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Stress and Immune Responses After Surgical Treatment for Regional Breast Cancer

Barbara L. Andersen*,
Department of Psychology, Institute for Behavioral Medicine Research, and Comprehensive Cancer Center, The Ohio State University, Columbus.

William B. Farrar,
Department of Surgery, College of Medicine, and Comprehensive Cancer Center, The Ohio State University, Columbus.

Deanna Golden-Kreutz,
Department of Psychology, The Ohio State University, Columbus.

Leigh Ann Kutz,
Department of Medical Microbiology and Immunology, College of Medicine, The Ohio State University, Columbus.

Robert MacCallum,
Department of Psychology and Institute for Behavioral Medicine Research, The Ohio State University, Columbus.

Mary Elizabeth Courtney, and
Department of Psychology, The Ohio State University, Columbus.

Ronald Glaser
Department of Medical Microbiology and Immunology, Institute for Behavioral Medicine Research, College of Medicine, and Comprehensive Cancer Center, The Ohio State University, Columbus.

Abstract

Background: Adults who undergo chronic stress, such as the diagnosis and surgical treatment of breast cancer, often experience adjustment difficulties and important biologic effects. This stress can affect the immune system, possibly reducing the ability of individuals with cancer to resist disease progression and metastatic spread. We examined whether stress influences cellular immune responses in patients following breast cancer diagnosis and surgery.

Methods: We studied 116 patients recently treated surgically for invasive breast cancer. Before beginning their adjuvant therapy, all subjects completed a validated questionnaire assessing the stress of being cancer patients. A 60-mL blood sample taken from each patient was subjected to a panel of natural killer (NK) cell and T-lymphocyte assays. We then developed multiple regression models to test the contribution of psychologic stress in predicting immune function. All regression equations controlled for variables that might exert short- or long-term effects on these responses, and we also ruled out other potentially confounding variables.

Results: We found, reproducibly between and within assays, the following: 1) Stress level significantly predicted lower NK cell lysis, 2) stress level significantly predicted diminished response

**Correspondence to:* Barbara L. Andersen, Ph.D., Department of Psychology, The Ohio State University, 1885 Neil Ave., Columbus, OH 43210-1222. Andersen.l@osu.edu.

of NK cells to recombinant interferon gamma, and 3) stress level significantly predicted decreased proliferative response of peripheral blood lymphocytes to plant lectins and to a monoclonal antibody directed against the T-cell receptor.

Conclusions: The data show that the physiologic effects of stress inhibit cellular immune responses that are relevant to cancer prognosis, including NK cell toxicity and T-cell responses. Additional, longitudinal studies are needed to determine the duration of these effects, their health consequences, and their biologic and/or behavioral mechanisms.

A diagnosis of cancer and cancer treatments are objective, negative events in an individual's life. Although negative events do not always produce stress and a lowered quality of life, data from many studies document severe, acute stress at cancer diagnosis (1) and during recovery (2). The negative psychologic responses of individuals with cancer to the diagnosis and treatment are important in their own right because these responses are targets for cancer control efforts (3,4). In addition, data suggest that stress responses are accompanied by nonrandom (i.e., correlated) negative changes in a broad range of immune responses. This study examines from a biobehavioral perspective whether stress influences cellular immunity in women with breast cancer after diagnosis of breast cancer and during the postsurgical period (5).

Meta-analyses (6,7) suggest that psychologic stress and the experience of life stressors are reliably associated with negative immune alterations in noncancer subjects; i.e., “higher” levels of stress (e.g., self-reports of stress or negative affects, such as sadness or clinical diagnoses of depression) are related quantitatively and functionally to “reduced” cellular immune responses, such as lowered natural killer (NK) cell lysis. This effect has been found regularly for individuals in the midst of chronic stressors, and some of the largest responses and changes have been found for lengthy stressors and those that have interpersonal components.

Illustrative data come from Kiecolt-Glaser, Glaser, and colleagues (8-11), who have followed individuals during the long, stressful experience of giving care to a spouse diagnosed with Alzheimer's disease. Not surprisingly, caregivers report high levels of distress and negative affect as they cope with their relative's difficult behavior and mental deterioration (8). Moreover, these researchers have found, for example, that NK cells obtained from caregivers are less responsive to the cytokine recombinant interferon gamma (rIFN γ) and recombinant inter-leukin 2 (rIL-2) than are cells obtained from matched community control subjects (9). In addition, these highly stressed subjects have a poorer proliferative response to mitogens (8), exhibit substantial deficits in the antibody and virus-specific T-cell responses to an influenza virus vaccine (10), and demonstrate stress-related defects in wound repair (11).

