Changes in Immune Parameters Seen in Gulf War Veterans but Not in Civilians with Chronic Fatigue Syndrome

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The purpose of this study was to evaluate immune function through the assessment of lymphocyte subpopulations (total T cells, major histocompatibility complex [MHC] I- and II-restricted T cells, B cells, NK cells, MHC II-restricted T-cell-derived naive and memory cells, and several MHC I-restricted T-cell activation markers) and the measurement of cytokine gene expression (interleukin 2 [IL-2], IL-4, IL-6, IL-10, IL-12, gamma interferon [IFN-γ], and tumor necrosis factor alpha [TNF-α]) from peripheral blood lymphocytes. Subjects included two groups of patients meeting published case definitions for chronic fatigue syndrome (CFS)—a group of veterans who developed the illness following their return home from participating in the Gulf War and a group of nonveterans who developed the illness sporadically. Case control comparison groups were comprised of healthy Gulf War veterans and nonveterans, respectively. We found no significant difference for any of the immune variables in the nonveteran population. In contrast, veterans with CFS had significantly more total T cells and MHC II+ T cells and a significantly higher percentage of these lymphocyte subpopulations, as well as a significantly lower percentage of NK cells, than the respective controls. In addition, veterans with CFS had significantly higher levels of IL-2, IL-10, IFN-γ, and TNF-α than the controls. These data do not support the hypothesis of immune dysfunction in the genesis of CFS for sporadic cases of CFS but do suggest that service in the Persian Gulf is associated with an altered immune status in veterans who returned with severe fatigue illness.

One explanation for the lassitude, malaise, and flu-like symptoms seen in chronic fatigue syndrome (CFS) is the existence of immune dysregulation with increased levels of cytokines. Supporting this hypothesis are a number of reports of reduced NK cell activity (2, 13), upregulation of immune activation markers (19, 36), and increases in levels of cytokines in serum (4, 21, 28). However, these results are far from uniform. In contrast, some researchers have found no evidence of immune dysfunction (29), while others have found support for the immune dysfunction hypothesis only in a subset of CFS patients (25) or only when functional tests are performed (40).

We have had the unique opportunity to evaluate further the immune dysfunction hypothesis in two groups of patients with CFS—a group of civilians who developed the illness sporadically and a group of veterans who developed the illness following their service in the Gulf War. We have assessed three hypotheses in exploring the immunological data obtained: first, that CFS patients would have evidence of immune dysregulation compared to controls and that this would be more marked in the civilians because of the increased frequency of sudden onset in this group (32); second, that more severely ill patients, those with a sudden onset of their illness, and/or those free of concurrent psychiatric illness would exhibit the most marked immune dysregulation; and third, that CFS patients in general and Gulf War veterans in particular would show a type 2 cytokine profile according to the hypothesis of Rook and Zumla (33).

MATERIALS AND METHODS

Sample and data. The patients were 43 Gulf War veterans on the Department of Veterans Affairs’ (DVA) Gulf War Registry and 68 nonveterans—all of whom initially completed health questionnaires indicating the symptom profile of CFS. These patients came to East Orange or Newark, N.J., respectively, where they provided a 3-h history and received a physical examination, a blood test, and a diagnostic psychiatric interview (24) by medical and psychological personnel to rule out medical and psychiatric (10) causes of chronic fatigue. Every CFS patient studied here was found to meet the 1994 case definition (10) of CFS (for details of the intake protocol, see reference 32). Based on the self-reported percentage of decrease in activity and the number and severity of their CFS minor symptoms, they were rated on a six-category CFS severity scale. The most severely affected patients, those with severe CFS, met the 1988 case definition (14) with the following modifications: seven symptoms were required to fulfill criteria, but symptoms were counted only if their severity was rated as 3 or higher on symptom intensity scales from 0 to 5 in the month prior to intake. In addition, patients were classified according to mode of illness onset, with “sudden” being defined as the development of the illness in 1 to 2 days and “gradual” being defined as a more delayed onset.

The healthy unmedicated controls were 34 Gulf War veterans on the Gulf War Registry and 53 civilians. We used a health survey to identify healthy Gulf War veterans and then recruited them for our studies. Healthy nonveterans were self-selected based on their response to newspaper advertisements and flyers placed throughout neighboring communities. Healthy nonveterans who exercised more than once a week or who had an Axis I psychiatric disorder were excluded. These criteria were not applied to healthy Gulf War veterans.

