Immune Responses Associated with Chronic Fatigue Syndrome: A Case-Control Study

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

Chronic fatigue syndrome (CFS) is characterized by debilitating fatigue accompanied by a variety of symptoms, including neurocognitive symptoms, myalgia, and arthralgia [1]. Several groups have suggested an infectious etiology for the disease on the basis of early observations that an elevated titer to the early antigen of Epstein-Barr virus (EBV) is associated with disease or that there is an increased frequency of seropositivity to Coxsackie B viruses [2]. Subsequent studies demonstrated that neither of these observations could be generalized to all CFS patients. However, the idea that CFS might be caused by an infectious trigger or other insult, which subsequently leads to a chronic activation of the immune system, became a major hypothesis in the etiology of CFS. This hypothesis was bolstered by the observation that clinical treatment with recombinant cytokines, such as interleukin (IL)-1, IL-2, and interferon (IFN)-γ, gave rise to many CFS symptoms [3–5]. It was thus postulated that a chronic immune activation could give rise to chronic production of cytokines that would in turn produce the symptoms of CFS.

Much CFS research has been directed towards identifying a lesion in the immune system. Many reports describe differences in immune parameters between CFS cases and healthy controls, including decreases in NK cell function [6–9], alterations in NK cell numbers [6, 7, 10, 11], decreased proliferative responses to mitogens [7, 12–14], altered cytokine production [7, 15–17], alterations in B and CD8 cell subpopulations [11, 18], increased expression of activation markers [7, 11, 18], altered naive and memory T cell populations [14], anergy in response to a delayed-type hypersensitivity (DTH) test [13], low levels of circulating immune complexes, and complement activation [19, 20]. However, none of these findings is consistently reproducible nor have they all been tested in a single group of patients.

We have assessed a comprehensive panel of immune function assays in a well-defined group of patients and in a set of randomly selected matched controls.

Materials and Methods

Case selection. Patients with CFS were recruited from the Atlanta component of the Centers for Disease Control and Prevention (CDC) surveillance study [21]. Persons in Atlanta who currently met the 1988 CFS research case definition [22] and who had been sick $\leq$10 years were eligible for the study. Twenty-six persons agreed to participate. To prevent bias of results against sicker patients, participants were allowed to continue taking any currently used medications, and medication use was recorded as part of a standardized medical history [23]. No subject was taking immunosuppressive drugs.

Subgrouping of cases. Analyses were done on combined cases and on 3 subgroups: timing of illness onset (sudden or gradual), health status on day of interview (well or sick), and duration of illness (short or long). Cases were divided into sudden onset (n = 9) or gradual onset (n = 17) on the basis of descriptions of the beginning of their illness. Persons who described a flu-like illness with an abrupt onset of 1–2 days were considered to have had sudden onset. The rest, all of whom had a more insidious progression to CFS, were considered to have had a gradual onset. Cases were categorized by health status on the basis of a self-reported wellness score (1–100) for how well they felt on the day of interview (1 was the worst, and 100 was the best they could feel). Since all controls reported a wellness score of $\geq$50 (range, 50–

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All study participants were volunteers and gave informed consent. Complete study protocols were approved by the CDC Human Subjects Committees. Human experimentation guidelines of the US Department of Health and Human Services were followed in the conduct of this study.

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100), cases with a score of >50 (range, 60–90) were defined as well on the day of interview (n = 13), and those with a score of ≤50 (range, 20–50) were defined as sick (n = 13). The subgrouping based on duration of illness categorized cases as above or below the median duration of 63 months (n = 13 in each category).

Control selection. We selected 2 controls for each case who were matched for age (±5 years), race, and sex. Controls were selected by random-digit dialing in the five-county Atlanta area covered by the CDC surveillance system. All controls were screened using a standardized questionnaire to eliminate confounding medical conditions. Controls who reported a history of cancer, heart disease, autoimmune diseases, tuberculosis, Lyme disease, brucellosis, CFS, chronic EBV infection, AIDS, hepatitis, multiple sclerosis, or a variety of other illnesses were eliminated. In addition, controls who reported taking corticosteroids, IL-2, IFN, zidovudine, or tamoxifen were eliminated [23].

