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Immunologic Abnormalities in Chronic Fatigue Syndrome

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The chronic fatigue syndrome (CFS), formerly known as chronic Esptein-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. The numbers of B cells, CD20 and CD21, were elevated, as were the numbers of a subset of B cells which coexpressed CD20 and CD5. The pattern of immune marker abnormalities observed was compatible with a chronic viral reactivation syndrome.

Several reports have noted the association between a chronic illness, which is characterized by a recurrent debilitating fatigue, and anomalous serological responses to Epstein-Barr virus (EBV) antigens (28, 56). The syndrome was designated by some as chronic EBV syndrome (5, 28, 54, 56). This designation was flawed by its implication that EBV, if not the etiologic agent, was at least uniformly present and a major factor in the pathophysiology of the syndrome. More recently, the Centers for Disease Control (Atlanta, Ga.) released a case definition for what is now called the chronic fatigue syndrome (CFS) (23). This syndrome is significantly heterogeneous not only in its clinical manifestations but also in its possible multifactorial etiologies. In the present study, we examined the hypothesis that CFS is, in fact, a form of acquired immunodeficiency in which the ability of the cellular immune system to deal normally with latent herpesviruses is impaired. This hypothesis is supported by a number of studies (6, 28, 29, 53, 55, 57) in which various immunologic impairments have been described in patients with CFS. However, the extent and pattern of cellular immune dysfunction in patients with clinically diagnosed CFS were undefined.

Because EBV is a ubiquitous herpesvirus, we designed our study to assess several markers related to the cellular immune response of a group of patients with clinical CFS and some serologic evidence of EBV reactivation. Although this patient sample might not represent the full spectrum of patients with CFS, it is surely a major subset. Many of the patients diagnosed as having CFS have high geometric mean titers of antibodies to EBV, including VCA and EA. Anti-EA is consistently found in patients with both recent and reactivation EBV infections (21). However, Hellinger and co-workers (20) have reported that 18% of 197 blood donors and 59% of 796 patients at the Mayo Clinic (Rochester, Minn.) had anti-EA titers of >1:10. The greater sensitivities of currently available assays suggest that the relationship of anit-EBV serology to clinical syndromes should be reassessed, as should antibody titers to the other viruses which have been linked to CFS, including herpes simplex virus type 1, herpes simplex virus type 2, measles virus, cytomegalovirus (24), human herpesvirus 6 (6–12, 36), and adenovirus type 2 (37).

The immune system appears to be the major mechanism by which EBV as well as many other herpesviruses are controlled. These primary lines of defense include T and B lymphocytes, natural killer (NK) cells, cytokines (e.g., gamma interferon and interleukin-2), and antibody production against EBV (11, 29). Alteration in immune function might permit viral reactivation with concomitant clinical disease expression and maintenance of chronicity. The purpose of this study was to evaluate several aspects of cellular immune system function in a single cohort of clinically defined patients with CFS and to compare them with healthy normal controls. A panel of immunologic tests was performed which measured phenotypic and functional parameters of the immune system in patients with CFS with evidence of chronic EBV infection.

MATERIALS AND METHODS

Patients. A total of 30 patients (12 males and 18 females; age range, 26 to 70; mean age, 39) who were diagnosed as having CFS were recruited and studied further. All patients met the criteria for CFS as set forth by the Centers for Disease Control (23). The frequency of symptoms in these

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TABLE 1. Clinical symptomatology of patients with CFS expressed as percentage of symptoms manifested