Veteran recruitment was limited to patients in the computer database of the DVA New Jersey Health Care System and individuals with major complaints of fatigue who were in the Gulf War Registry and resided in 10 states east of the Mississippi River. Civilian recruitment was of people residing within a 100-mile radius of our center. Because of their distance from our center, veterans were admitted to the VA Medical Center for their studies; nonveterans came to the center for limited times only.

After written informed consent was obtained, subjects underwent venipunc-
tured and blood was collected in EDTA anticoagulated tubes that were coded to disguise the identity of the subject group. Peripheral blood lymphocytes (PBLs) were labeled within 6 h of collection with commercially available combinations of monoclonal antibody and the following cell surface markers; CD45-CD4, CD3-CD4, CD3-CD8, CD3-CD20, CD45RA-CD16+56 (Simulset Reagents, Becton Dickinson [BDIS], San Jose, Calif.), CD8-CD3, CD8-CD58, CD8-CD54, CD4-CD20, CD4-CD45RA, and CD4-CD45RA (antibodies to CD11b from DAKO, Carpinteria, Calif.; all other antibodies from BDIS). After the samples had been fixed in 0.5 ml of 1% formalin (methanol free) and refrigerated overnight, all flow cytometric analyses were performed in the same laboratory with a FACScan cytometer (BDIS) equipped with a 15-mW air-cooled 488-nm argon ion laser and with standard techniques (9). Thus, the following cell populations were quantified for each group of subjects: total leukocyte (WBC) count; number (and percentage of total WBC) of lymphocytes; number (and percentage of total lymphocyte count) of CD3[CDS (total T cells), CD3D (major histocompatibility complex [MHC] II-restricted T cells), CD3D (CD8+ [MHC I-restricted T cells), CD1D (B cells), and CD3D (CD16+56) (NK cells); percentage of class II-restricted T cells that were CD45RO and CD45RA, and percentage of class I-restricted T cells that were CD28+, HLA-DR+, CD56+, and CD11b-. PBLs, harvested from additional aliquots of blood, were homogenized in RNAzol (Cine/BioTech, Friendswood, Tex.) at 50 mg per 0.2 ml of 10% fetal bovine serum. The quantitative reverse transcriptase PCR (RT-PCR) cytokine assay was used as previously described (11, 38, 39). mRNA samples were reverse transcribed with Superscript RT (Bethesda Research Labs, Rockville, Md.), and cytokine-specific primers were used to amplify the following cytokines (38): gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin 2 (IL-2), IL-4, IL-6, IL-10, and IL-12. Amplified PCR product was detected by Southern blot analysis (38, 39), and the resultant signal was quantified as the relative differences between samples with a PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.) (38, 39). Subjects had no missing data for any of the cell surface markers or cytokines in the final analysis.

### Analysis

Our goal was to determine if immune alteration or dysregulation existed in CFS patients, relative to the healthy controls (comprised of both Gulf War veterans and civilians) and to compare veteran and civilian data when possible. All problems in prior reports of immunological abnormalities in CFS lies in their use of multiple, separate, univariate inferential tests, even in the cases in which multivariate analysis of variance (MANOVA) techniques are used. An approach differed from a traditional repeated-measures analysis in that it is not appropriate for hypothesis testing of interaction effects because of the possibility that some immune variables in the patient groups would show increases while others showed decreases relative to controls or that differences from controls might occur preponderantly in only one of the CFS groups. Likewise, the effects of the covariates may have different impacts on the various immunological measures. For example, men may have lower levels than women of one cell surface marker but have higher levels than women of another. These specific differences must be accounted for in order to determine if a true difference exists between CFS patients and the controls. The model testing group differences in the percentages of cell surface markers was specified as follows:

\[

c_{ij} = b_0 + b_1(x_{ij}) + b_2(race_{ij}) + b_3(age_{ij}) + b_4(Axis I_{ij}) + b_5(CFS_{ij}) + b_6(veteran_{ij}) + b_7(cell surface marker_{ij}) + b_8(CFS \times veteran_{ij}) + b_9(CFS \times cell surface marker_{ij}) + r_j (i = 1, 2, \ldots, n, number of subjects; j = 1, 2, \ldots, k, number of cell surface markers)
\]

