# Fatigue and Proinflammatory Cytokine Activity in Breast Cancer Survivors

JULIENNE E. BOWER, PHD, PATRICIA A. GANZ, MD, NAJIB AZIZ, MD, AND JOHN L. FAHEY, MD

**Objective:** Fatigue is a common problem among cancer patients and survivors, yet the mechanisms underlying the occurrence and persistence of this symptom are not known. Activation of the immune system may evoke feelings of fatigue, which are mediated by proinflammatory cytokines. We examined whether fatigued breast cancer survivors would show elevations in proinflammatory cytokines and markers of cytokine activity compared with nonfatigued survivors. Differences in lymphocyte subsets, cortisol, and behavioral symptoms associated with proinflammatory cytokines were also assessed. **Methods:** Forty breast cancer survivors (20 fatigued, 20 nonfatigued) provided blood samples at visits scheduled to control for diurnal variability. Cytokines, soluble markers of cytokine activity, and cortisol were measured by immunoassay and lymphocyte subsets by flow cytometry. Participants also completed questionnaires measuring demographic, medical, and behavioral variables. **Results:** Fatigued breast cancer survivors had significantly higher serum levels of several markers associated with proinflammatory cytokine activity than nonfatigued survivors, including interleukin-1 receptor antagonist (IL-1ra), soluble tumor necrosis factor receptor type II (sTNF-RII), and neopterin. They were also more likely to report behavioral problems that co-occur with fatigue in the context of immune activation. Fatigued survivors had significantly lower serum levels of cortisol than the nonfatigued group as well as differences in two lymphocyte populations. **Conclusions:** Fatigued breast cancer survivors showed elevations in serum markers associated with proinflammatory cytokine activity an average of 5 years after diagnosis. Results suggest mechanisms through which enduring immune activation may occur, including alterations in cortisol and in lymphocyte subsets. **Key words:** fatigue, breast cancer, proinflammatory cytokines, sickness behavior, cortisol.

ANCOVA = analysis of covariance; HPA = hypothalamic-pituitary-adrenal; IL-1$\beta$ = interleukin-1 beta; IL-1ra = interleukin-1 receptor antagonist; IL–6 = interleukin–6; sTNF-RII = soluble tumor necrosis factor receptor II; TNF-$\alpha$ = tumor necrosis factor alpha.

Fatigue is a common problem among women undergoing treatment for breast cancer and may endure for months or years following completion of treatment in some patients. Recent studies have shown elevated levels of fatigue among breast cancer survivors relative to age-matched healthy controls (1, 2), with approximately one third of survivors continuing to report moderate to severe symptoms of fatigue 2 or more years posttreatment (3, 4). Fatigue has a negative impact on mood, social relationships, daily activities, and overall quality of life among both breast cancer patients and survivors (1–3, 5).

Despite its prevalence, the mechanisms underlying the onset and persistence of fatigue among cancer patients have not been determined. Although a variety of biological mechanisms have been proposed, the few studies to assess biological parameters (eg, hematocrit, hemoglobin, albumin, thyroid hormone) have typically not found a correlation with fatigue (6, 7). One possible mechanism for cancer-related fatigue is activation of the immune system in response to the tumor itself or to treatments for the disease. In particular, proinflammatory cytokines interleukin-1 beta (IL-1$\beta$), interleukin-6 (IL-6), and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) may be released as part of the host response to the tumor or in response to tissue damage or depletion of immune cell subsets associated with cancer treatment (8). These cytokines have a wide spectrum of peripheral and central effects that contribute to host defense, including effects on energy. Studies in laboratory animals have shown that injection of IL-1$\beta$, TNF-$\alpha$, or lipopolysaccharide (which induces IL-1$\beta$ and TNF-$\alpha$ synthesis) leads to fatigue as well as decreased activity, increased somnolence, anorexia, and social withdrawal (9). Fatigue has also been observed in clinical trials of cancer patients treated with cytokine therapy, occurring as part of a constellation of flu-like symptoms including lethargy, depressed mood, and cognitive disturbance (10, 11). These behavioral manifestations of sickness have been collectively referred to as sickness behavior.

To our knowledge, only two studies have specifically explored the possibility that proinflammatory cytokines may contribute to cancer-related fatigue. Greenberg et al. (12) found that both reports of fatigue and serum levels of IL-1$\beta$ tended to increase among prostate cancer patients receiving radiation therapy,

From the Division of Cancer Prevention and Control Research, Jonsson Comprehensive Cancer Center (J.E.B., P.A.G.), the Schools of Medicine and Public Health (P.A.G.), the Center for Interdisciplinary Research in Immunology and Disease (N.A.), and the Department of Microbiology and Immunology (J.L.F.), the University of California, Los Angeles, California.

Address reprint requests to: Julienne E. Bower, PhD, Division of Cancer Prevention and Control Research, Jonsson Comprehensive Cancer Center at UCLA, 650 Charles Young Drive South, Room A2-125 CHS, Box 956900, Los Angeles, CA 90095-6900. Email: jbower@ucla.edu

Received for publication March 27, 2001; revision received July 25, 2001.



