BRIEF COMMUNICATION

T-Cell Homeostasis in Breast Cancer Survivors With Persistent Fatigue

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Approximately 30% of women successfully treated for breast cancer suffer persistent fatigue of unknown origin. Recent studies linking inflammatory processes to central nervous system–mediated fatigue led us to examine cellular immune system status in 20 fatigued breast cancer survivors and 19 matched non-fatigued breast cancer survivors. Fatigued survivors, compared with non-fatigued survivors, had statistically significantly increased numbers of circulating T lymphocytes (mean 31% increase, 95% confidence interval [CI] = 6% to 56%; *P* = .015 by two-sided analysis of variance [ANOVA]), with pronounced elevation in the numbers of $CD4^+$ T lymphocytes (mean 41% increase, 95% CI = 15% to 68%; *P* = .003 by two-sided ANOVA) and $CD56^+$ effector T lymphocytes (mean 52% increase, 95% CI = 4% to 99%; *P* = .027 by two-sided ANOVA). These changes were independent of patient demographic and treatment characteristics. Absolute numbers of B cells, natural killer cells, granulocytes, and monocytes were not altered. The increased numbers of circulating T cells correlated with elevations in the level of serum interleukin 1 receptor antagonist (for $CD3^+$ cells, *r* = .56 and *P* = .001; for $CD3^+/CD4^+$ cells, *r* = .68 and *P*<.001, by Spearman rank correlation). Results of this study suggest that persistent fatigue in breast cancer survivors might be associated with a chronic inflammatory process involving the T-cell compartment. These results require confirmation in a larger study that is specifically designed to address this hypothesis. [J Natl Cancer Inst 2003;95:1165–8]

Fatigue is a common side effect of cancer treatment and may persist for months or years after treatment is completed *(1–5).* Approximately 30% of breast cancer survivors report persistent fatigue of unknown origin *(6–9).* Fatigue after cancer therapy is not consistently associated with treatment modality *(4,6,8,10),* and there is no evidence of residual or recurrent neoplastic disease in fatigued breast cancer survivors. Basic research on neuro-immune signaling has shown that inflammatory stimuli can signal the central nervous system to generate fatigue, as well as changes in sleep, appetite, social behavior, and reproduction *(11).* In a previous study of fatigued breast cancer survivors *(12),* we found elevated levels of several inflammatory markers in circulating blood, including interleukin 1 receptor antagonist (IL-1ra), soluble tumor necrosis factor receptor type II (sTNF-RII), and neopterin. We designed this study to identify the immunologic basis for these elevations. In particular, we evaluate the hypothesis that these soluble inflammatory markers and associated symptoms of fatigue stem from an underlying chronic cellular immune response involving the T-cell compartment.

We contacted 332 potential participants from a larger study of breast cancer survivors *(13,14)* and screened 132 responders for study eligibility. From this group, we identified 20 breast cancer survivors who reported enduring fatigue and a matched control group of 19 non-fatigued breast cancer survivors. Fatigue was assessed by use of the RAND SF-36 energy/fatigue scale *(15,16).* Survivors were considered eligible if they reported moderate-to-severe fatigue at the initial assessment (mean = 1.85 years after diagnosis, range = 1–5 years) and at the assessment for this study (mean = 5.25 years after diagnosis, range = 3–7 years; mean number of years between first and second assessment = 3.4 years, range = 2–5 years). Control group participants scored in the non-fatigued range at both assessment points. All participants had completed primary cancer treatments (surgery, radiation therapy, and/or chemotherapy) at least 2.5 years earlier, showed no evidence of recurrence, and had no history of an immunologic disease. Nine participants were still taking tamoxifen. Fatigued and non-fatigued breast cancer survivors did not differ by age, ethnicity, menopausal status, primary cancer treatment, and other medical comorbidities. Fatigued survivors, compared with non-fatigued survivors, had statistically significantly higher body mass indexes and lower incomes and reported higher levels of depressed mood. This sample of breast cancer survivors was the focus of a previous study *(12)* that includes more detailed information about recruitment and sample characteristics. In this communication, we report additional immune analyses conducted on blood samples collected from this cohort.

