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Psychosocial Adaptation and Cellular Immunity in Breast Cancer Patients in the Weeks After Surgery: An Exploratory Study

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Abstract

Background—The period just after surgery for breast cancer requires psychosocial adaptation and is associated with elevated distress. Distress states have been associated with decreased cellular immune functioning in this population, which could have negative effects on physical recovery. However little is known about relations between psychological status (negative and positive mood states and overall quality of life) and cellular signaling cytokines that could account for these associations in women undergoing treatment for breast cancer.

Methods—The present study examined associations between psychological adaptation indicators (mood, quality of life) and T-helper cell-type 1 (Th1) cytokine production from stimulated peripheral mononuclear cells in women who had recently undergone surgery for early-stage breast cancer but had not yet begun adjuvant therapy. These associations were evaluated while controlling for relevant disease/treatment, sociodemographic and health behavior covariates.

Results—Lower anxiety related to greater production of the Th1 cytokine interleukin-2 (IL-2) while greater positive mood (affection) related to greater production of the Th1 cytokines IL-12 and interferon-gamma (IFN- γ). Better quality of life (QOL) related to greater production of the Th1 cytokine, tumor necrosis factor-alpha (TNF- α).

Conclusion—Individual differences in psychosocial adaptation in women with breast cancer during the period after surgery relate to biological parameters that may be relevant for health and well-being as they move through treatment.

KEY TERMS

breast cancer; Th1 cytokines; mood; quality of life; anxiety

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Introduction

Over 200,000 women in the United States are diagnosed with breast cancer annually (1). This diagnosis and subsequent medical treatment can be stressful for women at a number of levels (2,3). There is also considerable distress surrounding medical procedures, (i.e., surgical mastectomy or lumpectomy), and adjuvant therapies (i.e., chemo- and radiation therapy) (4–8). Throughout this process, the women confront a variety of personal, psychological, and physical losses (9–14), which may affect their ability to adjust, a process that we have referred to as psychosocial adaptation (15). Recent work suggests that mood and quality of life in the months after diagnosis and surgery for breast cancer may be a harbinger for psychological well-being decades later (16), though effects on future physical health and well-being are less clear.

Attempts to examine a biobehavioral mechanism to explain the oft-noted association between psychosocial factors and disease outcomes in breast cancer patients several studies have shown a relation between distress and other aspects of psychosocial functioning and decrements in immune functioning in patients at varying points in treatment (for review see 17). Andersen et al. (18) reported, that high distress in newly diagnosed post-surgical breast cancer patients correlated with lower proliferation of T cells in response to anti-CD3 stimulation and lower natural killer cell cytotoxicity (NKCC) with or without IFN- γ activation. Breast cancer patients undergoing different types of psychosocial interventions designed to reduce distress have shown improved T cell proliferation, mixed lymphocyte reaction (MLR), and NKCC and reduced cortisol levels (19–21). Less is known about the signaling molecules that mediate these effects of stress and stress reduction in the context of breast cancer though some work suggests that neuroendocrine-cytokine interactions may play a role (22).

The effects of stressors and distress states such as depression and anxiety have been known to be immunosuppressive and anti-inflammatory in nature for many years (23,24). Associations between the distress states and immune parameters have been suggested to be explained by stress responses that involve activation of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of this system in response to different stressors either internally (i.e., blood-borne) or externally (i.e., psychological perception through the neurosensory system), can lead to the release of corticotrophin-releasing hormone (CRH) from the hypothalamus and ultimately the systemic secretion of glucocorticoids, like cortisol, which tend to down-regulate cellular immune responses (25–27).

Glucocorticoids can also affect the transcription of many cytokines, generally down-regulating pro-inflammatory (Th1) cytokines and upregulating anti-inflammatory (Th2) cytokines (28). Th1 immunity is characterized by secretion of cytokines such as IFN- γ , IL-2, TNF- α and IL-12, which promote differentiation of macrophages, NK cells and cytotoxic T cells. These cells are involved in the destruction of invading pathogens, as well as the antitumor response. The modulation of the expression of IL-12 or its receptor on T and NK cells is thought to be a major mechanism by which glucocorticoids mediate the Th1-Th2 switch (28).