The same specifications were used for the model testing group differences in the numbers of cell surface markers, except that the response function was log transformed. The model testing group differences in cytokine levels was specified as follows:

\[
\log\text{[cytokine]}_{ij} = b_0 + b_1(assay_{ij}) + b_2(sex_{ij}) + b_3(race_{ij}) + b_4(age_{ij}) + b_5(Axis I_{ij}) + b_6(CFS_{ij}) + b_7(cytokine_{ij}) + b_8(sex \times cytokine_{ij}) + b_9(race \times cytokine_{ij}) + b_{10}(age \times cytokine_{ij}) + b_{11}(CFS \times cytokine_{ij}) + r_j (i = 1, 2, \ldots, n, number of subjects; j = 1, 2, \ldots, k, number of cytokines)
\]

This approach differed from a traditional repeated-measures analysis in that the residual terms \( r \) in the equations were not assumed to be homogeneous within subject \( i \). Instead, error variances and covariances among subjects were fully estimated in every model by using the SAS mixed procedure (22) with an unconstrained error structure to reflect the interrelations among cell surface markers among cytokines.

Next, we did a stepwise elimination of nonsignificant interaction terms between the covariates and the immune status indicators from the models specified...
above. A nonsignificant interaction term indicates that the group differences of interest were not explained by a given covariate. Thus, for example, the interaction terms of Axis I versus surface markers and cytokines did not show statistical significance, and so they were not included in the specified models to be evaluated.

Finally, we systematically evaluated higher-order interactions testing group differences of interest. A higher-order interaction was eliminated from a model if it failed to reach statistical significance \( (P < 0.05) \), and then the model with only lower-order terms was evaluated. The final models retained are reported in the next section. The numbers of cell surface markers and cytokine measurements were log transformed before analyses to achieve normality and reduce undue influences from outlying measurements.

## RESULTS

Table 1 gives descriptive statistics about the sample. The veteran sample consists of predominantly white men, below age 50, with a high school or college education, while the civilian sample is predominantly white women of whom a higher percentage received postgraduate education than the veterans. For the healthy veterans, the chance of having an education higher than high school was three times that for the CFS veterans. The chance of having an Axis I diagnosis among the CFS veterans was over 2.5 times that among civilian CFS patients \( (\text{odds ratio} = 2.78, P < 0.05) \) and 12 times that of the healthy veterans \( (\text{odds ratio} = 12, P < 0.05) \). Sixteen percent of veteran patients were diagnosed in the most severe CFS category, while 87% of civilian patients were in that category \( (\text{odds ratio} = 0.11, P < 0.05) \). Sixteen percent of veteran patients reported a sudden onset of symptoms in comparison to 63% of civilians with CFS \( (\text{odds ratio} = 0.03, P < 0.05) \).

Eleven cell surface markers, the percentage and number of lymphocytes, WBC counts, and levels of seven cytokines were examined in the present study. We found no systematic group differences between patient groups and the respective controls for any of the following markers phenotypes: CD4+ CD45RO−, CD4+ CD45RA−, CD8− CD28−, CD8+ CD38−, or CD8+ CD11b−. Therefore, our report focuses on the group differences in the five parent cell surface markers and the seven cytokines.

Table 2 shows the means and standard errors for cytokines and CD cell surface markers.
TABLE 3. Linear contrasts of CD cell surface markers between Gulf War veteran and civilian groups from a mixed-effects model with three-way interaction between factors of veteran status (yes or no), CFS status (yes or no), and cell surface markers*.  

| Variable | Parameter estimates | t | P > t |
|----------|---------------------|---|------|
| Veteran with CFS versus veteran control |                  |   |      |
| CD(16+56) | -3.32* | -2.57 | 0.011 |
| CD19     | -1.56  | -1.36 | 0.176 |
| CD3      | 4.35*  | 2.74  | 0.007 |
| CD3 CD4  | 5.03*  | 3.66  | 0.000 |
| CD3 CD8  | -0.42  | -0.26 | 0.797 |

| Veteran with CFS versus civilian with CFS |                  |   |      |
| CD(16+56) | 2.22  | 1.84  | 0.068 |
| CD19     | -2.62* | -2.42 | 0.016 |
| CD3      | 0.65  | 0.44  | 0.662 |
| CD3 CD4  | 0.23  | 0.15  | 0.882 |
| CD3 CD8  | 1.21  | 0.79  | 0.428 |

* Surface marker percentages were compared to determine the statistical values shown.

b Parameter estimates are predicted differences in each of the cell surface markers between groups indicated by the subheadings.