Symptoms	% of patients
Fatigue	100
Low-grade fever	85
Sore throat	76
Adenopathy	40
Painful lymph nodes	30
Unexplained general muscle weakness	90
Myalgias	90
Prolonged fatigue >24 h after exercise	80
Headaches	76
Arthralgias	90
Photophobia	76
Inability to concentrate	90
Forgetfulness	90
Irritability	85
Confusion	90
Mood swings	90
Emotional lability	90
Depression	80
Difficulty sleeping	76
Ability to date onset of illness	100
Gastrointestinal complaints	30
Shortness of breath	33
Allergies	70
Hot flushes	30
Vertigo	40
Nausea	33
Palpitations	30
Chest pain	33
Night sweats	25
Wt loss	30
Wt gain	15
Rash	15

patients is given in Table 1. In addition to the clinical criteria, we added a requirement for the existence of anomalous serology to EBV antigens, comprising increased immunoglobulin G (IgG) titers to EBV viral capsid antigen (≥320) and EBV early antigen (≥40). Informed consents were obtained from the patients, and the study was approved by the Institutional Review Board of the University of Miami.

The assessments of the markers for cellular immune function were also done on blood samples from 86 healthy, normal controls (46 males and 41 females; ages, 18 to 65) who did not differ statistically in age or sex from the patients with CFS and who had no apparent chronic fatigue or other apparent illness.

Blood samples. Peripheral venous blood samples were obtained from each subject or normal control. Sodium heparin tubes were used for in vitro functional assays and flow cytometry. Because heparinized blood specimens are not optimally counted with automated hematology cell counters, a separate blood sample in EDTA was collected from each subject. These EDTA samples were used for leukocyte and differential counts, all of which were performed within 4 h of collection. In order to make the results comparable, whole-blood samples from all patients and controls were held overnight (18 h) at room temperature before preparation and fixation for the flow cytometry assay or for functional assays. The serum samples were separated from whole clotted venous blood (23°C for 30 min) and stored at −20°C until use.

Flow cytometer. A single laser flow cytometer (EPICS C; Coulter Epics, Inc., Hialeah, Fla.), which discriminates forward- and right-angle light scatter as well as two colors, was used with a software package (Quad Stat; Coulter).

Monoclonal antibody panel. Mononuclear cell populations were determined by two-color direct immunofluorescence by using a whole-blood staining technique with the appropriate monoclonal antibody and flow cytometry (12, 13). The following pairs of fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (Coulter Immunology) were selected: T11-FITC (CD2) or sheep erythrocyte receptor-bearing cells (42) and Ta1-PE (CDw26), to measure a surface marker with an alternate pathway of lymphocyte activation (14); T4-FITC (CD4) or helper-inducer cells (39) combined with either 4B4-PE (CD29), to measure the subset of CD4 which gives help to B cells in response to antigenic stimulation immunoglobulin synthesis (34), or with 2H4-PE (CD45RA), to measure the subset which activates CD8 cells to act as either suppressor cells or cytotoxic cells (51; S. C. Pflugfelder, J. Pepose, M. A. Fletcher, N. G. Klimas, and N. Font, Ophthalmology, in press); T8 (CD8) or suppressor-cytotoxic T cells (40) plus I2-PE to measure human lymphocyte antigen (HLA)-DR activation antigen (41) expression on CD8; B1-FITC (CD20) and B2-PE (CD21) for determining B-cell subsets (52); T1-PE for total T cells combined with B1, to measure the subset which has been described as being elevated in patients with certain autoimmune diseases (19); and NKH.1-PE (CD56), which defines the entire pool of large granular lymphocytes with potential NK cell activity (22). Isotypic controls were mouse IgG1, IgG2a, or IgM.

Sample preparation and flow cytometric analysis. Two-color flow cytometry was done by using a whole-blood procedure as described by Fletcher et al. (12, 13). To monitory lymphocyte markers, bit maps were set on the lymphocyte population of the forward-angle light scatter versus a 90° light scatter histogram. Cells positive for the surface marker NKH.1 were measured in a larger gate encompassing the lymphocyte and monocyte area of the forward-angle light scatter versus a 90° light scatter histogram. The granulocyte area was excluded. The percentage of positively stained cells for each marker pair as well as the percentage of doubly stained cells were determined. Peripheral lymphocyte counts were calculated by multiplying the total leukocyte count and the percentage of lymphocytes, as determined with a Cell Dyne 1500 (Sequoia-Turner). Estimates of absolute numbers of the lymphocyte or mononuclear cell populations that were positive for the respective surface markers were determined by multiplying peripheral lymphocyte or mononuclear cell counts by the percentage of positive cells for each surface marker (3).