There are fewer data on the relationship between stress and immunity among cancer patients. Levy et al. (12) reported on these relationships in 66 women with stage I or II breast cancer 3 months after treatment (lumpectomy or mastectomy with or without adjuvant therapy). In addition to finding that estrogen receptor status predicted NK cell lysis, these researchers found that social support—a variable hypothesized to *reduce* stress—contributed significantly to a regression model predicting *higher* NK cell activity. These findings suggest that how a person responds to stress may also influence how stress, in turn, influences the immune response.

There is considerable evidence that patients with cancer express abnormal cellular immune responses; these abnormal responses have been found in patients with many different types of cancer (13–15), including breast cancer (16,17). Stressors are not generic, and they would not be expected to have identical physiologic outcomes. So too, the immune response involves a cascade of responses and events that can occur over time. For these reasons, we used a homogeneous breast cancer subject sample and timing of assessment to test the relationship between stress and several components of the cellular immune response, including NK cell and T-cell functions.

Women who had been diagnosed with breast cancer and who had undergone surgery for the breast cancer were studied before they began adjuvant therapy. Since we were interested in the contribution of stress in predicting an immune response above and beyond known correlates, we controlled for naturally occurring factors in our statistical analyses that affect the immune responses—specifically, age, disease stage (lymph node status), and recovery (days since surgery) (18). Because the immune system contains a considerable amount of redundancy, we focused on three components that would each provide important, but complementary, information.

First, we measured NK cell lysis. We chose to measure NK cell lysis because those cells are believed to act early in the immune response and they have been demonstrated to play an important role in immune surveillance against tumors and virally infected cells (19–21). Second, we measured the ability of the NK cells to respond to rIFN γ and rIL-2. It has been shown that lymphokine-activated killer (LAK) cells are highly cytotoxic against a wider variety of tumor cells than those lysed by resting NK cells (22), an effect also observed in patients with breast cancer (23). Finally, to obtain information on the T-cell response, we measured the response of peripheral blood leukocytes (PBLs) to two mitogens—phytohemag-glutinin (PHA) and concanavalin A (Con A)—and we induced proliferation by stimulating the T cells with a monoclonal antibody (MAb) to the T-cell receptor.

Subjects and Methods

Patient Eligibility and Data Collection

Participants were 116 women who had been diagnosed with invasive breast cancer and who were surgically treated within the last 4 months but who had not yet begun adjuvant treatment. Women were from 14 to 101 days (mean = 37 days; median = 33 days) after surgery for stage II (70%) or III (30%) invasive breast cancer. We used the American Joint Committee on Cancer and the International Union Against Cancer staging system. The women ranged in age from 31 to 84 years (mean = 52 years). Recruited consecutively from mid-1994 to early 1997, the majority (82%) were being treated at a National Cancer Institute-designated, university-affiliated Comprehensive Cancer Center, and the remainder (18%) were receiving treatment at local community hospitals. All women came to the General Clinical Research Center at the university where psychologic, behavioral, and medical data were collected and a 60-mL blood sample was taken from them. Assessments were conducted between 8:00 AM and 12:00 AM to reduce diurnal variability.

Stress Measure

The Impact of Event Scale (IES) (24) is a standardized self-report questionnaire used to examine intrusive thoughts (“I had dreams about being a cancer patient,” “Other things kept making me think about cancer”) and avoidant thoughts and actions (“I tried not to talk about it,” “I was aware that I still had a lot of feelings about cancer, but I didn't deal with them”) concerning cancer. Fifteen items are used, and women rate each event or feeling in terms of the frequency of occurrence (i.e., “not at all,” “rarely,” “sometimes,” and “often”) during the previous 7 days. Scores range from 0 to 75. For this sample, descriptive statistics were as follows: range, 0–65; mean = 26; median = 25; and standard deviation = 15.2. The scale has satisfactory reliability with internal consistency of .78–.82 and a 2-week test-retest reliability of .79–.89, respectively. The validity of the measure is suggested by data indicating that individuals who experience involuntary, distress-related thoughts following traumatic life events are also those who suffer the greatest negative effects psychologically [e.g., (2)].