Statistical analysis. We analyzed data using matched analysis procedures. We used a nonparametric test (Cochran-Mantel-Haenszel statistic [24] with modified ridit scores [25]) to assess differences in continuous variables between cases and controls. Differences between proportions were tested by Fisher's exact test. Subgroup analyses were done by stratifying cases by either onset type, wellness score, or disease duration and testing whether they differed from their matched controls. For all statistical tests, $P \leq .05$ was considered significant. No attempt was made to adjust the significance level for multiple comparisons, since the study objective was to describe and explore the relationship between a variety of immunologic parameters and CFS cases [26]. Statistical analyses were done with SAS software (SAS Institute, Cary, NC).

Specimen collection. We collected blood and urine samples from each participant. Blood for functional studies was collected into Vacutainers (Becton Dickinson, San Jose, CA) containing either heparin or EDTA as anticoagulant. Serum was stored at −70°C for measurement of immune complex, serum immunoglobulin, and complement levels. We obtained blood from a case and 2 matched controls on the same day and did blinded functional studies on that day. All specimens were collected before 10 A.M. All participants completed a detailed symptom questionnaire and were asked to evaluate how they were feeling that day on a scale of 1–100 (wellness score).

Flow cytometric analysis. Lymphocyte populations were analyzed by flow cytometry using three-color direct immunofluorescence after whole blood lysis. Table 1 shows the panel of monoclonal antibodies (MAbs) used and their conjugates (fluorescein isothiocyanate, phycoerythrin, or peridinin chlorophyll protein). All MAbs were from Becton Dickinson, except S6F1 (Coulter, Miami). We placed 100 μL of whole blood in EDTA into each tube, added 5 μL of each MAb as appropriate, and incubated tubes for 15 min at room temperature. Red blood cells (RBCs) were lysed by incubating for 10 min with commercial lysing solution (FACSLyse; Becton Dickinson). Cells were pelletted by centrifugation and resuspended in PBS containing 1% paraformaldehyde. Analysis was done by FACScan with Consort 32 computer software (Becton Dickinson). Lymphocyte gates were determined using Leukogate and Paint-a-Gate software was used for analyzing three-color panels (Becton Dickinson).

Lymphocyte proliferation assays. Peripheral blood lymphocytes (PBL) were isolated from heparinized blood on lymphocyte separation medium (Organon Teknika, Durham, NC). Cells were washed three times with PBS by centrifugation and resuspended in RPMI 1640 (Life Technologies GIBCO BRL, Grand Island, NY) supplemented with 10% human AB serum, 2 m*M* glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells (10^5) were dispensed into each well of a round-bottomed microtiter plate and stimulated with a series of mitogens and antigens. These (with final concentrations or dilutions) included phytohemagglutinin (PHA; Difco, Detroit), 1:1000 and 1:4000; concanavalin A (ConA; Pharmacia, Piscataway, NJ), 80 μg/mL; pokeweed mitogen (PWM; Life Technologies GIBCO BRL), 1:2000; *Candida* antigen (Hollister-Stier Laboratories, Spokane, WA), 1:160; streptolysin O (Difco), 1:100; and tetanus and diphtheria toxoids (Connaught Laboratories, Toronto), both at 40 Lf/mL. A mixed lymphocyte reaction was also done using frozen PBL from a pool of 5 donors. The stimulator cells were thawed, irradiated (15 Gy), and used at a 1:1 ratio with fresh cells. All cultures were set up in triplicate. Microtiter plates were incubated at 37°C in a humidified incubator containing 5% CO_2. PHA- and ConA-stimulated cultures were harvested after 48 h. Four hours before harvest, 0.5 μCi of [^{3}H]thymidine (specific activity, 6.7 mCi/mL; NEN Research Products, Boston) was added to each well. All other cultures were incubated for 6 days, and [^{3}H]thymidine was added 18 h before harvest. Cultures were harvested onto glass-fiber filters and counted in a scintillation counter. Results are expressed as change in counts per minute (cpm) = stimulated cpm − unstimulated cpm or as stimulation index = stimulated cpm/unstimulated cpm.

Cytokine assays. We tested for the following cytokines in serum after culturing PBL for 48 h with no stimulation and after 48

Table 1. Three-color panels used for cell surface marker studies in CFS patients and controls.