Lymphocyte proliferation assay. Lymphocyte proliferation to mitogen stimulation, phytohemagglutinin (PHA) and pokeweed mitogen (PWM), was measured by using a whole-blood procedure as described previously (12). Briefly, 100 μl of diluted heparinized blood (1:5) was dispensed in triplicate to the wells of a U-bottom microdilution plate (Costar, Cambridge, Mass.). Mitogens were tested at the following levels to give final dilutions as follows: PHA (Wellcome Diagnostics, Dartford, England), 10 μg/ml; PWM (GIBCO Laboratories, Chagrin Falls, Ohio), 1:40. Portions of the appropriate dilution of each mitogen were dispensed into wells containing diluted blood, and three wells for each sample received only culture medium. Plates were incubated for 72 h. During the last 6 h of the appropriate incubation time, cultures were pulsed with 25 μl of tritiated thymidine (1 μCi per well; Dupont, NEN Research Products, Boston, Mass.). At the end of the pulse incubation, cultures were

harvested onto glass filter paper disks (Titertek Cell Harvester; Flow Laboratories, Inc., McLean Va.), immersed in scintillation fluid, and counted on a beta-scintillation counter (LKB Instruments, Inc., Rockville, Md.). Results were expressed as mean net counts per minute incorporated per 100,000 lymphocytes. Calculations were done by using a computer program that was written in-house.

NK cell cytotoxicity. NK cell function was evaluated by determining cytotoxicity by the whole-blood chromium release assay as outlined in detail by Baron et al. (3). The target cell line used was the NK cell-sensitive erythroleukemic K562 cell line. The assay was done in triplicate at four target-to effector-cell ratios with a 4-h incubation. NK cell-mediated cytotoxicity expressed as percent cytotoxicity (CYT) was calculated as follows:

$$\%CYT = \left[\frac{(ER - b) \times \frac{(V_t - (V_b \times H)}{V_t} - (SR - b)}{(TR - b) - (SR - b)} \right] \times 100$$

where ER is the mean counts per minute of experimental release of the specimen, SR is the mean counts per minute of spontaneous release, TR is the mean counts per minute of total release, V_t is the total volume in each well, V_b is the total volume of blood in well, H is specimen hematocrit, and b is instrument background. The percent cytotoxicity at the four target-to-effector-cell ratios and the number of NKH.1-positive cells per unit of blood were used to express the results as percent cytotoxicity at a target-to-effector ($CD56^+$)-cell ratio of 1:1 or as lytic units (LU) determined as the number of ($CD56^+$ cells × 1,000)/30% cytotoxicity. Calculations were done by using a computer program that was written in-house.

Callewaert and colleagues (7, 8) have demonstrated that estimates of V_{max} for NK cell activity are equal to the concentration of NK cells tested times the mean lytic activity per NK cell whether a single-step or multistep model of effector cell/target cell interaction and lysis was used. The percent cytotoxicity determined by the ^{51}Cr release assay for the dilutions of target cells to be used was transformed to the number of target cells killed in each dilution by the formula $V = \%CYT \times T$, where V (velocity) is the number of target cells lysed and T (substrate) is the number of target cells in the assay. The data were then fitted to an equation analogous to the Michaelis-Menten kinetic equation as described by Cleland (9) by using a computer program that was written in-house. $V = (V_{max} \times T)/(K_m + T)$, where V is the number of targets killed, T is the number of targets in the assay, V_{max} is the number of targets killed when T is infinite, and K_m is the number of targets required for one-half V_{max}. Kinetic LU/NK cell (KLU/NK) was expressed as the maximum number of targets lysed by each NK ($CD56^+$) cell during the 4-h assay. Functional efficiency per effector cell was thus compared in the study groups.