††, P < 0.05.

(mean = 7.200, SE = 472.7, P = 0.026). However, these differences were not mutually independent, in that the decrease in the percent lymphocytes was a result of an increased number of WBC. Indeed, we found very similar numbers of lymphocytes in the gradual-onset group (mean = 1,846) and the control group (mean = 1,874). An increase in the percent activated T suppressor cells (CD8+ CD38+) (mean = 58.9, SE = 2.3, P = 0.023), compared to the control (mean = 51.1 and SE = 1.8 [Table 2]), was also found in the gradual onset group. Patients with a sudden onset of CFS (n = 18) showed a decreased percentage of CD8+ CD11b- cells (P = 0.056). No differences were found for cytokines after these stratifications.

There were not enough veterans with a sudden onset of CFS (n = 7) to allow a similar stratified test.

Turning to the Gulf War veterans, we systematically tested the three hypotheses stated in the introduction by utilizing mixed-effects models. The veterans with CFS were found to have a significantly lower percentage of NK cells (P = 0.011) and a significantly higher percentage of CD3+ cells (P = 0.007) and CD3+ CD4+ cells (P < 0.0003) than the healthy veteran controls (Table 3). These differences were tested simultaneously and confirmed by a significant three-way interaction (P = 0.0019) between CFS status, veteran status, and the five cell surface marker phenotypes (CD3+ CD(16+56)+, CD3+ CD19+, CD3+, CD3+ CD4+, and CD3+ CD8+) while controlling for sex, race, age, and Axis I diagnosis. When the results were averaged across veterans and civilians, subjects who were age 40 or older had significantly more CD3+ CD4+ cells (P = 0.006) and significantly fewer CD3+ CD8+ cells (P = 0.0006) than younger subjects. These significant age differences could have confounded our results had we not controlled for this variable in our analysis.

Veterans with CFS had significantly more CD3+ cells (P = 0.021) and CD3+ CD4+ cells (P = 0.002) than veteran controls, but no significant difference in the number of NK cells was found between the groups (Table 4). The veterans with CFS had significantly more NK cells, total T cells, CD3+ CD4+, and CD3+ CD8+ cells, but not more B cells, than both civilian groups. This general elevation in the numbers of cell surface markers in veteran patients was tested again and confirmed by a significant three-way interaction (P = 0.048) between CFS status, veteran status, and the five cell surface markers.

Figures 1 and 2 show the observed and predicted numbers of cell surface markers, confidence intervals for the predicted cell numbers, and the normative ranges of the five cell surface markers in the general population for the samples of veterans with CFS and of healthy civilians. Both the healthy civilian group and the CFS veteran group had cell number values in the normative range; the confidence intervals of all four subject groups studied here (only two are depicted in the figures) were within the boundaries of normative ranges. However, Fig. 1 and 2 show that the confidence intervals were broader in the healthy civilian group than those in the CFS veteran group, suggesting greater heterogeneity in the former. Not depicted are the broad confidence intervals for CFS civilians and the narrow confidence intervals for the veteran controls—suggesting that all civilians are more heterogeneous than Gulf War veterans in this regard.

The levels of seven cytokines were examined in the veteran sample (Table 5). Consistent with the cell surface marker data, veterans with CFS showed a general tendency toward upregulation relative to the controls across all cytokines in this study, indicated by the nonsignificant two-way interaction between CFS status and cytokines (P = 0.642) and a highly significant main effect of CFS (P = 0.006). Linear contrasts indicated that the group differences were statistically significant for IL-2 (P =

TABLE 4. Linear contrasts of CD cell surface markers between Gulf War veteran groups and civilian groups from a mixed-effects model with three-way interaction between factors of veteran status (yes or no), CFS status (yes or no), and cell surface markers**

| Variable | Parameter estimate | t | P > t |
|----------|-------------------|---|------|
| Veteran with CFS versus veteran control |                  |   |      |
| CD(16+56) | 0.12  | 0.97  | 0.334 |
| CD19     | 0.06  | 0.44  | 0.661 |
| CD13     | 0.20* | 2.41  | 0.012 |
| CD3 CD4  | 0.20* | 4.24  | 0.000 |
| CD3 CD8  | 0.24* | 2.12  | 0.035 |