	Fluorescent conjugate		
	FITC	PE	PerCP
T cell subsets	CD3	CD4	CD8
	CD4	CD11b	CD8
	CD4	CD28	CD8
	CD8	S6F1	—
Activation markers	CD4	CD38	CD8
	CD71	CD4	CD8
	CD4	CD25	CD8
	CD26	CD4	CD8
	DR	CD4	CD8
Adhesion molecules	CD11a	CD4	CD8
	CD4	CD54	CD8
	CD4	CD11c	CD8
Naive and memory cells	CD45RA	CD4	CD8
	CD4	CD45RO	CD8
NK cell markers	CD25	CD56	CD3
	CD3	CD56	—
	CD57	CD56	CD8
	CD2	CD56	CD8
	CD71	CD56	DR
B cell subset	CD5	CD25	CD19

NOTE. FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

h with PHA stimulation: IL-1α, IL-1β, IL-2, IL-4, IL-6, IFN-γ, tumor necrosis factor (TNF)-α, TNF-β, and transforming growth factor (TGF)-β. In addition, we tested serum for the presence of soluble (s) CD4, sCD8, and sIL-2 receptor (sIL-2r).

Fresh PBL were cultured in RPMI 1640 supplemented with 10% AB serum, 2 m*M* glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 10^6 cells/mL in a 24-well tissue-culture plate. Eight wells with 2 mL/well were set up for each subject. Four wells were stimulated with PHA (Difco) at a final concentration of 1:1000, and four wells remained unstimulated. Plates were incubated at 37°C in a humidified incubator containing 5% CO_2 for 48 h. This time point had previously been determined to allow detection of all cytokines under investigation. At 48 h, cultures were harvested and centrifuged to remove the cells, and supernatants were aliquoted and stored at −70°C.

Cytokine quantitation was done using commercial ELISA kits. Kits for sCD4, sCD8, and sIL-2r were obtained from T Cell Diagnostics (Cambridge, MA), for IFN-γ from Genzyme (Cambridge, MA), and all others from R&D Systems (Minneapolis). All assays were done according to manufacturers' instructions. For each cytokine, all samples were run on the same day, and samples from each case and those of the 2 matched controls were run on the same plate. Standard curves and experimental values were determined using four-parameter fit with SOFTmax software (Molecular Devices, Menlo Park, CA).

NK cells. NK cell function was determined using a K562 ^{51}Cr release assay [27]. Briefly, fresh PBL isolated on ficoll-hypaque were incubated at effector-to-target (E:T) cell ratios from 50:1 to 3:1 with 10^4 ^{51}Cr-labeled K562 cells as targets in a 96-well microtiter plate. After 4 h at 37°C in a 5% CO_2 atmosphere, 100 μL of supernatant was removed and counted in a gamma counter. Total ^{51}Cr uptake was determined by adding 1% Triton X to the target cells. The percentage of killing was determined as follows: [(experimental release − spontaneous release)/(total release − spontaneous release)] × 100.

DTH. DTH was determined using the Mérieux multitest (Connaught Laboratories, Swiftwater, PA), which was given on the day of the interview and read 48 h later by trained nurses.

Radioallergosorbent (RAST) testing. Detection of specific circulating IgE against panels of individual and mixed antigens designed for the Atlanta area was done using the carrier polymer system (Pharmacia Diagnostics, Uppsala, Sweden) in a clinical allergy laboratory. We used the following individual allergens: Japanese cedar, sycamore, white ash, white pine, pecan, hickory, beech, Bermuda grass, bahia grass, rough pigweed, rough marsh elder, sheep sorrel weed, *Mucor racemosus, Fusarium moniliform, Stemphylium botryosum,* crab, tomato, orange, strawberry, fire ant, and mosquito. The mixed allergens included four separate food mixes, each containing five allergens (nuts, seafoods, cereal, pediatric), and mixes of mold, animal, feather, house dust, weeds, grasses, and trees. The laboratory also determined total serum IgE levels using the Pharmacia system.

Immune complexes. Levels of circulating immune complexes were determined using both the C1q binding assay and the *Staphylococcus* protein A binding assay [27].

Toxic metals. The levels of mercury and arsenic in urine and blood lead levels were determined by standard techniques [27–31].

Other tests. Serum levels of IgG, IgA, IgM, C3 and C4, and RBC levels of magnesium were determined at SmithKline Beecham Laboratories (Atlanta). These laboratories also did 50% complement hemolytic (CH_{50}) tests.

Table 2. Median laboratory findings in CFS cases and matched controls.