Immune Assays

Blood cell separation—PBLs were isolated from 60 mL of venous blood by use of Ficoll gradients (Pharmacia Biotech, Inc., Piscataway, NJ). The isolated leukocytes were then washed in calcium- and magnesium-free phosphate-buffered saline and counted on a Coulter counter (Coulter Corp., Miami, FL). Aliquots of 8×10^6 isolated PBLs were suspended again in 0.8 mL of RPMI-1640 medium supplemented with 10% fetal bovine serum, 0.75% sodium bicarbonate, 2 m*M* L-glutamine, and 10 μg/ mL of ciprofloxacin.

Quantification of total T lymphocytes, T-cell subsets, and NK cells—Isolated PBLs were absorbed with MAbs conjugated to either fluorescein isothio-cyanate or rhodamine according to the cell surface marker being studied: total T cells (CD3, fluorescein isothiocyanate), T4 subset (CD4, rhodamine), T8 subset (CD8, fluorescein isothiocyanate), and NK cells (CD56, rhodamine). All MAbs were purchased from Coulter Corp. Briefly, 0.5×10^6 cells were incubated with the MAb for 15 minutes at room temperature. After the incubation, the cells were fixed, and the red blood cells were lysed with Optilyse C, a buffered solution containing 1.5% formaldehyde, according to the manufacturer's instructions (Coulter Corp.). Samples were analyzed with the use of a Coulter EPICS Profile II flow cytometer as described previously (8).

NK cell cytotoxicity—To determine NK cell activity, a microtiter ^{51}Cr-release cytotoxicity assay was used as described previously (9,25). The target cells used were K-562 cells, an NK cell-sensitive human myeloid cell line. Target cells, labeled overnight for 16 hours with ^{51}Cr, were placed in triplicate wells of 96-well V-bottom plates, and PBLs were added, resulting in effector-to-target (E:T) cell ratios of 100:1, 50:1, 25:1, 12.5:1, and 6.25:1.

NK cell response to cytokines—Procedures for treatment of PBLs with rIFN γ and rIL-2 involved preparing isolated PBLs at a concentration of 3×10^6 cells/mL in complete RPMI-1640 medium and then seeding the cells into three replicate tissue culture tubes (Falcon, Becton Dickinson and Co., Lincoln Park, NJ) at 6×10^6 cells per tube. Cells were incubated in complete RPMI-1640 medium alone or complete medium supplemented with 250 IU/mL rlNF γ or 60 IU/mL rIL-2 (Genzyme, Boston, MA). Cell suspensions were gently mixed and then incubated at 37 °C in an atmosphere of 5% CO_2 for 65 hours. For the assay, triplicate aliquots of cell suspensions were placed in wells of V-bottom plates, with E:T cell ratios of 50:1, 25:1, 12.5:1, 6.25:1, or 3.13:1. In addition, six wells with target cells and medium only and target cells with detergent (5% sodium dodecyl sulfate in phosphate-buffered saline) were prepared to determine spontaneously released chromium and maximal lysis, respectively. The plates were centrifuged at 300g for 5 minutes at 20 °C to bring the effector and target cells into close contact; they were then incubated at 37 °C in an atmosphere of 5% CO_2 for 5 hours. After this incubation, the plates were centrifuged at 300g for 5 minutes at 20 °C, 100 μL of supernatant was collected from each well, and counts per minute were determined by use of a Beckman 9000 gamma counter (Beckman Instruments, Inc., Fullerton, CA) as described previously (9, 26).

Blastogenic response to PHA, Con A, and MAb to the T3 receptor—The concentrations for PHA and Con A used were 2.5, 5.0, and 10.0 μg/mL. To measure the blastogenic response to the MAb to the T-cell receptor, we used the following three dilutions of the purified MAb: 32:1, 64:1, and 128:1. For all three assays isolated, PBLs seeded in triplicate at 0.5×10^5 per well were incubated for 68 hours at 37 °C in 96-well flat-bottomed plates and then labeled for 4 hours with MTS, i.e., 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (Promega Corp., Madison, WI) to measure proliferative response. Briefly, the MTS procedure is a nonradioactive calorimetric procedure that labels metabolically active cells via reduction of a

colored substrate. The amount of proliferation was determined by optical density of the suspension in the well. Optical density determinations were performed by use of a Titertek Multiscan MCC microplate reader (Flow Laboratories, Inc., Finland) at a determination wavelength of 492 nm and a reference wavelength of 690 nm as has been noted (27,28).