0033-3174/02/6404-0604

although the correlation between these parameters was not reported. Another study, published in abstract form only, reported a correlation between IL-6 and fatigue in a sample of lung cancer patients treated with both radiation therapy and chemotherapy (13). The association between proinflammatory cytokines and fatigue in patients beyond the acute phase of treatment has not yet been examined.

The primary purpose of this study was to test the hypothesis that fatigue is associated with proinflammatory cytokine activity and behavioral markers of sickness among breast cancer survivors. This was done by determining serum levels of a proinflammatory cytokine, IL-1$\beta$, and of molecules associated with proinflammatory cytokine activity, including IL-1ra, sTNF-RII, and neopterin. Serum levels of these molecules were quantified because they can be measured more reliably in serum than the proinflammatory cytokines that induce their production and, in the case of sTNF-RII and neopterin, may provide a better measure of cytokine activity. IL-1ra is a member of the IL-1 family and antagonizes the effects of IL-1 in vivo and in vitro (14). IL-1ra is produced by many of the same cell types as IL-1 and is made in response to the same stimuli, including infection and inflammation (15). Soluble TNF-RII is released from activated cells, particularly monocytes/macrophages and lymphocytes, when TNF-$\alpha$ is produced (16). Soluble TNF-RII levels are highly correlated with TNF-$\alpha$ levels in serum and reflect TNF-$\alpha$ activity (16). Neopterin is secreted by activated macrophages, the primary source of proinflammatory cytokines, and provides a measure of macrophage activity (17). These molecules are elevated in conditions associated with proinflammatory cytokine activity, including autoimmune, inflammatory, and infectious diseases (15–17).

A secondary goal of this study was to explore other immune and hormonal parameters that may be influenced by cancer and its treatment, including lymphocyte subsets and cortisol. Changes in these parameters are relevant to proinflammatory cytokine activity and fatigue because they may underlie or follow from persistent activation of the inflammatory response. Surgical procedures, radiation therapy, and chemotherapy can all cause acute changes in immune parameters, most of which resolve following completion of treatment. However, prospective studies have shown prolonged deficits in certain lymphocyte populations among breast cancer patients treated with radiation and chemotherapy (18–20). Disturbances in other physiological systems associated with cancer and its treatment may also be important for immunological functioning. In particular, alterations in hypothalamic-pituitary-adrenal (HPA) axis function may have enduring immune effects, as glucocorticoids are powerful modulators of the immune system and are specifically known to inhibit production of proinflammatory cytokines (21). Interestingly, HPA abnormalities have been associated with fatigue in the context of chronic fatigue syndrome and other clinical disorders (22, 23). We examined both lymphocyte populations and serum levels of cortisol to determine whether these parameters were associated with enduring fatigue in cancer survivors.

The current study compared women who had vs. had not experienced prolonged fatigue following breast cancer diagnosis and treatment. We hypothesized that breast cancer survivors reporting enduring fatigue would show higher serum levels of IL-1$\beta$, IL-1ra, sTNF-RII, and neopterin than nonfatigued survivors. In addition, we hypothesized that fatigued survivors would show evidence of other sickness behaviors, including somnolence, decreased activity, social withdrawal, cognitive disturbance, and depressed mood.

## METHODS

### Participants

Subjects were former participants in a large survey study of breast cancer survivors focusing on quality of life, intimacy, and sexuality (24, 25). Participants in the prior survey study ($N$ = 1957) were recruited from two large metropolitan areas (Los Angeles, CA, and Washington, DC) between September 1994 and June 1997. Women were eligible for participation if they met the following criteria: 1) had been diagnosed with early, resectable breast cancer (stage 0, I, or II at diagnosis); 2) were between 1 and 5 years after initial breast cancer diagnosis; 3) had completed local and/or systemic adjuvant cancer therapy; 4) were currently considered disease-free and were not receiving any cancer therapy other than tamoxifen; 5) had no history of other cancers, with the exception of noninvasive skin cancer and cervical cancer; 6) could read and write English; 7) could provide informed consent; and 8) had no other major disabling medical or psychiatric conditions that would confound evaluation of health-related quality of life.

For the current study, we were interested in identifying two subgroups of survivors from the larger project: those who reported enduring fatigue following breast cancer diagnosis and treatment and those who did not report enduring fatigue. Potential participants were identified based on their scores on the energy/fatigue subscale of the RAND 36-Item Health Survey, which was completed as part of the original survey packet. Scores on this scale range from 0 to 100, with higher scores indicating better functioning (ie, higher levels of energy). Scores below 50 indicate limitations or disability related to fatigue (26, 27).