Natural Killer (NK) cells are a distinct subset of large granular lymphocytes that have the ability to spontaneously kill virally infected or tumor cells expressing low major histocompatibility complex class I molecules (MHC-I) in the absence of any stimulation. NK cells lack the T cell receptor complex and lyse target cells through receptors that do not recognize antigen in the context of class I MHC. NK cells are an important part of the innate immune response not only by eliminating virus infected cells and tumors but also by secreting cytokines upon activation, important for the development of cellular immune responses (29). Distress states have been associated with decreased NK cell activity and this is believed to be mediated in part by increases in cortisol and other corticosteroids, which have an inhibitory effect on NK cytotoxic

activity in humans (30–38). Importantly these associations are thought to be orchestrated through downregulation of the IL-12 receptor on these cells (32), as well as through downregulation of the surface expression and function of triggering receptors involved in NK cell cytotoxicity (33–34). Th1 cytokines such as IL-2 and IFN- γ are important in activating NK cells, as well as Lymphokine Activated Killer (LAK) cells and T cytotoxic cells, which are all believed to be important in cancer surveillance.

In sum, prior studies have related greater levels of distress to poorer cellular immune functioning (e.g., lymphocyte proliferation and cellular cytotoxicity) in women with early stage breast cancer, yet little is known about how mood states and psychosocial adaptation indicators relate to the ability of cells to produce Th1 cytokine in this population. The present study examined the association between indicators of psychosocial adaptation (mood, quality of life), and Th1 cytokine production in women who had recently undergone surgery for early-stage breast cancer but had not yet begun adjuvant therapy. We hypothesized that better psychosocial adaptation (reflected in less self-reported negative mood and more positive mood, and better quality of life) would relate to greater production of Th1 cytokines by stimulated PBMCs. Because active treatment for breast cancer introduced many potential confounds on both psychological and immunological indicators, these associations were evaluated while controlling for relevant disease, treatment and health behavior covariates.

Materials and Methods

Patient and Sample Collection

A total of 134 women with early/mid-stage breast cancer (Stage 0–III) were recruited from private practitioners' offices in the weeks after receiving surgical mastectomy or lumpectomy for non-metastatic breast cancer. The distribution of the patients by disease stage was: 25 patients in Stage 0, 58 patients in Stage I, 45 patients in Stage II and 6 patients in Stage III. Women had from 0 – 19 positive lymph nodes (57.5 % had no positive nodes), 39.5% had undergone mastectomy, 41% had lumpectomy (this data was missing on 19.4 % of cases), while 28.4 % had received reconstructive surgery. Among women who reported their menopausal status, 42.5% were pre-menopausal, 9.7% peri-menopausal, and 28.4% post-menopausal. In terms of demographic characteristics women were aged 25–69 years (mean age 49.5 yrs, SD = 7.63 years), nearly the majority were married or partnered (48.5%) and most employed (66.4%). Women reported a mean of 15.8 yrs of education and an average annual income of \$76,400 (SD = 49, 316). The major race/ethnic groups represented included 53.7 % non-Hispanic White, 18.7 % Hispanic White, and 5.9% Black, non-Hispanic). Of these 134 women we obtained psychosocial data on 108 cases¹. Sample sizes for cytokine analyses vary considerably and range from 67 to 124² (see Table 1).