Statistical Analyses

Preliminary analyses—Before conducting the principal analyses, we checked the data for the contribution of "nuisance" variables (covariates) that could potentially be related to psychologic stress, immune outcomes, or both [*see* (25) for a discussion]. The variables examined were measures of aspirin, alcohol, caffeine, and nicotine intake; amount of sleep; plasma albumin level (as an indicator of nutritional status); incidence of recent infectious illness; and the Karnofsky performance status rating. We examined the relationships between these variables and each of the three sets of outcome variables: NK cell lysis, ability of NK cells to respond to rlFN γ and rlL-2, and the blastogenic response of PBLs to Con A, PHA, and the T3 MAb. Analysis of variance was used for the categorical independent variables, and simple correlations were used for numerically scaled independent variables.

Screening of these potential covariates involved examination of the relationships between 11 covariates and 20 dependent variables, or a total of 220 bivariate associations. Of these 220 associations, 15 were found to be statistically significant at .05 significance level. This number of significant effects is only slightly more than would be expected by chance alone (i.e., 220 × .05 = 11). Inspection of the significant relationships showed that many of them were attributable to the influence of a few outliers in the data. To be conservative, all of the regression analyses described below were run twice, once including and once excluding those covariates that had significant bivariate associations with the relevant dependent variables. In no case were results of the regression analyses significantly altered by the inclusion of the covariates. Given this fact and the consistently weak relationships of the covariates to the dependent variables, we do not report further results involving the covariates.

Principal analyses—The principal analyses assess the relationship between the IES measure of psychologic stress and the following three sets of outcome measures: 1) NK cell lysis at five E:T ratios, 2) response of NK cells to rlFN γ and rlL-2 stimulation at five E:T ratios each, and 3) the PBL blastogenic response to PHA and Con A and proliferative response to the T3 MAb at three concentrations or dilutions each.

We were interested in the role of stress in predicting these outcomes, over and above the impact of disease and recovery variables on the immune response. Thus, we chose to control for three variables: 1) age, which is associated with down-regulation of the immune system; 2) disease stage, which is an indicator of the extent or burden of disease; and 3) days since surgery, which is an indicator of the degree of recovery from surgical stress and related factors (e.g., anesthesia).

Using hierarchical multiple regression (29), we tested the predictive value of psychologic stress for the measured immune outcomes. This procedure enters variables in a specified sequence and, at the final step, provides a test of the variance of the dependent variable (immune outcome) due to the predictor (stress), above and beyond the contribution of the control variables (age, stage, and days since surgery). In these regression analyses, age, days since surgery, and IES were considered as numerical variables. Stage was a categorical variable with two levels: II versus III.

For all of the analyses described below, any missing data were managed by the pairwise deletion technique, wherein each bivariate association is estimated with the use of all subjects for whom measures on both variables are available. This approach allows for more complete

usage of available data than do alternative procedures (e.g., listwise deletion). For all of the dependent variables except the response of NK cells to rIFN γ, the quantity of missing data was small—with never more than 10 observations missing for any bivariate association. Effective sample sizes for the regression analyses ranged from 113 for the NK cell lysis ratios to 103 for T3 MAb values. For rIFN γ measures, sample sizes varied from 85 to 49 across the range of concentrations employed.

For each analysis, we provided three regression models: models A, B, and C. Model A includes only the control (independent) variables (i.e., age, stage, and days since surgery) in predicting the immune outcome (e.g., NK cell lysis). Predictors in model A were introduced simultaneously because we had no basis for or a strong interest in investigating their effects in any particular sequence. Model B includes the three control variables as well as the psychologic stress variable (IES) in the prediction of the immune outcome. Of particular interest in this analysis was the increment in the squared multiple correlation (R^2) from model A to model B (i.e., R^2_{B-A}), indicating variance in a dependent variable (e.g., NK cell lysis) attributable to stress (IES) beyond that explained by the control predictors. In addition, the standardized regression beta (β) for the psychologic stress variable (IES) in model B (i.e., β_{stress}) indicates the magnitude and direction of the influence of this predictor on the dependent variable. The significance of the β weight was also tested. Finally, model C indicates the contribution of psychologic stress as the lone predictor; this third model provides the simple association between psychologic stress and immune function.