Fasting blood samples were drawn and subjected to a complete blood count and flow cytometric determination of circulating lymphocytes, including T lymphocytes ($CD3^+$), B lymphocytes ($CD19^+$), natural killer cells ($CD3^-/CD16^+$ or $CD3^-/CD56^+$), $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, activated T lymphocytes ($CD3^+/HLA\text{-}DR^+/CD38^+$), and effector T lymphocytes ($CD3^+/CD56^+$). Blood was not collected from three subjects (one fatigued and two control survivors) because of technical difficulties or subject refusal. The investigation was approved by the Institutional Review Board of the University of California, Los Angeles, and informed, written consent was obtained from all subjects. Immunologic parameters in fatigued breast cancer survivors were compared with those of non-fatigued control survivors by analysis of

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variance (ANOVA), and relationships among various immunologic parameters were determined by the Spearman rank correlation coefficient. Analyses of covariance (ANCOVA) were used to control for possible confounders in comparisons between fatigued survivors and controls. All statistical tests were two-sided.

Fatigued breast cancer survivors did not differ from non-fatigued survivors in total numbers of white blood cells, granulocytes, or monocytes (Fig. 1). Fatigued breast cancer survivors, compared with non-fatigued control survivors, had approximately 28% more lymphocytes per cubic millimeter of circulating blood (95% confidence interval [CI] = 7% to 49%; $P = .011$). Within the lymphocyte population, numerical expansion was confined to the T-cell subset. Fatigued breast cancer survivors, compared with non-fatigued survivors, had 31% more $CD3^+$ T lymphocytes (95% CI = 6% to 56%; $P = .015$), 41% more $CD4^+$ T lymphocytes (95% CI = 15% to 68%; $P = .003$), and 52% more $CD3^+/CD56^+$ lymphocytes (95% CI = 4% to 99%; $P = .027$), which are thought to represent terminally differentiated cytotoxic effector cells *(17).* Fatigued breast cancer survivors also had 31% more $CD8^+$ T lymphocytes, but this difference failed to reach statistical significance (95% CI = –9% to 80%; $P = .124$). The fractions of T lymphocytes expressing CD38 and HLA-DR were not statistically significantly different between fatigued and non-fatigued breast cancer survivors. In addition, no differences were observed in red blood cell count, hemoglobin level, or hematocrit values. Differences in T-cell subsets were maintained in analyses of covariance (ANCOVAs) controlling for potential confounders, including age, income, ethnicity, body mass index, depressed mood, and treatment type.

Previous analysis of this cohort revealed a 46% increase in circulating levels of IL-1ra (95% CI = 2% to 89%; $P = .006$), a 33% increase in levels of neopterin (95% CI = 6% to 59%; $P = .018$), and an 18% increase in levels of sTNF-RII (95% CI = 1% to 34%; $P = .005$) *(12).* More recent analyses at the individual patient level show a strong interrelationship among these soluble inflammatory markers (Fig. 2, A). Total blood lymphocyte counts and the numbers of T lymphocytes and $CD4^+$ T lymphocytes were elevated in direct proportion to the concentrations of IL-1ra (Fig. 2, B). These relationships were specific to T lymphocytes, because the concentration of IL-1ra showed no statistically significant correlation with the number of circulating $CD19^+$ B cells or $CD16^+/CD56^+$ natural killer cells. Despite moderately strong correlations between IL-1ra and other soluble markers, neither sTNF-RII nor neopterin showed a statistically significant association with lymphocyte subsets.

To determine whether alterations in circulating T-cell levels might be involved in the association of IL-1ra with fatigue (as opposed to an independent contributor), we conducted analyses of covariance controlling for differences in T lymphocyte numbers in comparisons of IL-1ra levels among fatigued and non-fatigued breast cancer survivors. Consistent with the hypothesis that altered T-cell homeostasis is involved in the relationship between increased IL-1ra concentrations and fatigue, statistical control for differences in circulating levels of $CD3^+$ or $CD3^+/CD4^+$ T lymphocytes rendered relationships between the levels of IL-1ra and fatigue statistically nonsignificant ($P = .253$ controlling for $CD3^+$ lymphocytes; $P = .591$ controlling for $CD3^+/CD4^+$ lymphocytes).