Blood samples (30–40 ml) were taken in the weeks after surgery, before adjuvant therapy started. We chose this period to avoid the effects of adjuvant therapies on immune parameters. It was also a point at which women were likely to be experiencing significant levels of anxiety and multiple concerns about impending adjuvant therapy (6). Since surgery itself could have

¹All women were part of a larger previously published trial of cognitive behavioral stress management effects on quality of life (15). Only women who had provided blood samples from that prior trial were included in the present study. Women from the parent study who did not provide blood samples (and were thus not included in this substudy) were more likely to have presented with a greater stage of disease ($p < .001$), and with a greater number of positive lymph nodes than women in the present study, $p < 0.01$. Finally, women from the parent study who did not provide blood samples were more likely to have been unemployed than those in the present study, $p = 0.01$. Despite these differences in disease status, there were no differences between women in the parent study who did not provide samples vs those who participated in the present study for any other clinicopathological (e.g., ER/PR status), demographic (e.g., age, education, marital status) or psychosocial variables.

²Sample sizes for cytokines varied due to the following reasons. We initially ran assays for IFN- γ and IL-2 to represent Th1 cytokines, but had to exclude a few IL-2 measurements when our kinetic studies showed a different optimal time. Subsequently, we expanded the Th1 cytokine panel to include TNF- α and IL-12.

affected immune parameters we carefully examined surgery dates when we characterized the sample. Although we had attempted to restrict recruitment to women who were 4 – 8 weeks post surgery there were some women who fell outside of this range. Approximately 59.8% of the sample fell within 4 – 8 weeks of surgery, 26.2% fell before 4 weeks and 14% fell after 8 weeks. Importantly, days since surgery was controlled for in analyses of associations between psychosocial and immunological variables. Blood was drawn in heparinized Vacutainer tubes (Becton-Dickinson, USA) between 4:00 pm and 6:00 pm, in order to avoid circadian variations, kept overnight at room temperature and then processed the following morning. Our previous studies showed that the immune assays measured here did not vary between samples immediately processed and those processed after holding overnight at room temperature.

Isolation of Peripheral Blood Mononuclear Cells (PBMC)

Blood was separated on Ficoll density gradient (Lymphocyte Separation Medium, ICN Biochemicals, USA). PBMC were collected from the gradient interface, washed twice with Phosphate Buffered Saline (PBS) (Gibco-BRL, USA) and resuspended in RPMI-1640 (Gibco-BRL, USA), supplemented with 10% fetal bovine serum, 100 U/ml penicillin (Gibco-BRL, USA), 100 ug/ml streptomycin (Gibco-BRL, USA), 1mM Sodium Pyruvate (Gibco-BRL, USA), 1mM non-essential amino acids (Gibco-BRL, USA) and 5×10^{-5} mM 2-mercaptoethanol, referred to as complete tissue culture media (cRPMI). Cell counts were performed by 0.4% Trypan blue dye exclusion and viability was always higher than 90%. All assays were performed with fresh (not frozen) samples (within 20 hours after the blood was drawn).

Phenotype Analysis

To examine whether our results were affected by the proportion of different cell types, PBMCs were stained and analyzed by flow cytometry. Three-color immunofluorescence was used to analyze total T cells (CD3+ CD19–), T helper (CD3+ CD4+), T cytotoxic (CD3+ CD8+), NK (CD56+CD3–) and B cells (CD19+). Antibodies used were: anti-CD3 conjugated with phycoerythrin-cyanine 5 (PC5), anti-CD4 conjugated to phycoerythrin (PE), anti-CD8 conjugated to fluorescein isothiocyanate (FITC), anti-CD19 conjugated to FITC and anti-CD56 conjugated to PE. Mouse IgG₁ conjugated to PE, PC5 and FITC were used for isotype controls (all antibodies were from Beckman-Coulter, USA). Briefly, 5×10^5 cells PBMC were labeled with antibody according to manufacturer's instructions in 50 µl of FACS buffer/tube (HBSS with 0.02% sodium azide and 0.1% BSA) and incubated in the dark on ice for 30 minutes; cells were washed once with ACK buffer and finally with FACS buffer then fixed with 0.25% paraformaldehyde and stored at 4°C in the dark until analyzed (1–2 days). Analyses were performed in a Becton Dickinson FACScan (San Jose, CA) equipped with FACScan research software.