Results

Analyses Predicting NK Cell Lysis

Table 1 provides the results from the three models, A, B, and C, predicting NK cell lysis. For model A, in which age, stage, and days since surgery are the independent variables, R^2_A was small and nonsignificant for every E:T ratio (all F ratios were <1.0). Because the percentage of NK cells available would influence the total NK cell activity as measured by lysis, we next added the percentage of NK cells, as determined by flow cytometry, into the analyses as an additional, independent control variable as shown (model AA). Across all E:T ratios, the R^2_{AA} values suggested that this variable added significant variance, as predicted, yielding R^2_{AA} values ranging from .085 to .250.

More important was the addition of the stress variable (IES) as a predictor, shown in model B. The value of R^2_B for lysis was noticeably larger than that of R^2_{AA}, and it provided a significant increment in prediction across the E:T ratios. These data indicate that the measure of psychologic stress that was used accounted for significant variance in NK cell lysis above and beyond that explained by age, stage, days since surgery, and percentage of NK cells. Moreover, the sign of the β regression coefficient for IES was negative, as predicted, indicating that an increase in measured stress was associated with a decline in NK cell lysis. The *t* tests for these coefficients were significant at five of the six E:T ratios. Also, no other predictor in model B had a significant regression coefficient.

We also provide the regression results when only IES was used as a predictor, eliminating the control predictors from the model (model C in Table 1). These results showed that the simple association between IES and NK cell lysis was statistically significant at five of the six E:T ratios.

Analyses Predicting Response of NK Cells to Cytokines

Results for the NK cell response to rIFN γ are provided in Table 2 and show a similar pattern. For model A, which used age, stage, and days since surgery as the independent variables, the

value of R^2_A was small to moderate, ranging from .025 to .138. When stress (IES) was added to the model B regression, the R^2 values were statistically significant at all but one E:T ratio (50:1). Furthermore, the increments in the prediction due to IES, $R^2_{B–A}$, were significant and ranged from .054 to .119. This value reflects the proportion of variance in the cell response accounted for by stress (IES) beyond that explained by the control variables. Again, the negative weight of β for IES in model B indicated a negative influence of psychologic stress on the response of the NK cells to rIFN γ. Again, no other predictor in model B had a significant regression coefficient. Finally, the results for model C in Table 2 showed a simple association between IES and the rIFN γ response. These correlations were significant at four of the five E:T ratios; the proportions of variance accounted for were in the range of .077 to .149.

We attempted to calculate a parallel set of regressions for the response of NK cells to rIL-2. However, cells from a large proportion of the patients (62%) had no response to rIL-2. When the regressions were conducted on data obtained from the remaining patients (38%), the addition of stress (IES) in model B produced a significant R^2 value at the 25:1 E:T ratio only. It appeared that the majority of the subjects' NK cells did not respond to treatment with rIL-2.

Analyses Predicting Blastogenic Response of PBLs to Con A, PHA, and the T3 MAb

Table 3 shows regression results for the Con A and PHA blastogenic responses across three concentrations each. Because the findings are similar for both assays, they will be discussed together. For model A, which used age, stage, and days since surgery as the independent variables, the value of R^2_A for Con A ranged from .035 to .054 and was of similar magnitude for PHA, ranging from .022 to .033. Since the number of total T cells available will affect the blastogenesis values, we next added the number of T3-positive cells into the analyses as an additional, independent control variable as shown by the step model AA. Across all concentrations for each mitogen, the value of R^2_{AA} suggested that this variable added variance, yielding the R^2_{AA} values ranging from .105 to .125 for Con A and from .023 to .033 for PHA.

The addition of stress (IES) to the regression for blastogenesis added significant variance, as indicated in model B. All of the R^2 values were statistically significant. Considering the increments in R^2 due to stress (IES), these were significant and ranged from .032 to .061 for Con A and from .047 to .060 for PHA, reflecting the proportion of variance in the blastogenesis accounted for by IES beyond that explained by the control variables. Again, the negative β weights for IES in model B indicated a negative influence of psychologic stress on the blastogenic responses across concentrations. Moreover, no other predictor in model B had a significant regression coefficient. Finally, results for model C in Table 3 showed a simple association between stress (IES) and the blastogenic response. These correlations were significant for each concentration of Con A and PHA.