Women were considered for participation in the current study if they scored in the lower (range = 0–50; high fatigue) or upper (range = 70–100; low fatigue) tertiles of the RAND scale at the time of original survey completion, lived in the Los Angeles area, and were not already participating in another follow-up project conducted by our group. A total of 332 survivors met these initial criteria and were sent letters describing the current project. They were asked to return a response form indicating their interest in the current study and were also asked to rate their current level of fatigue on the RAND

energy/fatigue scale. Women who again scored in either the lower or upper tertiles of the RAND scale were contacted by phone for determination of final eligibility. Exclusion criteria were as follows: 1) breast cancer recurrence; 2) diagnosis with other cancers; 3) history of immunologically related diseases or diseases that could affect the immune system; 4) regular use of immunosuppressive medication; 5) history of psychiatric hospitalization or severe psychological distress in last 6 months; and 6) consumption of more than 15 alcoholic beverages per week.

Response forms were received from 207 women, 137 of whom were interested in the study and provided contact and energy information. Fifty-one of these women were excluded because of a change in their energy tertiles; 13 were excluded because of a cancer recurrence, another cancer diagnosis, or other medical problems; and 15 were unable to come for an appointment at UCLA. Of the 58 eligible women remaining, 22 were high in fatigue at both assessment points and 36 were low in fatigue at both points. We were able to contact and recruit 20 of the fatigued women. To maintain equal sample sizes, we stopped recruitment of low-fatigue women at 20. The UCLA Institutional Review Board approved the study procedures, and written informed consent was obtained from all participants.

### Procedure

All assessments were scheduled within the same 2-hour time period in the morning (8–10 AM) to control for diurnal variations in immune and endocrine parameters. Participants were asked to refrain from consuming food, drinking alcohol and/or caffeine, using tobacco, taking nonprescription medication, and engaging in strenuous exercise during the 12-hour period before their appointment. Blood was collected by venipuncture into sterile blood collection tubes after a 15- to 20-minute rest period. Blood draws were not completed for three subjects due to technical difficulties ($N = 2$) and subject refusal ($N = 1$). Following the blood draw, a semistructured interview was conducted focusing on fatigue during and post breast cancer diagnosis and treatment. All participants also completed several self-report questionnaires the evening before their appointments.

### Cytokines, Immune Activation Markers, and Cortisol

Serum samples were separated according to standard procedures and stored at −70°C for subsequent batch testing. Serum levels of IL-1$\beta$ were measured using Quantikine High Sensitivity Immunoassay kits (R&D Systems, Minneapolis, MN). IL-1ra and sTNF-RII were measured with Quantikine Immunoassay kits (R&D Systems). Neopterin was measured using enzyme immunoassay kits (BRAHMS Diagnostics, Berlin, Germany). Cortisol was measured with enzyme immunoassay kits (Diagnostics Systems Laboratory, Webster, TX). The measurement of cytokine and activation marker levels was performed according to the manufacturer's instructions. Quality-control procedures for our laboratory were conducted in the usual manner (28, 29). The intraassay precision of all tests was less than or equal to 10% for in-house quality-control samples.

### Lymphocyte Subsets

Lymphocyte cell subsets were enumerated using FACScan flow cytometry with three-color immunofluoresence. CD4 cells were defined as $CD3^+CD4^+$, CD8 cells as $CD3^+CD8^+$, B cells as $CD19^+$, and NK cells as $CD3^-CD56^+/CD16^+$. Memory ($CD45RO^+$) and naive ($CD45RA^+$) subpopulations of CD4 cells were also assessed.

### Psychological and Behavioral Measures

Fatigue was assessed using the energy/fatigue subscale of the RAND 36-Item Health Survey (26, 27) and the Fatigue Symptom Inventory (FSI; 5). The RAND scale consists of four items assessing feelings of energy and tiredness during the past 4 weeks. The FSI is a 13-item measure that assesses fatigue intensity, duration, and interference with daily functioning during the past week. This scale has acceptable psychometric properties and has been shown to discriminate between cancer survivors and healthy controls (5).

Because there are no validated scales to assess behavioral manifestations of cytokine-related sickness in humans, we assessed sickness behaviors using a combination of items from the Breast Cancer Prevention Trial (BCPT) Symptom Checklist (30) and items designed specifically for this project. Items were included to assess somnolence (tendency to take naps, feeling like you need more rest that usual), activity level, social interest, and cognitive difficulties, all of which have been identified as behavioral components of illness (31). Respondents were asked whether or not they had experienced each symptom during the past 4 weeks. Depressed mood in the last 2 weeks was assessed using the Beck Depression Inventory II (BDI-II) (32). Health behaviors occurring over the past week that may influence immune and endocrine parameters (eg, use of alcohol, caffeine, cigarettes) were assessed using the Health Behavior Questionnaire. Demographic and medical variables were also assessed by questionnaire.

### Statistical Analyses

Differences between the fatigued and nonfatigued groups were evaluated using $\chi^2$ and independent-samples $t$ tests. Group comparisons were conducted on immune and hormonal measures as well as measures of fatigue, sickness behaviors, and demographic and medical factors (ie, age, race, education, income, relationship status, type of treatment received). For those comparisons that involved physiological parameters, we first computed the association between the physiological parameters and potential behavioral confounds using Pearson correlation coefficients. Confounds that were correlated with any of the physiological markers were controlled for using analysis of covariance (ANCOVA). All tests of statistical significance were two sided. Because of problems with insufficient samples, neopterin assays were not conducted for two subjects (one fatigued, one nonfatigued), and IL-1$\beta$, IL-1ra, and sTNF-RII assays were not conducted for six subjects (four fatigued, two nonfatigued). One subject was excluded from all immune analyses because of an abnormally high level of B lymphocytes.