Cytokine Production

PBMCs at a concentration of 2×10^6 cells/ml were stimulated with plate-bound anti-CD3 antibody (OKT-3, 1 µg/ml, from eBioscience) routinely or plate bound anti-CD3 plus soluble anti-CD28 (PharMingen, 2 µg/ml) in initial conditions and supernatants were collected at different times of stimulation, then frozen at – 20 °C until analysis. IL-2, IFN-γ, IL-12, and TNF-α were all measured using ELISA kits following the manufacturer recommendations, optimal conditions for stimulation and supernatant collection were established for each cytokine³. Kinetic experiments were performed to establish the optimum time point for

³We ran kinetic tests of cytokine secretion in response to CD3 stimulation. For the following cytokines, optimal stimulation time was 48 hours: IFN-γ, IL-12, and TNF-α; for IL-2 the optimal stimulation time was 24 hours (data available upon request). For all the cytokines there were no significant differences at optimal levels of cytokine secretion between anti-CD3 stimulation alone and anti-CD3 plus anti-CD28 co-stimulation for the PBMC samples (data available upon request).

collection and evaluation of the supernatants. The time points obtained (48 hrs for the cytokines IFN- γ , TNF- α , and IL-12, and 24 hours for IL-2) are in agreement with previous reports for healthy controls assayed under similar conditions (48–50). All kits were from Biosource International except for IL-2 (R and D Systems).

Psychosocial Adaptation Indicators

Positive and negative mood—We measured a range of emotional responses, both positive and negative, with the Affects Balance Scale (ABS; 39). This measure incorporates scales to assess negative affects of depression, hostility, guilt, and anxiety and scales to measure positive emotions of affection, contentment, vigor, and joy over the past week. Positive and negative subtotals were also computed. Items are self-descriptive adjectives, and respondents indicate the extent to which they have been feeling the emotional quality the item portrays. The reliability of ABS subscales is $\alpha > .85$ for all scales.

Quality of life—The Functional Assessment of Cancer Therapy–Breast Module (FACT-B; 40) assesses quality of life (QOL) across multiple domains. The instructions asked participants to indicate to what degree each statement has been “true” during the past week. The 5-point scale ranges from “Not at All” to “Very Much”. The FACT-B has been used extensively to assess post-treatment QOL in cancer patients and has demonstrated robust reliability and validity. In our current work, the FACT-B has had high internal consistency ($\alpha = .92$) in samples of women undergoing treatment for Stage I – III BRCA.

Statistical Analysis

All variables were analyzed for outliers and normality. The logarithmic transformation was applied to all immunologic variables to achieve normal distributions. The transformed variables were then used in all calculations. The Pearson Product-Moment correlation coefficient and multiple regression analyses were used to compute the associations between psychosocial and immune variables. Statistics were run using SPSS version 14 (Chicago, IL).

The following covariates were tested for their relationships with the psychosocial and immunologic variables: Disease stage, surgical procedure type, time since surgery, radiation therapy, chemotherapy, taking tamoxifen/aromatase inhibitors, Estrogen Receptor (ER) status, Progesterone Receptor (PR) status, hormone replacement therapy, age, income, ethnicity (coded Black, White, Hispanic), and reconstructive surgery. We also tested for relations with health behaviors such as caffeinated beverages, alcohol use, recreational use (marijuana and cocaine), cigarette smoking, and moderate physical exercise/activities (e.g., golf, doubles tennis, yard work, brisk walking, etc). Criteria for the use of a covariate in subsequent partial correlation analyses were those covariates significantly correlated with immunologic variables at $p < .05$. As a conservative strategy we also conducted multiple regression analyses relating psychosocial adaptation measures with immune parameters after controlling for all of these potential confounders. To minimize Type I error we only conducted regression analyses only on immunologic variables shown to be associated with psychosocial adaptation variables in preliminary analyses.