** Surface marker counts were compared to determine the statistical values shown.

† Parameter estimates are predicted differences in each of the cell surface markers between groups indicated by the subheadings. For activation markers, CFS veterans were found to have lower levels of several activation markers measured in percentages than CFS civilians: CD28 (9.79 [P = 0.0009]), CD38 (6.52 [P = 0.0325], and CD11bmn (6.25 [P = 0.0169]). In addition, CFS veterans had lower levels of CD28 than the civilian controls (7.01 [P = 0.0169]).

††, P < 0.05.
FIG. 1. Estimated and observed mean numbers of five cell surface markers in Gulf War veterans with CFS. Lines are drawn to connect the measurement points simply to help the reader see the pattern of the immunological variables. The predicted mean numbers of cell surface markers were derived from a three-way interaction model between CFS status (yes or no), veteran status (yes or no), and cell surface markers. The model was analyzed by a univariate, mixed-effects repeated-measures analysis that allowed more efficient, simultaneous, cross-group comparisons between sick and healthy veterans and sick and healthy civilians than regular multivariate analysis. All predicted values fell within the laboratory's normative range. Note that the 95% confidence interval is narrower for all five markers relative to that of civilian controls.

0.021), IL-10 ($P = 0.01$), IFN-$\gamma$ ($P = 0.014$), and TNF-$\alpha$ ($P = 0.002$). Since the veterans with CFS had uniformly higher levels of all the cytokines (Table 2) than the controls, the two-way interaction of CFS status versus cytokines was not expected to be significant.

We next evaluated the hypothesis of a shift from type I to type II cytokine responses in the Gulf War veterans, especially in those with CFS (33). This shift was not observed. Instead, the data indicated a type I response with significant upregulation in IL-2 and IFN-$\gamma$ and not IL-4 and IL-6 in CFS veterans. In fact, instead of finding negative correlations between IFN-$\gamma$ and IL-4 or IL-6, we found significant positive correlations. Similar positive correlations were found between IL-2, another type 1 cytokine, and IL-4 and IL-6 in both the veteran and civilian samples (Table 6). Significant increases in IL-10 were also detected; IL-10 elevations are characteristic of either a type 1 or type 2 response, at least partly because IL-12 induces IL-10 and IFN-$\gamma$ production (26, 31).

**DISCUSSION**

This paper reports important negative and positive findings. The important negative finding is that we cannot confirm immune dysregulation in this carefully controlled study of non-veterans who developed CFS sporadically. When we began collecting these immunological samples, we had several a priori hypotheses. One was that we would find immune dysregulation in the entire group of CFS patients. A second related to our finding of decreased cognitive function and brain magnetic resonance imaging abnormalities in the group of CFS patients devoid of DSM-III-R psychopathology (8, 20). Those findings led us to hypothesize that we would find immunological abnormalities in this subgroup—especially in the most symptomatic of these patients. Again, those hypotheses were not supported. Even after stratifying the data for presence or absence of Axis I psychiatric disorder, there were no significant differences between patients and controls with regard to any of the immunological variables under study.

Because a Centers for Disease Control and Prevention (CDC) group also found negative results for their entire CFS patient pool but then did find some differences following stratification based on illness severity and mode of illness onset (25), we did a similar analysis. The CDC group reported differences based on severity, which we did not confirm. Concerning the differences based on mode of illness onset, we obtained results different from those of Mawle et al. The one similarity was the results for the phenotype CD8$^+$ CD11b$^+$. However, we found this cell population to be altered in the gradual-onset group, while Mawle et al. reported finding this in the sudden-onset group. These differences across studies show the problems associated with using statistical methods that do not guard against type 1 errors. In contrast to our use of the mixed-effects model detailed in Materials and Methods, this analysis (both for our data and for the CDC data) did not adjust the significance level for multiple comparisons. Since our results were not the same as those noted by the CDC group, our inference is that these results are probably statistical artifacts.