## RESULTS

Analyses were first conducted to verify the accuracy of the fatigue group classifications. As expected given our classification system, fatigued women scored well below the nonfatigued women on the energy/fatigue subscale of the RAND Health Survey (fatigued group mean = 39.5; nonfatigued group mean = 80.5; recall that lower scores on this measure indicate more significant fatigue). There were also significant differences between groups on the FSI, with fatigued women reporting significantly higher levels of fatigue, more days spent fatigued, and more disruption due to fatigue than nonfatigued participants (all $p$ values $< .05$).

Demographic and treatment-related characteristics of fatigued and nonfatigued study participants are shown in Table 1. Significant differences between fatigue groups emerged only for relationship status and income level; fatigued women were significantly less likely to be married or in a committed relationship ($\chi^2 = 6.67$, $p = .01$) and had a lower income level than nonfatigued women ($\chi^2 = 6.65$, $p = .04$). No group differences in the type of breast cancer treatment received or in the length of time since diagnosis and treatment were observed. In addition, we found no differences between groups on menopausal status or a number of common medical conditions (ie, arthritis, headaches, high blood pressure, heart disease, osteoporosis, and/or lymphedema).

### Group Differences in Cytokines, Soluble Immune Activation Markers, and Cortisol

Fatigued women had significantly higher serum levels of IL-1ra, sTNF-RII, and neopterin than nonfatigued women, controlling for health behaviors associated with the soluble immune and hormonal markers (ie, caffeine and alcohol use; $p = .006$ for IL-1ra, .005 for sTNF-RII, and .018 for neopterin). Figure 1 shows levels for fatigued and nonfatigued survivors on each of these parameters. No group differences in IL-1$\beta$ were observed; however, it should be noted that serum levels of IL-1 were very low in this sample of women and fell outside of the detectable range for almost half of the study participants. Thus, our ability to detect differences between groups on this immune parameter may have been limited by assay sensitivity. Fatigued women had significantly lower serum levels of cortisol than the nonfatigued group, controlling for the same confounds (fatigued group mean = 11.88 $\mu$g/dl, SD = 3.7; nonfatigued group mean = 14.01 $\mu$g/dl, SD = 3.2; $F(1, 26) = 6.09$, $p = .02$).

In addition to controlling for behavioral factors, demographic factors that distinguished fatigued from nonfatigued participants were considered as potential confounds. Neither relationship status or income level was associated with any of the biological measures, and controlling for these variables did not influence the association between fatigue and immune and endocrine parameters. Exclusion of the three women who were currently menstruating (one fatigued, two nonfatigued) also had no effect on results.

**TABLE 1. Demographic and Medical Characteristics of Fatigued and Nonfatigued Breast Cancer Survivors**

| Characteristic | Fatigued ($n = 20$) | Nonfatigued ($n = 20$) |
|---|---|---|
| Age (mean ± SD) | 57.1 ± 8.7 | 58.4 ± 10.1 |
| Ethnicity (N) | | |
| White | 18 | 17 |
| Black | 2 | 1 |
| Asian | 0 | 2 |
| Married or in committed relationship (N)* | | |
| Yes | 8 | 16 |
| No | 12 | 4 |
| Education level (N) | | |
| High school or less | 2 | 2 |
| College graduate | 11 | 9 |
| Postgraduate | 7 | 9 |
| Employment status (N) | | |
| Employed full or part-time (including full-time homemaker) | 13 | 15 |
| Retired | 5 | 5 |
| Medical leave, unemployed | 2 | 0 |
| Income level (N)* | | |
| <$45,000 | 8 | 3 |
| $45,000–$75,000 | 7 | 4 |
| >$75,000 | 5 | 13 |
| Type of treatment (N) | | |
| Surgery only | 6 | 6 |
| Surgery + radiation | 7 | 6 |
| Surgery + chemotherapy | 4 | 1 |
| Surgery + radiation + chemotherapy | 3 | 7 |
| Tamoxifen use (N) | | |
| Current | 4 | 5 |
| Past | 9 | 4 |
| Never | 7 | 11 |
| Years since diagnosis (mean ± SD) | 5.5 ± 0.8 | 5.0 ± 1.0 |

* $p < .05$.