Results

Correlations Among Psychosocial and Immunologic Variables

Means and standard deviations for all psychosocial variables and cytokines are included in Table 1. Descriptive statistics for lymphocyte subpopulations are shown in Table 2. For the main analyses we computed associations between two types of psychosocial variables—mood, and quality of life—and the immune values.

Mood—Greater anxiety related to less IL-2 production, while higher anger scores were related to lower TNF- α production. On the other hand greater levels of affection, a measure of positive affiliative emotions, were associated with greater IFN- γ and IL-12 production. (see Table 3). Given these preliminary findings four multiple regression analyses were conducted between mood variables and cytokine indicators. Three regression analyses produced significant equations (Table 4). In each case we controlled for medical (stage, surgical procedure, and presence/absence of adjuvant therapy), demographic (age, income, ethnic group), and behavioral (cigarette smoking, alcohol use) controls. We found that greater levels of anxiety were associated with less production of IL-2 from stimulated PBMCs, $F(1,49) = 4.29, p = .044$. On the other hand greater levels of affection predicted greater IL-12 production, $F(1,47) = 4.89, p = .032$, and greater IFN- γ production, $F(1,78) = 4.79, p = .032$. There were no associations between mood and cell phenotypes.

Quality of life—We then examined correlations between FACT-B scores and immune variables. (Table 3). Greater FACT-B scores, indicating better quality of life, related to higher TNF- α levels. After controlling for medical, demographic and health behavior variables, greater FACT-B scores were associated with greater TNF- α responses in a multiple regression analysis, $F(1,47) = 8.20, p = .006$. (Table 5). No other associations were significant between FACT-B and the production of other cytokines. Also there was no association between the FACT-B and any cell phenotypes measured.

Discussion

This exploratory study describes cross-sectional associations between selected indicators of psychosocial adaptation and immunologic parameters in women who recently completed surgery for early-to-mid-stage breast cancer, a group that is under considerable stress from the physical and psychological effects of surgery and the anticipation of adjuvant therapy. We previously documented that this group of women reports many concerns at this point in time including fear of recurrence and concerns about the physical effects of adjuvant therapy (41), and significant disruptions in quality of life (42). Importantly, better psychosocial adaptation during breast cancer treatment predicts greater well-being up to 13 years after treatment (16).

While much is known about the psychosocial aspects of dealing with the stress of diagnosis and treatment for breast cancer, less is known about how individual differences in these psychosocial experiences relate to biological parameters that may contribute to disease outcomes. Th1 cytokine regulation may vary as a function of psychosocial stress factors, which may account in part for the associations previously noted between psychosocial factors and cancer outcomes (for review see 22). Animal studies have confirmed that experimental stressors are associated with lower NK cell cytotoxicity on the one hand and increased tumor progression on the other (43). After surgical treatment of tumors, immune function may be important in eliminating residual disease and micrometastases and some have suggested that intact perioperative immune function is involved in tumor control (44). This has led some to suggest the value of identifying stressed surgical cancer patients who might benefit from stress reduction interventions as a means to improve longer-term outcomes (45).

In one prior clinical study greater levels of cancer-specific anxiety were associated with lower levels of NK cell cytotoxicity in women recently treated for Stage II-III breast cancer (18). No prior work in humans has examined associations between other psychosocial variables such as mood and QOL and more specific indicators of immune functions that relate to cytokine signaling in women undergoing treatment for breast cancer. The purpose of this study was to report associations between mood and QOL on the one hand, and Th1 cytokine production on the other, in a sample of women who had received surgery for early-to-mid-stage disease within the past several weeks but who had not yet begun adjuvant therapy. Other work in the field has

established associations between psychosocial factors and lymphocyte proliferation in breast cancer patients (46,47) though less is known about how psychosocial factors relate to the production of important signaling molecules that are produced following lymphocyte stimulation.