Gamma interferon production. A whole-blood assay was used to study the capacity of mononuclear cells to produce gamma interferon. Briefly, 100 μl of diluted heparinized blood (1:5) was dispensed in triplicate into wells of a U-bottom microdilution plate. PHA (10 μg/ml) and PWM (diluted 1:40) were dispensed to wells containing both diluted blood and culture medium alone. The cells were incubated for 48 h 37°C in 5% CO_2 in air, after which they were centrifuged, and the supernatants were stored at −70°C until there were assayed.

Gamma interferon determination. Gamma interferon levels were determined by a solid-phase, sandwich immunoradiometric assay by using a commercially available kit (Centocor, Malvern, Pa.). Briefly, polystyrene beads coated with mouse anti-human gamma interferon were incubated with 200 μl of test samples in an assay tray at room temperature for 2 h without shaking. The beads were washed with distilled water and incubated with 200 μl of ^{125}I-labeled anti-gamma interferon antibody (mouse monoclonal antibody) at room temperature for 2 h. After incubation, the beads were washed and assayed for ^{125}I in a gamma counter. Assay standards and controls were determined with human gamma interferon diluted in normal defibrinated human plasma.

EBV serology. Antibodies to specific EBV antigens were determined in serum samples by the indirect immunofluorescence method described by Henle et al. (21) with a commercially available kit (Organon Teknika Corp.).

Data analysis. Many of the immunological measures were not normally distributed. Since sample sizes between control and test groups were also different, nonparametric procedures based on rank sums were used to evaluate the magnitudes of between-group differences.

Given the large number of potential statistical tests, the immunological parameters were clustered into four distinct subgroups. Four overall, nonparametric multivariate analyses were performed to determine, on a molar level, whether the clusters showed evidence of statistically significant between-group differences. The nonparametric multivariate procedure used was an extension of the Kruskal-Wallis one-way analysis based on rank sums (50). χ^2 values for performing hypothesis tests were easily obtainable by using Statistical Analysis System software (46) and following the procedures outlined by Zwick (59).

Following each statistically significant multivariate χ^2 test, individual Kruskal-Wallis tests were performed on each measure to determine which one reflected the overall statistical significance.

RESULTS

The overall nonparametric multivariate analyses were significant for the NK cell functional assays, T-cell subsets, B-cell subsets, lymphoproliferative assays, and gamma interferon production. The mulitvariate results, as well as the results of the individual Kruskal-Wallis analyses, are presented in Tables 2 through 6.

Lymphocyte phenotypic analysis. A substantial difference in the distribution of lymphocyte subsets in peripheral blood of patients with CFS was found when compared with the distribution of lymphocyte subsets in peripheral blood of normal controls (Tables 2 and 3). Statistically reliable elevations of several lymphocyte subpopulations were observed. The percentage of CD8 cells, i.e., the suppressor-cytotoxic T lymphocytes, was elevated by 19%. There was a 67% elevation in a subset of CD8 cells, those coexpressing the HLA/DR class II marker, $CD8^+$ $I2^+$ cells. Of the 30 patients with CFS, 14 had values for the percentage of $CD8^+$ $I2^+$ cells which were ≥1 standard deviation (SD) above the mean value for the normal controls. Substantial elevation (71%) was also noted in the percentage of CD2 cells that coexpressed the CDw26 marker, with 20 of the 30 subjects having values ≥1 SD above the mean for the normal controls. Although overall CD4 cell counts for patients with CFS were not different from those for normal controls, a significant decrease (31%) in the inducer subset, $CD4^+$ $CD45RA^+$, was detected. Among the B cells, the CD21 subset was elevated by 40%, as were the $CD20^+$ $CD21^+$ cells. The subset of

TABLE 2. Phenotypic marker analysis of T lymphocytes[a]