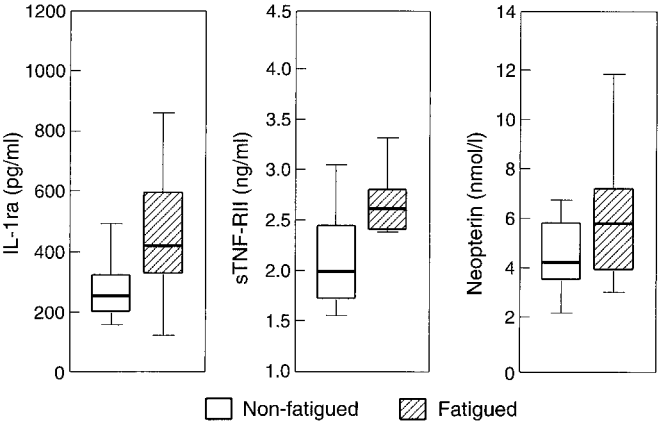


Fig. 1. Levels of serum immune markers for fatigued and nonfatigued breast cancer survivors. Fatigued survivors showed significantly higher levels of interleukin 1 receptor antagonist (IL-1ra), soluble tumor necrosis factor receptor II (sTNF-RII), and neopterin than nonfatigued survivors, controlling for relevant health behaviors (all $p$ values $< .05$). The lower boundary of each box represents the 25th percentile, and the upper boundary represents the 75th percentile. The line inside each box represents the median. Lines outside each box extend to the largest and smallest observed values within 1.5 box lengths.

### Group Differences in Lymphocyte Subsets

Analyses were conducted to examine differences in lymphocyte subsets between fatigue groups. As shown in Table 2, fatigued women had a significantly lower percentage of NK cells than nonfatigued women, controlling for health behaviors associated with cell subsets (ie, cigarette use; $F(1, 33) = 4.33$, $p < .05$). There was also a higher ratio of $CD45RO^+$ to $CD45RA^+$ CD4 T cells in the fatigued group than in the nonfatigued group, controlling for cigarette use ($F(1, 33) = 4.01$, $p = .05$); fatigued women had a higher percentage of CD45RO+ cells and a lower percentage of $CD45RA^+$ CD4 cells than nonfatigued women. No other significant differences in lymphocyte distribution were observed.

### Group Differences in Sickness Behaviors

Fatigued women were significantly more likely to report experiencing a number of sickness behaviors than nonfatigued women. Fatigued women reported a higher rate of somnolence (ie, needing more rest and taking more naps), decreased activity level, decreased interest in planning or initiating social activities, forgetfulness, and distractibility (all $p$ values $\leq .05$). Fatigued women also reported significantly higher levels of depressed mood (BDI-II mean score = 12.9, range = 1–34) than nonfatigued women (BDI-II mean score = 3.05, range = 0–15; $t(38) = 3.87$, $p = .001$; BDI-II scores of 13 and below are considered to fall within the minimally depressed range). These differences were maintained in analyses excluding the two items on the BDI-II that assess loss of energy and fatigue.

Because elevations in markers of immune activation have been observed in patients with clinical depression (33, 34), we conducted analyses to determine whether the immune differences between fatigue groups might be attributable to differences in depression. None of the immune parameters assessed were correlated with scores on the BDI-II, nor did we find immune differences between participants categorized as minimally, mildly, and moderately to severely depressed on the BDI-II. In addition, controlling for depression scores did not affect the association between fatigue and the immune variables.

**TABLE 2. Lymphocyte Subsets in Fatigued and Nonfatigued Breast Cancer Survivors**

| Cell Subset | Fatigued ($n$ = 19) | Nonfatigued ($n$ = 17) |
|---|---|---|
| % CD4+ | | |
| Mean (SD) | 47.3 (9.1) | 42.6 (7.2) |
| % CD8+ | | |
| Mean (SD) | 23.6 (6.8) | 24.5 (8.3) |
| % CD19+ | | |
| Mean (SD) | 14.7 (9.4) | 13.5 (4.3) |
| % CD56 + 16 (NK)* | | |
| Mean (SD) | 11.7 (5.0) | 15.7 (4.8) |
| % CD45RO + (mature) in CD4+ | | |
| Mean (SD) | 81.1 (9.2) | 75.4 (11.4) |
| % CD45RA + (naïve) in CD4+ | | |
| Mean (SD) | 43.8 (16.8) | 48.9 (11.0) |
| CD45RO:CD45RA ratio* | | |
| Mean (SD) | 2.3 (1.4) | 1.6 (0.5) |

* $p \leq .05$.

## DISCUSSION

Activation of the immune system by infection, injury, or trauma leads to the release of proinflammatory cytokines and other immune factors, including receptor antagonists, soluble receptors, and products of cellular activation. These cytokines orchestrate local and systemic immune responses and also mediate neural symptoms such as fatigue (31, 35). We found elevated levels of immune markers associated with proinflammatory cytokine activity in breast cancer survivors reporting persistent fatigue an average of 5 years after cancer diagnosis. Fatigued survivors showed significantly higher levels of IL-1ra, sTNF-RII, and neopterin than nonfatigued survivors and also reported a number of behavioral symptoms associated with cytokine activity. Proinflammatory cytokines are known to be involved in many autoimmune and infectious diseases (36–38) and have also been implicated in disorders characterized by chronic fatigue, including chronic fatigue syndrome (39) and vital exhaustion (40). To our knowledge, this is the first study to document an association between persistent fatigue and markers of cytokine activity in cancer patients.