Results of the analyses of psychosocial adaptation factors suggest that mood states and QOL indicators generally reflecting better adaptation were associated with several of the immune parameters measured in this cohort of breast cancer patients. In general, lower levels of negative mood state of anxiety was associated with greater production of the Th1 cytokine IL-2 while greater levels of positive mood states such as affection were associated with greater production of the Th1 cytokines IL-12 and IFN- γ . To our knowledge this is the first study to show associations between greater positive mood states and Th1 cytokine production in women being treated for breast cancer. The fact that greater levels of affection were associated with greater Th1 cytokine production suggests the importance of both positive mood states and the quality of personal relationships in this population. Since prior work from our group has shown that stress management can increase reports of positive affect and positive social interactions in women being treated for breast cancer (15) future studies should examine how changes in positive affect and social relationships during stress management relate to alterations in cytokine production in these women as they move through treatment.

Interestingly, the pattern of results suggests that association observed between negative mood and Th1 cytokine production involved activated mood states (anxiety), which may be more likely to associate with elevated sympathetic nervous system hormones like norepinephrine (NE), which has been associated with immune alterations and other tumor-promoting changes (22,51). Specifically, NE has been shown to inhibit Th1 cytokine secretion, target binding and apoptotic programming (52). We plan to collect information on NE output in the future. Regardless of the biobehavioral pathways implicated, the present work is among the first to show a specific association between lower negative mood and greater positive mood and the ability of PBMCs of breast cancer patients to produce Th1 cytokines upon stimulation. Studies in ovarian cancer patients have shown associations between depressed and anxious mood, and lower ratios of IFN- γ vs. IL-4 production in T-helper and T-cytotoxic lymphocytes from peripheral blood and the tumor microenvironment (tumor-infiltrating lymphocytes and ascites), among ovarian cancer patients on the day of surgery (53). Other studies have shown increased perceived stress, anxiety, and mood disturbance in women undergoing breast biopsy, accompanied by a persistent reduction in natural killer cell activity and IFN- γ production, as well as an increased production of IL-4, IL-6 and IL-10 (54). We also found that greater breast cancer - specific quality of life was associated with greater production of the Th1 cytokine TNF- α . In women with advanced-stage ovarian cancer, Costanzo et al. found associations between psychosocial factors such as social attachment and quality of life, and plasma levels of IL-6 before surgery (55). We are unaware of prior studies demonstrating associations between specific quality of life indicators and cytokine production in early-stage breast cancer patients after surgery. These findings converge on a pattern of better mood status and quality of life—indicators of psychosocial adaptation—being associated with greater Th1 production in these women undergoing treatment for breast cancer. Future work examining the effects of psychosocial interventions that modulate quality of life and indicators of psychosocial adaptation in women with breast cancer (46,47,56) in the peri-surgical period may show parallel effects on Th1 cytokine regulation that could have implications for disease outcomes and well-being over time (44).

The present findings should be considered in view of a few caveats. The women recruited for this study were a convenience sample that is not necessarily reflective of all women undergoing treatment for breast cancer. In the present study women were required to provide blood samples for assays. Analyses of this subsample of women versus those who participated in the parent

study examining the effects of psychosocial intervention (15) revealed that the present sample represented a group with less advanced disease than those women from the parent sample who did not provide blood samples. This difference may be due to the fact that women with more advanced disease were likely to have received neo-adjuvant (pre-surgery) treatments, which excluded them from the present substudy of biological outcomes. These advanced cases are also more likely to have been unemployed due to health reasons. These factors singly or in combination may have made them less willing or able to provide blood samples for the study. Thus caution is in order in generalizing the present results to all women being treated for non-metastatic breast cancer. Generally, the present sample tended to be largely White, middle-class and well-educated women who were receiving surgery at private practices in the study area. Future studies will need to recruit women across different ethnic groups and a broader range of socioeconomic status who may be receiving their treatments at public hospitals and community clinics. Also there is the fact that almost 40 % of the blood samples were obtained outside the time frame of 4 to 8 weeks after surgery. Although this variable was controlled for in our analyses, the wide range of days since surgery might have lead us to underestimate our findings on associations between psychosocial and immunological variables. Future studies should attempt to minimize this range by restricting recruitment to a shorter time frame.