	Cases (n = 26)	Controls (n = 50)
White blood cells (10^3/mm^3)	5.60 (4.4–6.5)	6.05 (5.4–7.3)
Lymphocytes (%)	35.0 (31–40)	32.0 (27–36)
PMNL (%)	56.0 (49–61)	59.0 (53–59)
Monocytes (%)	7.0 (5–8)	7.0 (6–7)
Eosinophils (%)	2.5 (2–4)	2.0 (1–4)
Basophils (%)	1.0 (0–1)	1.0 (0–1)
Serum		
IgG (mg/dL)	798.0 (614–940)	792.5 (699–866)
IgM (mg/dL)	91.0 (49–141)	103.5 (67–154)
IgA (mg/dL)	132.5 (60–186)	130.0 (87–195)
IgE (U/mL)	26.4 (9.5–41.6)	34.8 (11.6–80.5)
Complement (mg/dL)		
C3	120.0 (105–153)	124.0 (104–156)
C4	22.5 (16–27)	22.0 (18–27)
CH_{50} (%)	36.0 (28–46)	40.0 (21–59)
Immune complexes		
C1q binding assay (%)	7.0 (5–9)	7.0 (5–9)
Protein A binding assay (Eq/mL)	24.5 (11–60)	38.0 (7–65)
Red blood cell magnesium (mEq/mL)	3.55 (3.2–4.1)	3.55 (3.1–4.0)
Urine		
Mercury (μg/L)	6.2 (2.5–8.6)	4.5 (2.9–7.3)
Arsenic (μg/L)	0.0 (0–0)	0.0 (0–0)
Blood lead (μg/100 mL)	1.5 (1.2–2.0)	1.7 (1.1–2.3)

NOTE. Data in parentheses are 25th–75th percentiles.
PMNL, polymorphonuclear leukocytes; CH_{50}, 50% complement hemolytic.

Results

Demographics. We recruited 26 CFS patients (23 female, 3 male) and 52 matched controls. Two controls withdrew, leaving 2 cases with only 1 matched control. All participants were white. The median duration of illness was 5.3 years, and the median age at illness onset was 33.5 years (range, 16–49). Occupation, income, and education were comparable for cases and controls.

Negative laboratory measurements for cases and controls. CFS patients did not differ from the matched controls with respect to the following parameters (table 2): number of white blood cells, total lymphocytes, monocytes, or neutrophils; serum levels of IgG, IgA, IgM, IgE, complement component C3 or C4, CH_{50} levels, or circulating immune complexes measured by C1q binding or by *Staphylococcus* protein A binding, RBC magnesium levels, blood lead levels, or urine levels of arsenic and mercury. There were no differences in any parameters when cases were subgrouped by sudden or gradual onset, duration of illness, or health status at the time of the assay.

Negative immune function findings. No differences were seen between cases and controls in DTH responses to any of

Table 3. Median NK cell function of CFS cases and matched controls.

Effector-to-target ratio	Cases (n = 26)	Controls (n = 50)
50:1	32.5 (20–45)	29.0 (18–41)
25:1	26.5 (16–38)	23.0 (16–37)
12.5:1	15.5 (10–25)	16.0 (9–26)
6:1	10.0 (8–17)	9.0 (7–15)
3:1	6.0 (5–9)	6.0 (3–9)

NOTE. Results expressed as % killing. Data in parentheses are 25th–75th percentiles.

the antigens tested or in the number of positive DTH responses. Patients and controls did not differ in the numbers of allergic responses measured by RAST or by severity of responses. There were no differences in NK cell function at any E:T ratio tested (table 3). Subgrouping of cases by sudden or gradual onset, duration of illness, or health status at the time of the assay detected no differences in any of these parameters.

Proliferative responses. Proliferative responses to antigens and mitogens did not differ overall between cases and controls (table 4), nor were there differences in the numbers of positive responders to each antigen. However, when CFS cases were analyzed by subgroup, cases with sudden onset had a lower response to *Candida* antigen (2500 vs. 3000, P = .006) than their matched controls.

Cytokines and soluble receptors. Five of the 9 cytokines tested were detected in serum: IFN-γ, IL-1α, IL-1β, IL-6, and TGF-β. IFN-γ was detected in both cases and controls, but there was no difference between the 2 groups. IL-6 was detected in 3 cases and 1 control and TGF-β was detected in 3 cases and no controls (Fisher's exact test, P = .040). There was no overlap between the cases with circulating IL-6 and those with circulating TGF-β. We also assayed for serum levels

Table 4. Median proliferative responses of CFS cases and controls to stimulation with mitogens and antigens.