The profile of immunologic alterations observed in these fatigued breast cancer survivors is consistent with the hypothesis that a T-cell–mediated inflammatory process is driving fatigue symptomatology via systemic distribution of cytokines. Elevated prevalence of $CD56^+$ T lymphocytes has been observed in chronic viral infections with cytomegalovirus or human papilloma virus *(18–23),* suggesting that treatment-induced reactivation of a latent viral infection could explain these results. The terminally differentiated effector T cells showing selective expansion in fatigued survivors are distinct from the proliferative T-cell phenotype marked by CD38 and HLA-DR *(24),* frequencies of which were not altered in fatigued breast cancer survivors. Although the present results are consistent with a chronic viral infection, they could also be induced by a generalized alteration in homeostatic set points that control T-cell development, survival, proliferation, or maturation. The $CD56^+$ T-cell subset, in particular, is known to be resistant to proliferative and apoptotic signals and to include an appreciable fraction of senescent $CD57^+$ cells *(20).* These alterations may be related to changes in immune regulatory systems, including the autonomic nervous system and the hypothalamic–pituitary–adrenal axis. For example, fatigued survivors have lower levels of morning serum cortisol *(12)* and flatter diurnal cortisol slopes (Bower J, Ganz PA, Dickerson SS, Petersen L, Aziz N, Fahey JL: unpublished data) than non-fatigued survivors, which could conceivably play a role in the inflammatory phenotype observed in fatigued breast cancer survivors. The processes that initiate and maintain immune alterations associated with fatigue are an important topic for future research.

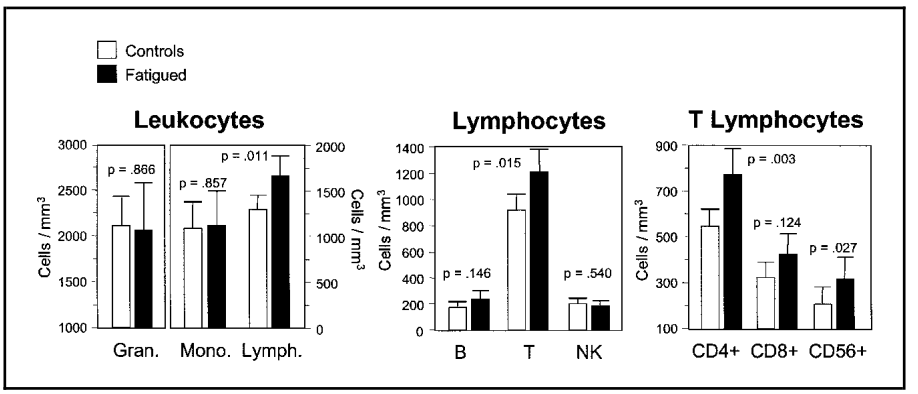

Fig. 1. Distribution of circulating leukocyte subsets in fatigued and non-fatigued (control) breast cancer survivors. Cells obtained from antecubital venipuncture were assessed by complete cell blood counts and flow cytometry for major lymphocyte subsets. Difference between persistently fatigued (**solid bars**) and non-fatigued (**open bars**) breast cancer survivors were analyzed by two-sample *t* test (after logarithmic transformation when necessary to normalize distributions). All statistical tests were two-sided. Gran. = granulocytes; Mono. = monocytes; Lymph. = lymphocytes; B = B cells; T = T cells; NK = natural killer cells. **Error bars** = 95% confidence intervals for the mean value of each parameter.