In summary, this work indicates that greater negative mood states (anxiety) correlate with lower Th1 cytokine (IL-2) production, while greater positive mood (affection) correlates with higher Th1 (IL-12, IFN- γ) cytokine production. Better quality of life was also associated with greater Th1 cytokine production. These findings support the hypothesis that achieving better psychosocial adaptation in women with breast cancer under treatment may contribute to their health status by affecting cell-signaling molecules that orchestrate immunologic responses. It is reasonable to hypothesize that psychosocial interventions that increase positive adaptation by changing individuals' appraisals of stress (cognitive behavioral-based interventions) in order to decrease negative mood states and increase positive mood and perceived quality of life during the period of active treatment for breast cancer might preserve immune system functioning as they begin adjuvant therapy. Such interventions may promote physiological "recovery" after surgery in order to interrupt the "window of opportunity" for micro-metastases to progress into full-blown recurrence of disease (17,45). Longitudinal studies tracking psychosocial adaptation and immune parameters across the recovery period after surgery and during and after adjuvant therapy may provide insight into biobehavioral processes that can explain the influence of psychosocial functioning on health outcomes in women treated for breast cancer.

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Table 1

Descriptive Statistics For Study Variables.

	N	Range	Minimum	Maximum	Mean	Std. Deviation
FACT-B	108	23.11	3.89	27.00	18.41	4.44
ABS-Neg Affect	108	2.75	1.00	3.75	2.08	.55
ABS-Pos Affect	108	3.65	1.35	5.00	3.36	.62
ABS-depressed	108	3.20	1.00	4.20	1.99	.66
ABS-guilt	108	2.40	1.00	3.40	1.69	.61
ABS-anger	108	2.60	1.00	3.60	1.96	.62
ABS-happy	108	4.00	1.00	5.00	3.39	.69
ABS-content	108	4.00	1.00	5.00	3.37	.70
ABS-vigor	108	3.80	1.20	5.00	3.07	.76
ABS-affection	108	3.20	1.80	5.00	3.61	.64
ABS-anxiety	108	3.40	1.00	4.40	2.70	.70
Log γ -IFN	124	4.89	.34	5.23	3.60	.88
Log-TNF- α	68	1.93	2.53	4.46	3.73	.46
Log IL-2	84	2.13	.85	2.98	2.12	.50
Log IL-12	67	2.62	.43	3.05	2.46	.50

Abbreviations: FACT-B (Functional Assessment of Cancer Treatment)-Breast Cancer version, ABS (Affect Balance Scale), IL-2 (Interleukin-2), γ -IFN (interferon-gamma), TNF- α (tumor necrosis factor-alpha)

Table 2
Descriptive Statistics For Lymphocyte Subpopulations (%)^a

	N	Minimum	Maximum	Mean	Std. Deviation
CD3+ (T cells)	128	55.80	92.90	75.14	7.19
CD4+ (T helper cells) ^a	125	34.40	81.70	59.98	9.82
CD8+ (T cytotoxic cells)	129	2.70	31.40	10.97	5.93
CD56+/CD3- (NK cells)	131	2.20	24.40	9.42	4.02
CD19+ (B cells)	131	1.60	30.90	8.78	4.84
CD3+/CD56+ (NK-T cells)	78	0.10	16.80	3.40	3.02

^a A comparison of the mean values of lymphocyte subpopulations in our study sample agrees with reference ranges previously reported for normal healthy adults (57), except for a slight increase in the percent of CD4+, and a more significant decrease in the percent of CD8+. An increased CD4+/CD8+ ratio in peripheral blood from early breast cancer patients post-surgery, compared to healthy controls, has been reported by Nicolini et al. (58). These investigators hypothesize that the localization of circulating CD8+ cells at the site of micrometastases could be the reason for an increased CD4+/CD8+ ratio in their apparently disease-free patients.