Table 4 shows regression results for the proliferative response of T cells to three different dilutions of the T3 MAb. For model A, the control R^2 values were not significant for any dilution. Addition of number of T3-positive cells available as a control increased the variance accounted for as shown by the step model AA. The R^2_{AA} values ranged from .088 to .143. However, increments in R^2 due to the addition of stress (IES), as shown by $R^2_{B–AA}$, were significant, ranging from .056 to .067. This indicates that about 6% of the variance was accounted for by stress (IES) beyond that explained by the control variables. Once again, no other predictor in model B had a significant regression coefficient. Results for model C again showed the simple, significant association of stress (IES) with the response to the T3 MAb at all dilutions, with R^2_c values of .092 to .102.

Discussion

Any immune response involves a complex cascade of events that occur over time. Studies suggest that the peripheral products of stress can play numerous roles in regulating immunity, and so the effects of stress will, necessarily, be variable. Current research suggests, for example, that the acute stressors, both real stressors [e.g., parachute jumps (30)] and artificial stressors [e.g., experimental tasks including speech or math stress (31)], are correlated with the mobilization (increase) of NK cells. These changes are thought to be a result of alterations in cell trafficking. In contrast, studies of chronic stressors [e.g., bereavement, caregiving, or divorce (7,9)] suggest that stress can have an effect on the ability of NK cells to lyse a target cell, the ability of NK cells to respond to rIFN γ and rIL-2 *in vitro*, and other aspects of the cellular immune response.

Our results suggest that stress, as assessed via a self-report measure of intrusive and avoidant thoughts and behaviors about cancer, was related to a negative effect on NK cell lysis, the ability of NK cells to respond to two cytokines, the blastogenic response of PBLs to two mitogens, and the proliferative response to MAb T-cell receptor. These effects were inhibitory and of similar magnitude (i.e., reliable), both between the assays and within an assay (i.e., across E:T ratios and mitogen concentrations). The analyses controlled for variables that might also be expected to exert short-term or long-term effects on immunity—such as age, stage of disease, and days since surgery—and ruled out other potentially confounding variables (e.g., nutritional status) that might also be influential. These controls reduced the plausibility of alternative, rival hypotheses for these consistent findings.

It is recognized that NK cells mediate natural immunity, but some researchers (32) suggest that their role in health generally has been underestimated. For example, there is evidence to suggest that the NK cells participate either directly or indirectly in multiple developmental, regulatory, and communication networks of the immune system. Furthermore, NK cells are efficient effector cells that not only are equipped for cell killing, but also are capable of rapid responses to exogenous or endogenous signals by producing cytokines and other factors involved in interactions between immune and nonimmune cells (20).

The ability to spontaneously lyse a broad range of infected cells or tumor cells is the best known functional attribute of NK cells (20,22). Consistent with previous reports, these data suggest that stress may impair this important process. Our findings highlight the specific effect of cancer stress on immune function, whereas prior data obtained by Levy et al. (33) had suggested that women's reports of fatigue were related to lower levels of NK cell lysis. Chronically low levels of NK cell activity occur in patients with cancer, particularly when there are large tumor burdens or disseminated metastases (32). In general, patients with low NK cell activity appear to be at higher risk for infections, to have more prolonged diseases, or to suffer more severe symptoms than patients whose NK cell activity remains normal (32,34).

A variety of biologic response modifiers are known to increase the activation, proliferation, or cytotoxicity of NK cells (20). Among the best known activators of NK cells are IL-2 and IFN γ. Our data show that the physiologic changes associated with psychologic stress inhibited NK cell lysis. Stress also affected the ability of NK cells to respond to rIFN γ, a finding that is consistent with two previous reports involving another life stressor [i.e., caregiving for a spouse with Alzheimer's disease (9,26)]. It is interesting that NK cells from 62% of the women did not respond to rIL-2. In subsequent analyses comparing women who did have an rIL-2 response with those who did not, no stress or disease variable differentiated the two groups. Further studies will need to be performed to explore this result, although it is possible that the lack of responsiveness of NK cells to rIL-2 may be due to an overproduction of prostaglandin E_2 by monocytes. It has been suggested that in breast cancer patients prostaglandin E_2 decreases IL-2

production in effector cell populations, resulting in the down-regulation of the expression of the IL-2 receptor on NK cells (23). Follow-up studies will need to pursue and clarify this difference in cytokine responses.

