	47.2 (13.0)	40.6 (15.0)	0.14
Memory ($CD45RA^-R0^+$) $CD4^+$ T cells	40.0 (10.4)	47.9 (14.5)	0.051
$CD45RA^+R0^+$ $CD4^+$ T cells	11.5 (5.6)	9.7 (6.2)	0.34
Naïve ($CD45RA^+R0^-$) $CD8^+$ T cells	62.4 (13.0)	61.2 (17.7)	0.91
Memory ($CD45RA^-R0^+$) $CD8^+$ T cells	19.7 (11.7)	16.3 (9.3)	0.31
$CD45RA^+R0^+$ $CD8^+$ T cells	17.2 (6.7)	16.9 (11.0)	0.92

TABLE 7

Comparison of Natural Killer Cells and Complement Receptor$^-$ 3$^+$ CD8 T Cells in CFS Patients Finishing the Study Improved or Unimproved, Measured at Entry to Study

Variable	Improved [mean % (SD)]	Unimproved [mean % (SD)]	*P* value
Natural killer cells	21.6 (10.5)	19.8 (10.5)	0.57
CD11b+ CD8 T cells	60.5 (12.8)	61.7 (13.8)	0.77

since similar findings have been made in patients with depression (8), it may be such that activation is not directly related to CFS.

Our findings differ in some respects from those of Landay and colleagues (28). They found that although levels of activated CD8 cells (CD38$^+$ and HLA$^-$DR$^+$) were normal in the study group as a whole, a subgroup of patients with the most severe disease, classified as having 25% of daily activities, had significantly higher levels of these cell populations. The authors also found abnormally low levels of suppressor CD11b$^+$ CD8 cells in the same subgroup, but again not in the group of CFS patients as a whole. This has led to the hypothesis that severe CFS patients are characterized by excessive immune activation, possibly related to a loss of T cell suppression, since the CD11b$^+$ CD8 cell population has been shown to down-regulate other lymphocyte functions *in vitro* (29). However, the function of this cell population remains poorly characterized. CD11b is the α chain of the Mac-1 integrin adhesion molecule and is expressed on a wide range of immune cell types. It is not clear why possession of high levels of CD11b should confer or denote suppressor activity. Intriguingly, we found an elevation of CD11b$^+$ CD8 cells in our subjects, while others find no difference (6). There is no obvious explanation for these differences, since monoclonal antibodies were derived from similar resources and analyses carried out in an identical fashion. Like others we found no evidence of activation of CD8 cells (6).

Numerous studies have focused on the natural killer population, since it has been reasoned that defects in these cells could lead to a persistent viral infection, one of the proposed etiological events in CFS. Studies have analyzed either NK cell function or phenotype, or both, with varied results (3, 7, 30–33). None of the previous phenotypic studies have examined the population of NK cells using the approach employed in the current study. In a phenotypic study such as this, we defined NK cells using the approach employed in the current study. In a phenotypic study such as this, we defined NK cells as non-T cells (CD3-negative) expressing either or both of the classical NK cell markers, CD56 (NKH-1) and CD16 (FcγRIII), both of which are involved in NK cell-mediated killing (34). Since both CD56 and CD16 may be found on a small population of conventional T cell receptor and CD3$^+$ T cells which do not mediate NK-type killing, our approach is superior to most previous studies which have either used a single marker or failed to distinguish CD3-negative cells (5, 28, 30, 32, 35).

There has also been a growing interest in the possible relevance of disturbances in the balance of so-called naïve and memory populations of CD4 and CD8 cells in CFS. However, we did note a modest but significant positive association between CD4$^+$ CD45RA$^-$RO$^+$ cells and scores on a depression inventory. This has also been observed in a study of depression per se (8). It is therefore possible that the previously reported findings in CFS reflect confounding by mood disorder.

The main objective of this study was to examine possible links between immunological variables and clinical status. To date there has been little evidence of any substantial correlations between markers of immune dysfunction and those of illness activity (such as duration, disability, or numbers of symptoms) (36), although this may be because few immunologically orientated researchers have attempted to make any such links, and fewer still have used reliable measures of clinical and functional status. As already discussed, Landay and colleagues found that the worst affected group tended to have the most abnormal values and also noted that in two cases in which a decrease in symptoms occurred, this was "accompanied by a return to normal of some immunological variables," though no further details are given (28). Ojo-Amaize and colleagues also categorized subjects into three groups on the basis of severity, a composite measure of symptom duration. Again, no appropriate statistical analysis was performed (33). In contrast, Straus and colleagues did use recognized measures of depression, psychological distress, and functional impairment, but were unable to find any correlation with any immune measure (6). Two larger studies have also reported essentially negative findings. Wilson and colleagues found no correlation between measures of cell-mediated immunity and outcome (37). Clark and colleagues found no associations between outcome and either absolute number or percentage of CD4, CD8, CD11, or B4 lymphocytes, nor percentages of natural killer cells (38).