The question arises as to why we were unable to confirm immune dysregulation in the CFS patients studied. However, the literature makes it clear that inconsistency of results from laboratory to laboratory is the rule rather than the exception. Thus, some but not all laboratories find decreases in NK cell numbers (13, 29), T-cell populations (23, 34), and activation markers (19, 29, 40). Concerning cytokines, Strober’s 1994 conclusion seems applicable (37): “Studies of circulating levels of various lymphokines and cytokines in CFS have not yielded evidence of a clear-cut and reproducible abnormality of lymphokine/cytokine secretion in CFS.”

Cannon et al. have indicated that assay methods and type of tissue are crucial in evaluating cytokines (3). This is the first study of cytokine gene expression in CFS. Furthermore, we measured cytokine RNA from PBLs without in vitro restimulation in order to avoid artifacts resulting from mitogen activation of lymphocytes. Using RT-PCR, we are unable to confirm the prior reports of elevated IL-6 levels associated with
CFS (1, 12); however, elevated IL-10 levels, seen in our veterans with CFS, has also been reported in a group of patients with a clinical picture resembling severe, chronic infectious mononucleosis (16).

Besides the difference in assay methodology, there is another major difference between our study and those of others. In our work, we excluded healthy controls who exercised regularly. It is known that some immune parameters are sensitive to state of fitness. For instance, NK cell numbers increase with fitness (30). Thus, reductions in NK cell numbers may reflect the inactivity inherent in CFS rather than the underlying illness itself. A rather different explanation is that our group is simply studying the wrong immunological markers. Consistent with this interpretation are unpublished results of a study on transforming growth factor B in serum in collaboration with Chun Chao, in which we found a small but significant increase in 20 subjects in the nonveteran CFS group compared to 19 of the sedentary controls (CFS = 187 ± 13 pg/ml; control = 141 ± 11 pg/ml [*P*, 0.01]). Thus, it is possible that the evaluation of other cytokines or the assessment of the function of stimulated lymphocytes might provide more consistent evidence of immune dysfunction in CFS patients than tests of lymphocytes in the static condition.

A second reason for the study was to test the hypothesis of Rook and Zumla (33) that CFS patients in general and Gulf War veterans with CFS in particular would show a type 2 pattern of cytokine activation. Our data did not support that hypothesis for either patient group. In fact, the pattern of cytokine activation exhibited by the veterans with CFS was type 1, or inflammatory—with increases in IL-2 and IFN-γ possibly related to the concurrent increases in CD3+ CD4+ T-cell numbers. This type 1 shift was, if anything, suppressed, as it is known that IL-10, which was also present at elevated levels in the veterans with CFS, can suppress IFN-γ synthesis (27).

Since both these cytokines are induced by IL-12 (26, 31), IL-10 is commonly associated with type 1 immune responses.

When we began these studies, we assumed that if we found any evidence of immune dysfunction in either patient group, it would be in the civilians. We based that line of thinking on the published data suggesting that sporadic CFS often involved a postinfectious process (17), while severe fatigue in Gulf War veterans usually did not have an infectious or sudden type of onset. In fact, our own data supported this belief, in that we found that civilians with CFS reported a significantly higher rate of sudden, rather than gradual, illness onset than Gulf War veterans (Table 1). However, we found evidence of altered immune status in Gulf War veterans with CFS and not in the civilian patients.

How do we interpret this result? There are two quite different answers to this question. One possibility is that something specific to service in the Gulf War altered the normal control

TABLE 5. Linear contrasts of cytokine levels between Gulf War veterans with CFS and healthy controls from a mixed-effects model with interaction terms between group membership and cytokine measurements

| Variable | Parameter estimate | t  | *P > t* |
|----------|--------------------|----|--------|
| IL-2     | 0.504*             | 2.34 | 0.021 |
| IL-4     | 0.428              | 1.61 | 0.111 |
| IL-6     | 0.598              | 1.73 | 0.086 |
| IL-10    | 0.754*             | 2.61 | 0.010 |
| IL-12    | 0.362              | 0.92 | 0.359 |
| IFN-γ    | 0.539*             | 2.51 | 0.014 |
| TNF-α    | 0.659*             | 3.07 | 0.002 |

*a Cytokine levels (in units) were compared to determine the statistical values shown.