Lymphocyte marker	Median % of cells reacting with monoclonal antibodies in[b]: Patients with CFS (n = 30)	Median % of cells reacting with monoclonal antibodies in[b]: Normal controls (n = 69)	% Difference between median values[c]	Kruskal-Wallis χ^2	Kruskal-Wallis P
CD4	44 (39/51)	46 (41/50)	3−	0.28	>0.50
CD8	31 (26/36)	26 (23/31)	19+	4.87	<0.03
CD4/CD8 ratio	1.40 (1.18/1.81)	1.72 (1.47/2.05)	19−	3.72	<0.06
$CD2^+$ $CDw26^+$	29 (21/36)	17 (13/21)	71+	24.37	<0.001
$DC4^+$ $CD45RA^+$	9 (7/12)	13 (9/21)	31−	10.17	<0.002
$CD4^+$ $CD29^+$	25 (21/28)	22 (18/27)	14+	2.86	<0.1
$CD8^+$ $I2^+$	5 (4/8)	3 (2/5)	67+	14.06	<0.001

[a] By multivariate rank analysis, there were 7 degrees of freedom, χ^2 was 40.16, and P was <0.001.
[b] Values in parentheses are 25th percentile/75th percentile.
[c] Percent differences were calculated by using the normal controls as a reference; the + or − sign indicates the direction of change.

CD20 cells expressing the T-cell marker CD5 was measured in a subset of patients (n = 12) and was also elevated in patients with CFS compared with the level in normal controls (data not shown).

Lymphocyte functional studies. Lymphocyte proliferation after PHA and PWM stimulation was significantly decreased in patients with CFS (by 47 and 67%, respectively) compared with that in normal controls (Table 4). Values for PWM-stimulated proliferation of ≥1 SD below the mean value for normal controls was seen for 19 patients with CFS. The ability of lymphocytes from patients with CFS stimulated in vitro by PHA or PWM to produce gamma interferon was decreased (67 and 45%, respectively) when compared with that of lymphocytes from normal controls (Table 5). Following stimulation with PHA, 17 patients had gamma interferon production ≥1 SD below the control mean.

Phenotypic and functional characterization of NK cells in patients with CFS. Patients with CFS had 50% more $CD56^+$ cells than normal controls did (Table 6), with 14 of the 30 subjects having values of ≥1 SD above the control mean. There was a significant reduction (64%) in the percent cytotoxicity to the K562 tumor cell line, at an effector-to-target-cell ratio of 1:1, with 22 subjects having values of ≥1 SD below the mean of the normal controls. In patients with CFS, 30% LU, i.e., the number of effector cells, represented by the $CD56^+$ cells, that resulted in 30% cytotoxicity, was significantly elevated (289%) compared with that in normal controls. This suggested a significant defect in NK cell cytotoxicity in patients with CFS; this conclusion is supported by the finding that NK cells from these patients had a significant reduction in maximal killing velocity. Kinetic analysis indicated that patients with CFS, as a group, compared with normal controls lysed a median of 0.109 versus 0.645 target cells per NK cell in 4 h, which was a deficit of 83% compared with that in normal controls.

TABLE 3. Phenotypic marker analysis of B lymphocytes[a]

Lymphocyte marker	Median % of cells reacting with monoclonal antibodies[b]: Patients with CFS (n = 30)	Median % of cells reacting with monoclonal antibodies[b]: Normal controls (n = 75)	% Difference between median values[c]	Kruskal-Wallis χ^2	Kruskal-Wallis P
$CD21^+$ $CD20^+$	7 (5/11)	5 (3/7)	40+	8.83	<0.003
CD21	7 (5/11)	5 (3/7)	40+	7.96	<0.005
CD20	12 (8/16)	11 (8/13)	14+	2.29	>0.10

[a] By multivariate rank analysis, there were 3 degrees of freedom, χ^2 was 9.02, and P was <0.05.
[b] Values in parentheses are 25th percentile/75th percentile.
[c] Percent differences were calculated by using the normal controls as a reference; the + sign indicates the direction of change.