Unlike Landay and colleagues we find no relationship between CD11b$^+$ CD8 cells and clinical status, using reliable clinical and psychological measures. We further report that not only was there no evidence of any correlation between clinical measures of CFS and immune parameters, but also no immune parameter changed significantly over time. In contrast, the clinical variables did change over time—some patients made substantial improvements in terms of symptoms and disability, but this was not reflected in any similar

changes in laboratory variables. Equally, immune parameters did not predict response to the treatment intervention. As far as we know this is the first such longitudinal study of immune function in CFS with the power to detect such relations, were they to exist.

Finally, could our findings be explained by methodological shortcomings? Overall, 71% of those taking part in the treatment study gave an initial sample. A very unexpected finding was that failure to give a sample was a predictor of poor response—partly because treatment drop outs, who were automatically classed as nonimproved, came from this group. However, failure to give an initial sample was not associated with any differences in clinical measures at entry into the study. Of those who did participate, 50% were in the much improved group and 50% in the unimproved group, ensuring there was sufficient variation in functional impairment to study the relationship between that impairment and immune function.

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REFERENCES

1. Strober, W., Immunological function in chronic fatigue syndrome. *In* "Chronic Fatigue Syndrome" (S. Straus, Ed.), pp. 207–240, Dekker, New York, 1994.
2. Kibler, R., Lucas, D., Hicks, M., Poulos, B., and Jones, J., Immune function in chronic active Epstein–Barr virus infection. *J. Clin. Invest.* **5,** 46–54, 1985.
3. Caliguri, M., Murray, C., Buchwald, D., *et al.,* Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. *J. Immunol.* **139,** 3303–3313, 1987.
4. Klimas, N., Salvato, F., Morgan, R., and Fletcher, M., Immunologic abnormalities in chronic fatigue syndrome. *J. Clin. Microbiol.* **28,** 1403–1410, 1990.
5. Gupta, S., and Vayuvegula, B., A comprehensive immunological analysis in chronic fatigue syndrome. *Scand. J. Immunol.* **33,** 319–327, 1991.
6. Straus, S., Fritz, S., Dale, J., Gould, B., and Strober, W., Lymphocyte phenotype and function in the chronic fatigue syndrome. *J. Clin. Immunol.* **13,** 30–40, 1993.
7. Tirelli, U., Marotta, G., Improta, S., and Pinto, A., Immunological abnormalities in patients with chronic fatigue syndrome. *Scand. J. Immunol.* **40,** 601–608, 1994.
8. Maes, M., Jacobs, J., and Lambreckhts, J., Evidence for a systemic immune activation during depression: Results of leucocyte enumeration by flow cytometry in conjunction with antibody staining. *Psychol. Med.* **22,** 45–53, 1992.
9. Maes, M., Meltzer, H., Bosmans, E., *et al.,* Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *J. Affect. Disord.* **34,** 301–309, 1995.
10. Sharpe, M., Archard, L., Banatvala, J., *et al.,* Chronic fatigue syndrome: Guidelines for research. *J. R. Soc. Med.* **84,** 118–121, 1991.
11. Wilson, A., Hickie, I., Hadzi-Pavlovic, D., *et al.,* What is chronic fatigue syndrome? Heterogeneity within an international, multicenter study. Submitted for publication.
12. Euba, R., Chalder, T., Deale, A., and Wessely, S., A comparison of the characteristics of chronic fatigue syndrome in primary and tertiary care. *Br. J. Psychiatry* **168,** 121–126, 1996.
13. Deale, A., Chalder, T., Marks, I., and Wessely, S., A randomised controlled trial of cognitive behaviour versus relaxation therapy for chronic fatigue syndrome. *Am. J. Psychiatry,* in press.
14. Butler, S., Chalder, T., Ron, M., and Wessely, S., Cognitive behaviour therapy in chronic fatigue syndrome. *J. Neurol. Neurosurg. Psychiatry* **54,** 153–158, 1991.
15. Sharpe, M., Hawton, K., Simkin, S., *et al.,* Cognitive behaviour therapy for chronic fatigue syndrome: A randomized controlled trial. *Br. Med. J.* **312,** 22–26, 1996.
16. Chalder, T., Berelowitz, G., Pawlikowska, T., *et al.,* Development of a fatigue scale. *J. Psychosom. Res.* **37,** 147–153, 1993.
17. Goldberg, D., "The Detection of Psychiatric Illness by Questionnaire." Oxford Univ. Press, London, 1972.
18. Beck, A., Ward, C., Mendelson, M., Mock, J., and Erbaugh, J., A scale for measuring depression. *Arch. Gen. Psychiatry* **4,** 561–571, 1961.
19. Stewart, A., Hays, R., and Ware, J., The MOS Short-form General Health Survey: Reliability and validity in a patient population. *Med. Care* **26,** 724–732, 1988.
20. Schluederberg, A., Straus, S., Peterson, P., *et al.,* Chronic fatigue syndrome research: Definition and medical outcome assessment. *Ann. Int. Med.* **117,** 325–331, 1992.
21. Fukuda, K., Straus, S., Hickie, I., Sharpe, M., Dobbins, J., and Komaroff, A., The chronic fatigue syndrome: A comprehensive approach to its definition and study. *Ann. Int. Med.* **121,** 953–959, 1994.
22. Wittenborn, J., and Buhler, R., Somatic discomforts among depressed women. *Arch. Gen. Psychiatry* **36,** 465–471, 1979.
23. Wessely, S., and Powell, R., Fatigue syndromes: A comparison of chronic "postviral" fatigue with neuromuscular and affective disorder. *J. Neurol. Neurosurg. Psychiatry* **52,** 940–948, 1989.
24. Tun, R., Peakman, M., Alviggi, L., *et al.,* The importance of persisting cellular and humoral immune changes in the prediabetic period: A prospective identical twin study. *Br. Med. J.* **308,** 1063–1068, 1994.
25. Peakman, M., Alviggi, L., Husain, M., *et al.,* Increased expression of T cell markers of immunological memory in Type 1 diabetes: A study of identical twins. *Diabetes* **43,** 712–717, 1994.
26. Levy, J., Introduction: Viral studies of chronic fatigue syndrome. *Clin. Infect. Dis.* **18**(Suppl. 1), S117–S120, 1994.
27. Masuda, A., Nozoe, S., Matsuyama, T., and Tanaka, H., Psychobehavioral and immunological characteristics of adult people with chronic fatigue and patients with chronic fatigue syndrome. *Psychosom. Med.* **56,** 512–518, 1994.
28. Landay, A., Jessop, C., Lennette, E., and Levy, J., Chronic fatigue syndrome: Clinical condition associated with immune activation. *Lancet* **338,** 707–712, 1991.
29. Landay, A., Garland, G., and Clement, L., Characterization of phenotypically distinct subpopulation of Leu2+ cells which suppress T cell proliferative responses. *J. Immunol.* **131,** 2757–2761, 1983.
30. Barker, A., Fujimura, S., Fadem, M., Landay, A., and Levy, J., Immunologic abnormalities in association with chronic fatigue syndrome. *Clin. Infect. Dis.* **18**(Suppl. 1), S136–S141, 1994.
31. Ho-Yen, D., Billington, R., and Urquhart, J., Natural killer cells