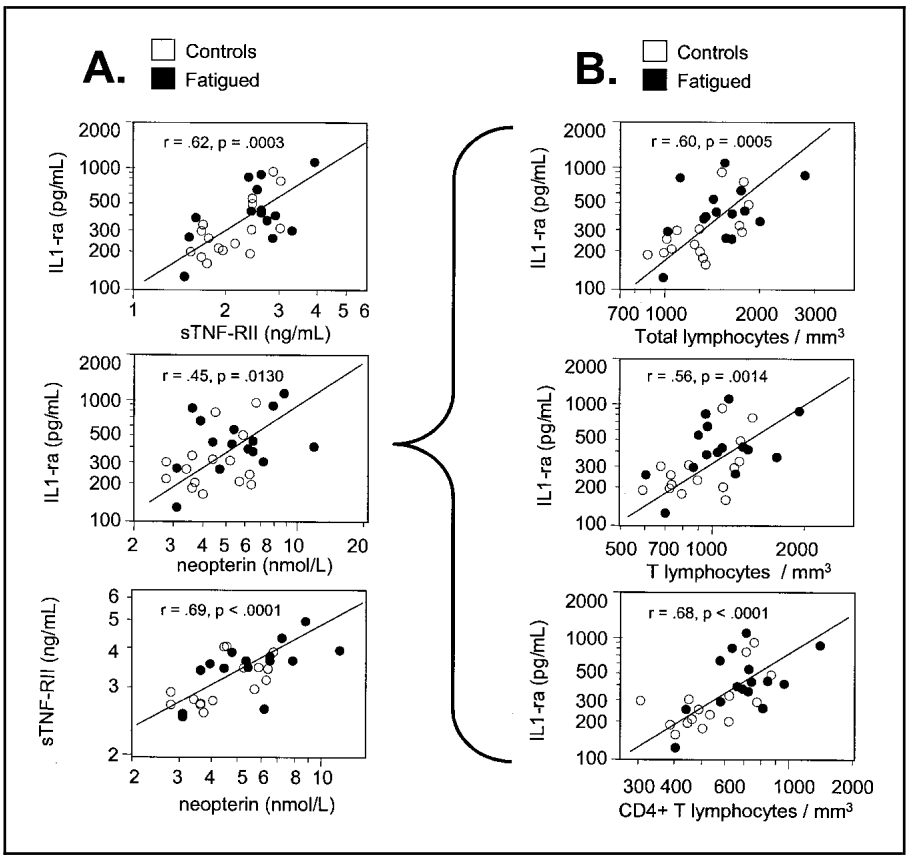

Fig. 2. Relationships between soluble inflammatory markers and leukocyte distributions. **A**) Serum levels of interleukin 1 receptor antagonist (IL-1ra), soluble tumor necrosis factor receptor type II (sTNF-RII), and neopterin were quantified by enzyme-linked immunosorbent assay, and relationships among these markers were quantified by Spearman rank correlation. **Solid circles** = fatigued patients; **open circles** = non-fatigued control patients. **B**) Relationships between circulating leukocyte subsets and serum inflammatory markers were analyzed by Spearman rank correlation. Leukocyte subset frequencies were obtained as the product of complete blood leukocyte counts and determination of subset percentages by flow cytometry ($CD3^+$ T lymphocytes or $CD3^+/CD4^+$ T lymphocytes). **Solid circles** = fatigued breast cancer survivors; **open circles** = non-fatigued breast cancer survivors. Relationships between serum IL-1ra concentrations were statistically significant, as indicated. Neither serum neopterin nor sTNF-RII concentration showed a statistically significant relationship to prevalence of any leukocyte subset (data not shown). All statistical tests were two-sided.

It has long been known that some physiologic responses to infection such as fever originate in brain structures that receive input from circulating cytokines *(25).* In the past three decades, it has also become clear that inflammatory mediators can regulate more complex central nervous system and behavioral processes including affective, motivational, and cognitive variables *(26–28).* Results presented in this paper lead us to establish the hypothesis that subclinical immunologic alterations may underlie cancer-related fatigue syndromes. Priority should be given to larger studies that specifically address this hypothesis.

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NOTES

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