Stimulant	Cases (n = 26)	Controls (n = 50)
PHA 1:1000	12,328 (122.0)	13,526 (146.0)
PHA 1:4000	8884 (91.0)	7464 (49.0)
Con A	939 (6.0)	942 (7.5)
PWM	12,612 (128.5)	13,195 (151.5)
MLR	7309 (46.9)	8013 (56.9)
Tetanus toxoid	2928 (24.0)	1832 (23.5)
SLO	3444 (32.5)	3117 (33.5)
Diphtheria toxoid	287 (2.0)	390 (6.0)
Candida	2418 (19.5)	2330 (25.8)

NOTE. Data expressed as changes in cpm (stimulated − control). Nos. in parentheses = stimulation index.

PHA, phytohemagglutinin; ConA, concanavalin A; PWM, pokeweed mitogen; MLR, mixed lymphocyte reaction; SLO, streptolysin O.

Table 5. Phytohemagglutinin-stimulated cytokine responses in CFS subgroups and matched controls.

Cytokine, subgroup	Median (range) Cases	Controls	P*
IL-2			
All	290 (220–390)	260 (180–350)	.121
Sick	300 (270–430)	265 (190–380)	.040
Well	270 (200–340)	245 (170–345)	.907
Gradual	290 (230–390)	270 (200–390)	.614
Sudden	320 (220–390)	210 (170–300)	.052
IL-1α			
All	130 (80–180)	185 (80–270)	.060
Sick	130 (60–180)	195 (120–270)	.038
Well	130 (110–150)	145 (55–245)	.561
Gradual	130 (80–170)	190 (90–300)	.011
Sudden	110 (90–270)	120 (40–210)	.782
IL-1β			
All	400 (170–650)	600 (350–1140)	.080
Sick	270 (150–580)	675 (380–1250)	.059
Well	470 (320–650)	545 (330–975)	.561
Gradual	320 (170–580)	670 (350–1170)	.039
Sudden	540 (200–650)	540 (200–1000)	.890

NOTE. Results are expressed in pg/mL. IL, interleukin. Gradual and sudden indicate onset of CFS.

* Cochran-Mantel-Haenszel statistic with modified ridit scores.

of sCD4, sCD8, and sIL-2r. Circulating levels of sCD8 were lower in cases than controls (430 vs. 500 pg/mL, P = .028). Several cytokines were produced spontaneously in culture: IL-1α, -1β, and -6 and TNF-α, but there were no detectable differences between cases and controls. All cytokines tested for were detected after lymphocyte stimulation with PHA, except for TGB-β. There were no differences in cytokine production between cases and controls. However, subgroup analyses showed that persons with gradual onset of CFS produced lower levels of IL-1α and IL-1β than their matched controls. In addition, serum levels of IL-6 and TGF-β segregated with gradual onset. Sudden onset cases produced higher levels of IL-2 than their matched controls (table 5). Cases who were sick (wellness score $\leq$50) produced significantly higher levels of IL-2 and lower levels of IL-1α than their matched controls (table 5). However, the differences in IL-1α production in these 2 subgroups were clearly driven by high IL-1α production in the control groups. When cases were subgrouped by duration of illness, there were no differences in cytokine production.

Cell surface markers. CFS patients did not differ from their matched controls for any of the lymphocyte subsets tested; however, analysis by subgroup showed differences between cases and controls. Gradual onset cases had a lower percentage of CD56 cells (NK cells) expressing CD2 (30.8% vs. 38.5%, P = .009), while sudden onset cases had more CD8 cells expressing CD11b (suppressor cells; 34.5% vs. 20.6%, P = .018). Cases with a wellness score $\leq$50 had fewer CD8 cells expressing CD25 (10.4% vs. 19.8%, P = .042). Cases with a

wellness score >50 also differed from their matched controls with more bright CD8 cells (21.5% vs. 19.2%, $P = .042$). Cases who had been sick >63 months had increased percentages of CD19 cells (B cells) expressing CD5 (19.8% vs. 13.0%, $P = .019$). They also had more CD8 cells expressing S6F1 (64.5% vs. 56.5%, $P = .043$), fewer CD56 cells expressing CD2 (32.6% vs. 38.2%, $P = .051$), and fewer CD8 cells expressing CD45RA (66.5% vs. 73.5%, $P = .051$). There were no differences in marker populations in patients who had been sick <63 months.