One plausible cause of immune disturbance and subsequent fatigue in breast cancer survivors is enduring effects of cancer treatment. However, we found no evidence that a specific type of treatment accounted for group differences in fatigue or immune status, although it is important to note that the small number of subjects receiving each type of treatment may have limited our ability to detect differences between groups. These results are consistent with previous studies that have failed to find an association between fatigue and treatment status among patients several years posttreatment (1, 3).

Another important factor that may be associated with both fatigue and immune status is depression. Fatigue is one of the most common symptoms of a depressive episode and may occur secondary to depression following cancer diagnosis and treatment. Our results showed that fatigue was associated with

depressive symptomatology in this group of survivors, consistent with previous research (1–3). However, because of the cross-sectional nature of the study design, it is impossible to determine which of these states came first; it is equally likely that fatigue may have precipitated feelings of depression due to its negative impact on quality of life and interference with normal functioning in the aftermath of the cancer experience.

We did assess whether depression acted as a mediator of the association between fatigue and the proinflammatory markers. Depression has been associated with elevations in proinflammatory cytokines (33, 34, 41) as well as disturbances in other aspects of the immune system (42) and dysregulation of the HPA axis (43). Our results did not support a mediating role for depression, as scores on a depression rating inventory were not correlated with the inflammatory markers assessed, and controlling for these scores did not influence the association between fatigue and the immune outcomes. These findings suggest that the association between fatigue and immune activation is not entirely due to elevated levels of depressed mood among the fatigued survivors, although the small size of this sample may have limited our ability to test this relationship. We speculate that rather than being secondary to depression, fatigue and depression may cooccur as part of a coordinated response elicited by cytokine actions on the central nervous system; indeed, our results suggest that fatigue might actually be more proximally linked to these cytokine changes, at least among individuals identified based on fatigue symptoms. The association between fatigue, depression, and cytokine changes in cancer patients and survivors is an important area for future research.

The serum markers assessed in this study provide a window on the activity of the immune system and specifically on those cells involved in the synthesis and release of proinflammatory cytokines such as monocytes and macrophages. Under normal conditions, these cells are tightly regulated by multiple inhibitory mechanisms, including negative feedback by glucocorticoids (21). Our results suggest dysregulation of this feedback system in breast cancer survivors experiencing enduring fatigue, as this group showed significantly lower levels of cortisol during the typical morning peak of the circadian cycle. The potential importance of glucocorticoids for regulating immune function and general health in cancer patients was highlighted in a recent study, which found that altered diurnal cortisol rhythms were associated with suppression of NK cell activity and decreased survival time in patients with metastatic breast cancer (44). Alterations in cortisol-immune interactions have also been observed in patients with chronic fatigue syndrome (45). One limitation of the current study is the assessment of cortisol at a single time point, which provides limited information about cortisol rhythm and responsiveness to neuroendocrine mediators. We are currently examining diurnal cortisol profiles and cortisol sensitivity in these patients to better evaluate the association between cortisol and markers of immune activation.

Another window on immune system functioning is provided by the distribution of lymphocyte subsets circulating in peripheral blood. We found preliminary evidence for changes in the prevalence of two cell populations among fatigued survivors, including a lower percentage of naive CD4 T cells and natural killer cells. These changes may reflect a delay in the recovery of these cell types following cancer treatment. Indeed, prospective studies conducted with breast cancer patients have shown deficits in naïve CD4 T cells for up to 5 years after intensive chemotherapy (18) and radiation therapy (19). It is possible that prolonged decreases in particular cell populations may underlie the immune activation seen in fatigued survivors if the immune system stays activated in response to homeostatic mechanisms that turn on production of cells when they get depleted. Alternatively, decreases in circulating levels of immune cells may reflect changes in lymphocyte trafficking that occur secondary to immune activation elsewhere in the body. In this case, the changes in lymphocyte subsets observed among fatigued survivors would follow from, rather than precipitate, elevations in proinflammatory cytokines.

We have focused here primarily on biological mechanisms for fatigue, although many other factors may influence this complex and multidimensional symptom. In particular, social and demographic factors can play a significant role in the onset and maintenance of fatigue. Fatigued women in this study were more likely to be unmarried and of lower income than nonfatigued survivors, consistent with results from our larger survivor study (3). These women may have experienced a lack of practical, emotional, and financial support during cancer diagnosis and treatment, increasing the burden of the experience and potentially making recovery more difficult. They may also have been under greater strain before cancer diagnosis; studies have shown a higher incidence of stressful life events and a lower level of social support among individuals of lower socioeconomic status (46–49). Both stress and social support are known to influence immune status (50, 51) and could potentially play a role in the fatigue-immune association found in the current study.