Table 3

Associations Between Psychosocial Adaptation Variables and Immunologic Indices.

Variables	r^a	When controlling for:
ABS Negative Composite X NKCC	.21	
ABS Affection X γ -IFN	.21	
ABS Affection X IL-2	.25	PR status: .27*
ABS Anxiety X IL-2	-.26	
ABS Anger X TNF- α	-.31	
FACT-B X TNF- α	.30	

^a All r values are statistically significant at $p < .05$.

Abbreviations: FACT-B (Functional Assessment of Cancer Treatment)-Breast Cancer version, ABS (Affect Balance Scale), IL-2 (Interleukin-2), γ -IFN (interferon-gamma), TNF- α (tumor necrosis factor-alpha), PR (progesterone receptor)

Table 4
Multiple Regression Relating Mood To Th1 Cytokine Production From Stimulated PBMCs

$\text{Log}_{10} \text{IL-2}$	R^2	β	t	p	Equation
Stage		.040	.285	.777	
Surgical Procedure		-.101	-.741	.462	
Adjuvant Therapy		-.114	-.777	.441	
Time Since Surgery		.202	1.410	.165	
Income		-.118	-.862	.393	
Ethnic Group		-.054	-.378	.707	
Age		-.072	-.507	.615	
Alcohol Use		-.273	-1.947	.057	
Cigarette Smoking		.095	.622	.537	
Anxiety	.239	-.283	-2.071	.044	$F(1,49) = 4.29, p = .044$
$\text{Log}_{10} \text{IL-12}$					
Stage		-.127	-.810	.422	
Surgical Procedure		.145	.962	.341	
Adjuvant Therapy		-.075	-.489	.627	
Time Since Surgery		-.081	-.528	.600	
Income		-.076	-.540	.592	
Ethnic Group		-.071	-.499	.620	
Age		-.152	-.956	.344	
Alcohol Use		-.023	-.161	.873	
Cigarette Smoking		.024	.174	.863	
Affection	.157	.344	2.211	.032	$F(1,47) = 4.89, p = .032$
$\text{Log}_{10} \text{IFN-}\gamma$					
Stage		.091	.783	.436	
Surgical Procedure		-.071	-.632	.529	
Adjuvant Therapy		-.130	-1.104	.273	
Time Since Surgery		.052	.457	.649	
Income		.076	.700	.486	
Ethnic Group		-.044	-.397	.692	
Age		.050	.432	.667	
Alcohol Use		-.205	1.831	.071	
Cigarette Smoking		.200	1.693	.094	
ABS-Affection	.154	.251	2.188	.032	$F(1,78) = 4.79, p = .032$

Abbreviations: FACT-B (Functional Assessment of Cancer Treatment)-Breast Cancer version, ABS (Affect Balance Scale), IL-2 (Interleukin-2), γ -IFN (interferon-gamma)

Table 5
Multiple Regression Analyses Relating FACT-B scores To Th1 Cytokine Production From Stimulated Peripheral Mononuclear Cells.

Log ₁₀ TNF- α	R ²	β	t	p	Equation
Stage		.270	1.757	.086	
Surgical Procedure		.000	.000	1.000	
Adjuvant Therapy		-.375	-2.543	.014	
Time Since Surgery		-.062	-.468	.642	
Income		.137	1.097	.278	
Ethnic Group		.047	.334	.740	
Age		.120	.812	.421	
Alcohol Use		.090	.679	.501	
Cigarette Smoking		.155	1.250	.218	
FACT-B	.307	.465	2.864	.006	$F(1,47) = 8.20, p = .006$

Abbreviations: FACT-B (Functional Assessment of Cancer Treatment)-Breast Cancer version, TNF- α (tumor necrosis factor-alpha)