DISCUSSION

Depression of cell-mediated immunity, as evidenced by a significant decrease in lymphoproliferative responses after PHA and PWM stimulation, was noted in our study population, with over 80% of patients having values of ≥1 SD below the normal mean. The values obtained were closely similar to those we observed in a group of human immunodeficiency virus type 1-seropositive intravenous drug users (N. G. Klimas, G. C. Baron, and M. A. Fletcher, *in* T. Fields, P. McCabe, and N. Schneiderman, ed., *Stress and Coping in Disease*, in press). Other investigators have found significant depression of in vitro lymphocyte responsiveness and cutaneous anergy during the acute phase of infectious mononucleosis, although restoration of normal immunological responses were noted during the convalescent period (32).

NK cells have been demonstrated to participate in the inhibition of EBV-induced B-cell transformation in vitro (17). Kibler et al. (29) have found diminished in vitro production of two major NK cell activators, gamma interferon and interleukin-2, by cultured lymphocytes, as well as diminished NK cell cytotoxicity in 13 patients diagnosed as having chronic EBV syndrome. However, Borysiewicz et al. (4) have described normal NK cell activity in their patients. In an earlier study (45) of a smaller sample of patients, our laboratory reported low levels of NK cell activity but an elevated number of NK cells, as measured by the NKH.1 monoclonal antibody. Caliguri et al. (6) have reported impaired NK cell function and a decreased number of $NKH.1^+$ $T3^-$ lymphocytes in patients with CFS who had evidence of EBV reactivation. The present report confirms that a qualitative defect is present in these patients' NK cells. The number of cells with the phenotype $CD56^+$ was elevated. It should be noted that NK cells are a heterogeneous group. It is possible that certain subsets, such as $CD56^+$ $CD3^-$ and $CD56^+$ $CD3^+$ cells, may be present in unequal proportions. This could account for differences in our results and other results presented previously (6). However, our observation of a low value of KLU and $CD56^+$ cells does suggest impairment of NK cell kinetic activity.

Mitogenically stimulated mononuclear cells from our patients exhibited a diminished ability to produce gamma interferon in vitro after stimulation with either PHA or PWM. This observation might represent cellular exhaustion

TABLE 4. Lymphocyte response to mitogens[a]

Mitogen	Median net cpm in[b]:		% Difference between normal values[c]	Kruskal-Wallis	
	Patients with CFS (n = 24)	Normal controls		χ^2	P
PHA	53,776 (23,037/91,047)	93,329 (57,525/136,118) (n = 88)	42−	11.35	<0.001
PWM	3,564 (2,132/4,968)	10,468 (6,509/13,893) (n = 25)	66−	19.78	<0.001

[a] Because of differences in sample sizes, only univariate analyses were done.
[b] Values in parentheses are 25th percentile/75th percentile.
[c] Percent differences were calculated by using the normal controls as a reference; the − sign indicates the direction of change.

as a consequence of persistent viral stimulus, a theory that is supported by the elevation of leukocyte 2′,5′-oligoadenylate synthetase in lymphocytes of patients with CFS (55). This enzyme is known to be activated during acute viral infections, including infectious mononucleosis (48). Gamma interferon is thought to be an immunoregulatory substance, enhancing both cellular antigen presentation to lymphocytes (58) and NK cell cytotoxicity (55). It could be concluded that the NK cell defect is due to the impaired ability of mononuclear cells from patients with CFS to produce gamma interferon.