The present data provide preliminary evidence for a

link between activity of proinflammatory cytokines and cancer-related fatigue and suggest mechanisms through which ongoing immune activation may occur, including alterations in immune regulatory systems and persistent changes in lymphocyte subsets. At this point, it is unclear why some patients may experience these changes and not others in the aftermath of a cancer diagnosis. Psychological and socioeconomic factors may play a role, as suggested by our data linking fatigue with depression, marital, and income status. In addition, these changes may be due to specific characteristics of the disease or treatment not assessed here. To clarify the contribution of proinflammatory cytokines to fatigue in cancer patients, prospective studies are needed to assess the association between changes in cytokines induced by cancer treatment and changes in fatigue levels. In addition, other immune, endocrine, and psychological factors should be assessed to determine how these variables influence immune activation and fatigue during and posttreatment.

## REFERENCES


1. Andrykowski MA, Curran SL, Lighner R. Off-treatment fatigue in breast cancer survivors: a controlled comparison. J Behav Med 1998;21:1–18.
2. Broeckel JA, Jacobsen PB, Horton J, Balducci L, Lyman GH. Characteristics and correlates of fatigue after adjuvant chemotherapy for breast cancer. J Clin Oncol 1998;16:1689–96.
3. Bower JE, Ganz PA, Desmond KA, Rowland JH, Meyerowitz BE, Belin TR. Fatigue in breast cancer survivors: occurrence, correlates, and impact on quality of life. J Clin Oncol 2000;18:743–53.
4. Lindley C, Vasa S, Sawyer WT, Winer EP. Quality of life and preferences for treatment following systemic adjuvant therapy for early-stage breast cancer. J Clin Oncol 1998;16:1380–7.
5. Hann DM, Jacobsen P, Azzarello LM, Martin SC, Curran SL, Fields KK, Greenberg H, Lyman G. Measurement of fatigue in cancer patients: development and validation of the Fatigue Symptom Inventory. Qual Life Res 1998;7:301–10.
6. Greenberg DB, Sawixka J, Eisenthal S, Ross D. Fatigue syndrome due to localized radiation. J Pain Symptom Manage 1992;7: 38–45.
7. Irvine D, Vincent L, Graydon JE, Bubela N, Thompson L. The prevalence and correlates of fatigue in patients receiving treatment with chemotherapy and radiotherapy. Cancer Nurs 1994; 17:367–78.
8. Herskind C, Bamberg M, Rodemann H. The role of cytokines in the development of normal-tissue reactions after radiotherapy. Strahlenther Onkol 1998;174 Suppl III:12–5.
9. Dantzer R, Bluthe RM, Kent S, Goodall G. Behavioral effects of cytokines: an insight into mechanisms of sickness behavior. Methods Neurosci 1993;17:130–50.
10. Adams F, Quesada JR, Gutterman JU. Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. JAMA 1984;252:938–41.
11. Denicoff KD, Rubinow DR, Papa MZ, Simpson C, Seipp CA, Lotze MT, Chang AE, Rosenstein D, Rosenberg SA. The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. Ann Intern Med 1987;107:293–300.
12. Greenberg DG, Gray JL, Mannix CM, Eisenthal S, Carey M. Treatment-related fatigue and serum interleukin-1 levels in patients during external beam irradiation for prostate cancer. J Pain Symptom Manage 1993;8:196–200.
13. Rigas JR, Hoopes PJ, Meyer LA, Smith EL, Hammond S, Leopold K, Maurer LH, Cole BF. Fatigue linked to plasma cytokines in patients with lung cancer undergoing combined modality therapy. Proc ACSO 1998;17:68A.
14. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991;77:1627–52.
15. Arend WP, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. Annu Rev Immunol 1998; 16:27–55.
16. Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. Eur J Haematol 1995;54:1–8.
17. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dierich MP, Wachter N. Neopterin as a marker for activated cell-mediated immunity: application in HIV infection. Immunol Today 1988; 9:150–5.
18. Hakim FT, Cepeda R, Kaimei S, Mackall CL, McAtee N, Zujewski J, Cowan K, Gress RE. Constraints on CD4 recovery postchemotherapy in adults: thymic insufficiency and apoptotic decline of expanded peripheral CD4 cells. Blood 1997;90:3789–98.
19. De Ruysscher D, Waer M, Vandeputte M, Aerts R, Vantongelen K, van der Schueren E. Changes of lymphocyte subsets after local irradiation for early stage breast cancer and seminoma testis: long-term increase of activated (HLA-DR+) T cells and decrease of "naïve" (CD4-CD45RA) T lymphocytes. Eur J Cancer 1992;28A:1729–34.
20. Petrini B, Wasserman J, Baral E, Blomgren H, Strender LE. Adjuvant radiation therapy compared with cyclic chemotherapy in patients with mammary carcinoma. Acta Radiol Oncol 1982; 20:137–41.
21. McEwen BS, Biron CA, Brunson KW, Bulloch K, Chambers WH, Dhabhar FS, Goldfarb RH, Kitson RP, Miller AH, Spencer RL, Weiss JM. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine, and immune interactions. Brain Res Rev 1997;23:79–133.
22. Clauw DJ, Chrousos GP. Chronic pain and fatigue syndromes: overlapping clinical and neuroendocrine features and potential pathogenic mechanisms. Neuroimmunomodulation 1997;4: 134–53.
23. Sternberg EM. Hypoimmune fatigue syndromes: diseases of the stress response? J Rheumatol 1993;20:418–21.
24. Ganz PA, Rowland JH, Meyerowitz BE, Desmond KA. Impact of different adjuvant therapy strategies on quality of life in breast cancer survivors. Recent Results Cancer Res 1998;152:396–411.
25. Ganz PA, Rowland JH, Desmond KA, Meyerowitz BE, Wyatt GE. Life after breast cancer: understanding women's health-related quality of life and sexual functioning. J Clin Oncol 1998;16: 501–14.
26. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. Health Econ 1993;2:217–27.
27. Ware JE Jr, Sherbourne CD. A 36-item Short Form Health Survey (SF-36): I. Conceptual framework and item selection. Med Care 1992;30:473–83.
28. Aziz N, Nishanian P, Fahey JL. Levels of cytokines and immune activation markers in plasma in human immunodeficiency virus infection: quality control procedures. Clin Diagn Lab Immunol 1998;5:755–61.
29. Aziz N, Nishanian P, Mitsuyasu R, Detels R, Fahey JL. Variables that affect assays for plasma cytokines and soluble activation markers. Clin Diagn Lab Immunol 1999;6:89–95.
30. Ganz PA, Day R, Ware JE Jr, Redmond C, Fisher B. Base-line