It has been shown that the ability of PBLs to respond to PHA is reduced, in general, in cancer patients (35); this lowered response is related to tumor burden and declines in the ability of PBLs to respond to PHA with disease progression (36). The negative effect of stress on blastogenesis was replicated in this study across two mitogens, PHA and Con A, as well as in the response of T cells to an MAb against the T-cell receptor. These findings are consistent with correlational and experimental studies indicating that stress impairs the blastogenic response of PBLs to mitogens and virus-specific T-cell responses (8,10,37–39). Mitogen-induced proliferation has been used to indicate the immune system's ability to respond to antigens from pathogens. Chronically stressed, but healthy, individuals showing decrements in the cellular immune response (including NK cell lysis and the response of the PBLs to mitogens) subsequently reported a higher incidence of infectious illnesses (8). If this effect is reliable, these data would suggest that cancer patients who experience high levels of stress, lowered levels of responsive T lymphocytes, and decreased NK cell function may be at greater risk for infectious illnesses as they begin adjuvant therapy.

It is interesting that evidence is accumulating to suggest that psychologic and/or behavioral stress reduction interventions may *enhance* certain aspects of the cellular immune response, including NK cell lysis. In an early investigation, Kiecolt-Glaser et al. (40) studied 61 healthy adults living in a retirement home. After receiving 1 month of training in progressive muscle relaxation, the subjects showed evidence of a 30% increase in NK cell lysis in comparison with those who received no treatment or only social contact. Fawzy et al. (41) studied 61 patients with melanoma and reported that, 6 months after treatment, subjects receiving intervention had significantly higher levels of IFN alfa-augmented NK cell activity than those who received no treatment. These data suggest that, if behavioral interventions can reduce stress and enhance the cellular immune response, then health outcomes might improve.

In conclusion, these data show a down-regulation of different aspects of the cellular immune response associated with the psychologic stress that accompanies the diagnosis and initial surgical treatment of cancer. We note that these study participants are part of a larger effort testing the biobehavioral aspects of stress, immunity, and disease course (5). It will be important to document the longitudinal nature of these findings, and future studies will provide such data. Moreover, half of the women who participated have been randomly assigned to receive a psychologic/behavioral intervention specifically designed to reduce stress, enhance quality of life, and test for the biologic mechanism—such as immune responses—that may mediate any positive effects of stress reduction on health and disease outcomes.