*b Parameter estimates are predicted differences in each of the cytokines between groups. *P < 0.05.
of the immunological system in veterans with CFS. We have been careful up to this point not to use words such as abnormal or dysfunctional in describing these changes, because the standardized laboratory cell surface marker data for the veterans were within the normative range. However, the variability of each measurement for both sick and healthy veterans is substantially less than that exhibited by nonveterans (Fig. 1 and 2). This suggests that the veterans are immunologically more homogeneous than the nonveterans. As Rook and Zumla have hypothesized (33), a reduction in immunological heterogeneity may result from multiple vaccinations received by deployed troops (33), an interpretation supported by a recent study of children vaccinated with Mycobacterium bovis BCG (35). Finding such a degree of homogeneity would suggest that it is inappropriate to compare Gulf War veterans’ immunological data with those of a normative nonveteran group. If this view were taken, it would indicate that Gulf War veterans with CFS indeed do show evidence of immune dysfunction relative to the respective Gulf War veteran controls.

An alternative explanation is that the putative immune abnormalities reflect differences between patients and controls that are independent of illness. An example would focus again on NK cell numbers. Improved fitness can increase this lymphocyte population (30), while partially disturbed sleep can result in immunological heterogeneity (32) and thus should have fewer problems with inactivity and poor sleep than nonveterans with CFS, whose immunological data are similar to those for carefully selected nonveterans. As Rook and Zumla have hypothesized (33), a reduction in immunological heterogeneity may result from multiple vaccinations received by deployed troops (33), an interpretation supported by a recent study of children vaccinated with Mycobacterium bovis BCG (35). Finding such a degree of homogeneity would suggest that it is inappropriate to compare Gulf War veterans’ immunological data with those of a normative nonveteran group. If this view were taken, it would indicate that Gulf War veterans with CFS indeed do show evidence of immune dysfunction relative to the respective Gulf War veteran controls.

We have presented two very different possible explanations for our finding of differences in immunological profiles between Gulf War veterans with CFS and healthy Gulf War veterans. Obviously the determination of which answer is correct will require further research. However, we believe that the data favor the first possibility—that something about serving in the Gulf War altered the immune status of that group of veterans who also have CFS. We conclude this because the veteran immunological data are relatively homogeneous but nonetheless show a clear difference between sick and healthy subjects and because veterans with CFS are less severely ill than civilians (32) and thus should have fewer problems with inactivity and poor sleep than nonveterans with CFS, whose immunological data are similar to those for carefully selected controls.

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**TABLE 6. Correlations among cytokines and T-cell and T-helper-cell surface markers**

| Surface marker or cytokine | Correlation to: | Veterans | Civilians |
|---------------------------|-----------------|---------|---------|
|                           | % CD3 | % CD3 CD4 | No. of CD3 | No. of CD3 CD4 | IL-2 | IL-4 | IL-6 | IL-10 | IL-12 | IFN-γ |
|                           |       |           |           |               |      |      |      |       |       |       |
| % CD3                     | 0.33* |           |           |               |      |      |      |       |       |       |
| % CD3 CD4                 | 0.20  | −0.02     |           |               |      |      |      |       |       |       |
| No. of CD3                | 0.10  | 0.36*     | 0.89*     |               |      |      |      |       |       |       |
| No. of CD3 CD4            | 0.04  | −0.02     | −0.09     | −0.08          |      |      |      |       |       |       |
| IL-2                      | 0.05  | 0.06      | 0.08      | 0.09           | 0.37*| 0.49*|      |       |       |       |
| IL-6                      | −0.01 | 0.34*     | −0.05     | 0.11           | 0.51*|      |      |       |       |       |
| IL-10                     | −0.01 | 0.23*     | −0.09     | 0.03           | 0.55*| 0.33*| 0.48*|       |       |       |
| IL-12                     | −0.07 | 0.06      | −0.08     | 0.04           | 0.14 | −0.10| 0.06 | 0.35*  |       |       |
| IFN-γ                     | 0.17  | 0.11      | 0.30*     | 0.30*          | 0.33*| 0.41 | 0.53*| 0.51*  | 0.15  |       |
| TNF-α                     | 0.05  | 0.27*     | −0.15     | −0.03          | 0.75*| 0.70*| 0.62*| 0.61*  | 0.11  | 0.41*  |

* p < 0.05.
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