quality of life assessment in the National Surgical Adjuvant Breast and Bowel Project Breast Cancer Prevention trial. J Natl Cancer Inst 1995;87:1372–82.
31. Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol Rev 1998;195: 83–107.
32. Beck AT, Steer RA, Brown GK. BDI-II manual. San Antonio (TX): The Psychological Corporation; 1996.
33. Maes M, Bosmans E, De Jongh R, Kenis G, Vandoolaeghe E, Neels H. Increased serum IL-6 and interleukin-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine 1997;9:853–8.
34. Maes M, Vandoolaeghe E, Ranjan R, Bosmans E, Bergmans R, Desnyder R. Increased serum interleukin-1-receptor-antagonist concentrations in major depression. J Affect Disord 1995;36: 29–36.
35. Hart BL. Biological basis of the sickness behavior of animals. Neurosci Biobehav Rev 1988;12:123–37.
36. Fahey JL. Cytokines, plasma immune activation markers, and clinically relevant surrogate markers in HIV infection. Clin Diagn Lab Immunol 1998;5:597–603.
37. Giovannoni G, Lai M, Kidd D, Thorpe JW, Miller DH, Thompson AJ, et al. Daily urinary neopterin excretion as an immunological marker of disease activity in multiple sclerosis. Brain 1997;120: 1–13.
38. Hahn G, Stuhlmuller B, Hain N, Kalden JR, Pfizenmaier K, Burnester GR. Modulation of monocyte activation in patients with rheumatoid arthritis by leukapheresis therapy. J Clin Invest 1993;91:862–70.
39. Buchwald D, Wener MH, Pearlman T, Kith P. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J Rheumatol 1997;24:372–6.
40. Appels A, Bar FW, Bar J, Bruggeman C, De Baets M. Inflammation, depressive symptomatology, and coronary artery disease. Psychosom Med 2000;62:601–5.
41. Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, Pollmacher T. Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry 2001;58:445–52.
42. Herbert TB, Cohen S. Depression and immunity: a meta-analytic review. Psychol Bull 1993;113:472–86.
43. Owens MJ, Nemeroff CG. The role of CRF in the pathophysiology of affective disorders: laboratory and clinical studies. Ciba Found Symp 1993;172:293–16.
44. Sephton SE, Sapolsky RM, Kraemer HC, Spiegel D. Diurnal cortisol rhythm as a predictor of breast cancer survival. J Natl Cancer Inst 2000;92:994–1000.
45. Kavelaars A, Kuis W, Knook L, Sinnema G, Heijnen C. Disturbed neuroendocrine-immune interactions in chronic fatigue syndrome. J Clin Endocrinol Metab 2000;85:692–6.
46. Adler NE, Boyce T, Chesney MA, Cohen S, Folkman S, Khan RL, Syme SL. Socioeconomic status and health. Am Psychol 1994; 49:15–24.
47. Anderson NB, Armstead CA. Toward understanding the association of socioeconomic status and health: a new challenge for the biopsychosocial approach. Psychosom Med 1995;57:213–25.
48. Ranchor AV, Bouma J, Sanderman R. Vulnerability and social class: differential patterns of personality and social support over social classes. Personality Individual Diff 1996;135:229–37.
49. Turner RJ, Marino F. Social support and social structure: a descriptive epidemiology. J Health Soc Behav 1994;35:193–212.
50. Herbert TB, Cohen S. Stress and immunity and humans: a meta-analytic review. Psychosom Med 1993;55:364–79.
51. Uchino BN, Cacioppo JT, Kiecolt-Glaser JK. The relationship between social support and physiological processes: a review with emphasis on underlying mechanisms and implications for health. Psychol Bull 1996;119:488–531.