Discussion

We studied a large number of parameters of immune function in a small but well-characterized group of patients with CFS. Many of the parameters have been found by at least one group of investigators to differ in persons with CFS from those in healthy controls, although the findings are not universal. These differences have been used to suggest an immune etiology for CFS. We wanted to see if there was a consistent pattern of immune perturbation by studying all of the parameters in a single group of patients and by comparing the findings with a randomly selected matched control group. Our findings are noteworthy for two reasons: the lack of differences between all CFS cases and controls and in the differences seen when cases are subgrouped either by disease onset or by how well they were feeling on the day of the assay.

We found no evidence for anergy among the CFS patients, although anergy has been consistently reported in Australian CFS patients [13]. We also did not find any increase in the incidence or severity of allergies, as measured by RAST, although CFS patients reported more allergy-associated symptoms than did controls [23]. A history of allergy has been reported anecdotally to be associated with CFS. We also found no evidence of low levels of circulating immune complexes or of complement activation.

As a part of this study, we assessed RBC magnesium levels, since these have been reported to be low, and magnesium supplementation has been suggested as a possible therapy [32]. We found no evidence of low RBC magnesium. We also assessed levels of lead, mercury, and arsenic, since fatigue is a symptom of toxicity with all three. There was no evidence of accumulation of any of these elements in the CFS patients studied.

Several groups have reported that NK cell numbers and function are depressed in persons with CFS [7–12]. In this study, there was no evidence of impaired NK cell function, nor were NK cells decreased overall. However in cases with gradual onset, the percentage of CD56 cells also expressing CD2 (i.e., the TNK cell populations), was decreased. The significance of this finding remains unclear. There was no difference in NK cell function in this subgroup of patients.

Since cytokines have been proposed as mediators of the symptoms of CFS, it is important to be able to detect cytokine differences between cases and controls. There was no evidence of circulating cytokines in the majority of CFS patients; however, 3 cases had detectable circulating TGF-β and another 3 had detectable circulating IL-6. Both of these groups had gradual onset of disease. It has been suggested that if there is chronic immune activation in CFS, then PBL may secrete cytokines in vitro without further stimulation, due to a prior activation event. We found no evidence to support this suggestion. After stimulation with PHA, there was no difference in cytokine production between CFS cases and matched controls. However, in subgroup analyses, there were some interesting differences. Cases with gradual onset produced lower levels of IL-1β than their matched controls, whereas cases with sudden onset produced higher levels of IL-2. Cases who were sicker on the day of the assay produced higher levels of IL-2 than did controls. This suggests that different CFS subgroups may have different disease mechanisms, as reflected in cytokine production.

Subgrouping was also seen in the cell surface marker studies. Cases with sudden onset had increased percentages of CD8 cells expressing CD11b, a characteristic of the T suppressor cell population. Elevated levels of this population are seen in acute infections. Coupled with the increase in IL-2 production, this is suggestive of an infectious process. In contrast, CFS patients with gradual onset of illness had a lower percentage of CD2 CD56 NK cells than did controls, although NK cell function did not differ. Perturbations of total NK cells and of NK cell subpopulations are often reported for CFS patients. The gradual onset group also showed a decrease in IL-1β production and detectable circulating IL-6 and TGF-β. This pattern of alterations is not usually seen in infectious disease and may reflect a different pathogenic process. It is also noteworthy that patients who had been sick >63 months had increased levels of B cells expressing CD5. This subpopulation of cells is responsible for autoantibody production and may be an indication of a pathogenic mechanism in a subset of patients.

Our study was undertaken to explore the immunologic differences between CFS patients and healthy controls. Since the cases were selected from the CDC surveillance system, the numbers in this study are small, which limits its power to detect differences. Thus, it is all the more striking that when the cases were subgrouped, giving even smaller numbers, that differences were seen between cases and their matched controls that paralleled those seen by other researchers. Since this was an exploratory study, we chose not to correct for multiple comparisons. This approach allows for all differences between cases and controls to be considered, even though some of these differences may be due to chance. The findings in this study must be confirmed in a second case-control study specifically set up to test the hypothesis that these findings are not due to chance [26].

In summary, we could not confirm the findings of immune differences between CFS cases and controls when we assessed our total CFS population. However, when we broke down the population by type of disease onset, we detected subtle immu-

nologic differences. The patients with sudden onset had a cytokine and cell surface marker profile reminiscent of that seen in acute infection [18, 33]. By contrast, the differences seen in persons with gradual onset of CFS were similar to those reported by several other groups. This exploratory study suggests that disease onset might be an important factor to consider when selecting CFS cases for study and should be used for stratification in analysis of data sets. This approach may help explain the differing results seen in different research centers.

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