and the post-viral fatigue syndrome. *Scand. J. Infect. Dis.* **23,** 711–716, 1991.

32. Morrison, L., Behan, W., and Behan, P., Changes in natural killer cell phenotype in patients with post-viral fatigue syndrome. *Clin. Exp. Immunol.* **83,** 441–446, 1991.
33. Ojo-Amaize, E., Conley, E., and Peter, J., Decreased natural-killer cell activity is associated with severity of chronic fatigue syndrome immune dysfunction syndrome. *Clin. Infect. Dis.* **18,** S157–S159, 1994.
34. Yokoyama, W., Natural killer cell receptors. *Curr. Op. Immunol.* **7,** 110–120, 1995.
35. Rasmussen, A., Nielsen, H., Andersen, V., *et al.*, Chronic fatigue syndrome: A controlled cross sectional study. *J. Rheumatol.* **21,** 1527–1531, 1994.
36. Buchwald, D., and Komaroff, A., Review of laboratory findings for patients with chronic fatigue syndrome. *Rev. Infect. Dis.* **13**(Suppl. 1), 12–18, 1991.
37. Wilson, A., Hickie, I., Lloyd, A., *et al.*, Longitudinal study of the outcome of chronic fatigue syndrome. *Br. Med. J.* **308,** 756–760, 1994.
38. Clark, M., Katon, W., Russo, J., Kith, P., Sintay, M., and Buchwald, D., Chronic fatigue: Risk factors for symptom persistence in a 2.5-year follow up study. *Am. J. Med.* **98,** 187–195, 1995.

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