There have been reports of alterations in the distribution of T-cell subsets in patients with CFS. In reference to CD4 and CD8 cells, discrepant results have been reported. Strauss et al. (54) reported a statistically higher percentage of CD4 lymphocytes with a normal number of CD8 cells and a normal CD4/CD8 ratio, but Jones et al. (28) found normal percentages of CD4 and CD8 cells as well as a normal CD4/CD8 ratio. Borysiewicz et al. (4) reported normal CD4 and CD8 populations as well as normal immunoglobulin levels but a reduced EBV-specific cytotoxic T-cell activity. In the present study, most patients with CFS had a normal number of CD4 cells and a small elevation in CD8 cells. These conflicting results may be due to the fluctuation and oscillation in the clinical manifestations of disease in these patients, as well as to the time at which the lymphocytes were obtained. The increase or decrease in a particular subset may represent different stages in the evolution of this syndrome.

Results from the present study indicate that there is an elevation in activated T cells. CD2 cells expressing the activation marker CDw26 were elevated. A strikingly similar elevation in $CD2^+$ $CDw26^+$ cells has been reported in patients with multiple sclerosis (18). There were also elevated numbers of CD8 cells expressing the class II marker, I2. Similar increases in $CD8^+$ $I2^+$ cells have been reported in patients with anti-human immunodeficiency virus type 1-positive congenital clotting disorder (12).

TABLE 5. Gamma interferon production after stimulation with mitogens[a]

Mitogen	Median production (U/ml) in[b]:		% Difference between normal values[c]	Kruskal-Wallis	
	Patients with CFS (n = 24)	Normal controls (n = 18)		χ^2	P
PHA	162 (125/318)	487 (340/772)	42−	15.92	<0.001
PWM	23.4 (11.9/28)	42.6 (32/73)	66−	14.917	<0.001

[a] By multivariate rank analysis, there were 2 degrees of freedom, χ^2 was 37.37, and P was <0.001.
[b] Values are per 10^5 mononuclear cells. Values in parentheses are 25th percentile/75th percentile.
[c] Percent differences were calculated by using the normal controls as a reference; the − sign indicates the direction of change.

No change was seen in the overall numbers of CD4 cells or in the number of one cell subset, CD29, which is thought to define the subset of T4 cells which gives help to B cells in response to exogenous antigen (34). The one category of lymphocytes which was significantly lower in patients with CFS was another subset of CD4 cells, CD45RA, with the majority of patients having absolute counts of ≥1 SD below the mean observed in the normal controls. Franco et al. (15) have also described a decrease in the number of $CD4^+$ $CD45RA^+$ cells in two patients with severe chronic active EBV infection, with one of the patients showing a persistent diminished number of cells, despite clinical improvement with interleukin-2 treatment. This subset of CD4 cells has been identified as the inducer subset (51). It may, in part, represent a naive T-cell subset as opposed to the memory T cells ($CD4^+$ $CD29^+$). Functionally, the $CD45RA^+$ CD4 cells, also termed Tinf, for inflammatory CD4 cells, can transfer delayed-type hypersensitivity, respond to mitogen stimulation, produce interleukin-2 and gamma interferon, active cytotoxic cells, and suppress specific B-cell responses to antigen (26). A drop in the number of such inducer subset lymphocytes could result in B-cell dysregulation because of the lack of effective suppressor cells. It might also decrease the number of activated cytotoxic T cells, thus promoting a state of chronic viral reactivation. Recent studies have associated decreases in this cell subset with a number of clinical entities. Selective depletion of $CD4^+$ $CD45RA^+$ cells was noted during the active phases of multiple sclerosis (44), but not in patients in remission or with inactive multiple sclerosis or other neurological diseases (43). Deficiencies quantitatively similar to those observed in patients with CFS were also reported in patients with other autoimmune diseases (10, 35, 47).

Several hypotheses can be proposed to explain the depletion of $CD4^+$ $CD45RA^+$ cells in patients with CFS. The simplest would be to attribute the relative paucity of these cells to the presence of somewhat higher percentages of $CD29^+$ memory T cells as a consequence of chronic virally induced antigenic stimulation. Alternative possibilities include the following: (i) a lytic virus such as 6 human herpesvirus 6 or another as yet unrecognized virus might preferentially infect these cells; (ii) this T-cell subset may preferentially migrate to another tissue, such as the central nervous system, as has been described in patients with multiple sclerosis (44); (iii) failure to express the CD45RA antigen may occur as a manifestation of T-cell activation (31); (iv) patients with CFS may be exposed to an environmental factor with specific destructive tropism for this cell subset (27); (v) patients with CFS are reported to have low levels of IgG1; immunoglobulins of the IgG1 subclass are reported to augment the generation of Tinf cells (26); or (vi) antibodies which are preferentially reactive with the $CD45RA^+$ cells could be produced (2).

Alterations in the humoral response of patients with CFS

TABLE 6. NK cell function[a]

Function	Patients with CFS (n = 30)	Normal controls (n = 73)	% Difference between median values[b]	Kruskal-Wallis	
				χ^2	P
NK cytotoxicity (%)[c]	9 (5/14)	25 (19/31)	64−	36.93	<0.001
CD56 cells (%)	12 (9/18)	8 (6/10)	50+	19.84	<0.001
LU[d]	148 (89/291)	38 (24/68)	289+	37.71	<0.001
V_{max}[e]	5,553 (3,296/11,300)	20,448 (12,474/39,269)	73−	31.24	<0.001
KLU[f]	0.11 (0.05/0.23)	0.64 (0.36/1.32)	83−	41.13	<0.001

[a] Values are expressed as medians. Values in parentheses are 25th percentile/75th percentile. By multivariate rank analysis, there were 6 degrees of freedom, χ^2 was 48.06, and P was <0.001.
[b] Percent differences were calculated by using the normal controls as a reference; the + or − sign indicates the direction of change.
[c] Percent lysis of K562 target cells at effector (CD56) to target cell ratio of 1:1.
[d] Number of CD56 cells × 10^3 to give 30% lysis.
[e] Number of target cells killed at infinite target cell concentration.
[f] Maximum number of target cells lysed per CD56 cells in a 4-h assay.

have also been described, including mild IgA deficiency (55); elevated immune complexes (28, 55); and the presence of cold agglutinins, rheumatoid factor, and antinuclear antibodies and false serologic positivity for syphilis (55). These aberrations might represent polyclonal B-cell activation, as EBV is a potent polyclonal stimulator of B cells (2). We found elevated B cells, including B cells that coexpressed the T1 (CD5) marker, a subset that has been reported to be elevated in patients with autoimmune disorders (19). B-cell activity is regulated by an intricate balance between T-helper and T-suppressor lymphocytes, as well as by NK cells. The depletion of the $CD4^+$ $CD45RA^+$ Tinf cell subset in our patients may favor an alteration in B-cell regulation as a result of inactive suppressor cells. The NK cell deficiency observed in most subjects would also contribute to B-cell regulatory disturbance, since a primary physiologic role of the NK cell is thought to be B-cell regulation (1).

Still unanswered is the question of the etiology of CFS. Candidates include known or unknown virus(es), environmental factors (e.g., recalling the abrupt onset in many patients), or both (27). The role of psychoneuroimmunological variables in both the onset and the perpetuation of this syndrome must be given strong consideration (30). Certain psychosocial conditions are known to influence immune function, such as depression (49), hostility associated with depression (16), and stress (38). All of these were found not infrequently in the cohort of patients with CFS who were studied (33). Of interest are studies showing that the convalescent periods of certain viral diseases, such as influenza, were altered by certain premorbid psychoneurotic conditions (25).

In summary, the results of the present study, in which a number of the attributes of immunologic function were determined in a single cohort of patients, as well as the combined results of a number of studies in the literature that was reviewed, suggest that CFS is a form of acquired immunodeficiency. This deficiency of cellular immune function was present in all the subjects that we studied. It has several manifestations, with NK cell dysfunction being the most consistent abnormality. The type of immune dysfunction observed would be anticipated to result in a propensity for chronic and recurrent EBV infection as well as other herpesvirus infections.

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