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References

1. Andersen BL, Anderson B, deProsse C. Controlled prospective longitudinal study of women with cancer: II. Psychological outcomes. J Consult Clin Psychol 1989;57:692–7. [PubMed: 2600239]
2. Moyer A, Salovey P. Psychosocial sequelae of breast cancer and its treatment. Ann Behav Med 1996;18:110–25.
3. Andersen BL. Surviving cancer. Cancer 1994;74(4 Suppl):1484–95. [PubMed: 8062180]
4. Shalala, DE. Proceedings: Secretary's Conference to Establish a National Action Plan on Breast Cancer; National Institutes of Health; Bethesda (MD). Dec 14–15, 1993; 1993. Chair
5. Andersen BL, Kiecolt-Glaser JK, Glaser R. A biobehavioral model of cancer stress and disease course. Am Psychol 1994;49:389–404. [PubMed: 8024167]
6. Herbert TB, Cohen S. Depression and immunity: a meta-analytic review. Psychol Bull 1993;113:472–86. [PubMed: 8316610]
7. Herbert TB, Cohen S. Stress and immunity in humans: a meta-analytic review. Psychosom Med 1993;55:364–79. [PubMed: 8416086]
8. Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R. Spousal caregivers of dementia victims: longitudinal changes in immunity and health. Psychosom Med 1991;53:345–62. [PubMed: 1656478]
9. Esterling BA, Kiecolt-Glaser JK, Bodnar JC, Glaser R. Chronic stress, social support, and persistent alterations in the natural killer cell response to cytokines in older adults. Health Psychol 1994;13:291–8. [PubMed: 7957007]
10. Kiecolt-Glaser J, Glaser R, Gravenstein S, Malarkey WB, Sheridan J. Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc Natl Acad Sci USA 1996;93:3043–7. [PubMed: 8610165]
11. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. Lancet 1995;346:1194–6. [PubMed: 7475659]
12. Levy SM, Herberman RB, Lee J, Whiteside T, Kirkwood J, McFeeley S. Estrogen receptor concentration and social factors as predictors of natural killer cell activity in early-stage breast cancer patients. Confirmation of a model. Nat Immun Cell Growth Regul 1990;9:313–24. [PubMed: 2077396]
13. Former JG, Kim DK, Hopkins L, Barrett MK, Pinsky CM, Day NK. Immunologic function in patients with carcinoma of the pancreas. Surg Gynecol Obstet 1980;150:215–8. [PubMed: 6965431]
14. Monson JR, Ramsden C, Guillou PJ. Decreased interleukin-2 production in patients with gastrointestinal cancer. Br J Surg 1986;83:483–6. [PubMed: 3487366]
15. Feo Figarella E, Morillo F, Blanca I, Bianco NE. Failure of cell-mediated effector mechanisms in lung cancer. J Natl Cancer Inst 1984;73:1–6. [PubMed: 6330420]
16. Anastasopoulos E, Reclos GJ, Baxevanis CN, Tsilivakos V, Panagiotopoulos N, Fotiou S, et al. Monocyte disorders associated with T cell defects in cancer patients with solid tumors. Anticancer Res 1992;12:489–94. [PubMed: 1533752]
17. Steinhauer EH, Doyle AT, Reed J, Kadish AS. Defective natural cytotoxicity in patients with cancer: normal number of effector cells but decreased recycling capacity in patients with advanced disease. J Immunol 1982;129:2255–9. [PubMed: 7119445]
18. Jubert AV, Lee ET, Hersh EM, McBride CM. Effects of surgery, anesthesia and intraoperative blood loss on immunocompetence. J Surg Res 1973;15:399–403. [PubMed: 4544256]
19. Herberman RB, Ortaldo JR. Natural killer cells: their roles in defenses against disease. Science 1981;241:24–30. [PubMed: 7025208]
20. Trinchieri G. Biology of natural killer cells. Adv Immunol 1989;47:187–376. [PubMed: 2683611]
21. Hersey P, Edwards A, Honeyman M, McCarthy WH. Low natural-killer-cell activity in familial melanoma patients and their relatives. Br J Cancer 1979;40:113–22. [PubMed: 314301]
22. Whiteside TL, Herberman RB. Characteristics of natural killer cells and lymphocyte-activated killer cells. Immunol Allerg Clin North Am 1990;10:663–704.
23. Baxevanis CN, Reclos GJ, Gritzapis AD, Dedousis GV, Missitzis I, Papamichail M. Elevated prostaglandin E_2 production by monocytes is responsible for the depressed levels of natural killer

and lymphokine-activated killer cell function in patients with breast cancer. Cancer 1993;72:491–501. [PubMed: 8319179]

24. Horowitz M, Wilner N, William A. Impact of Event Scale: a measure of subjective stress. Psychosom Med 1979;41:209–18. [PubMed: 472086]
25. Kiecolt-Glaser JK, Glaser R. Methodological issues in behavioral immunology research with humans. Brain Behav Immun 1988;2:67–78. [PubMed: 3052653]
26. Esterling BA, Kiecolt-Glaser JK, Glaser R. Psychosocial modulation of cytokine-induced natural killer cell activity in older adults. Psychosom Med 1996;58:264–72. [PubMed: 8771626]
27. Gieni RS, Li Y, Hay Glass KT. Comparison of [^{3}H]thymidine incorporation with MTT- and MTS-based bioassays for human and murine IL-2 and IL-4 analysis. Tetrazolium assays provide markedly enhanced sensitivity. J Immunol Methods 1995;187:85–93. [PubMed: 7490461]
28. Shobitz B. Steroids and central regulation of immune response. Meth Neuro Sci 1994;22:510–52.
29. Cohen, J.; Cohen, P. Applied multiple regression/correlation analysis for the behavioral sciences. Erlbaum; Hillsdale (NJ): 1983.
30. Schedlowski M, Jacobs R, Stratmann G, Richter S, Hadicke A, Tewes U, et al. Changes in natural killer cells during acute psychological stress. J Clin Immunol 1993;13:119–26. [PubMed: 8320310]
31. Uchino BN, Cacioppo JT, Malarkey W, Glaser R. Individual differences in cardiac sympathetic control predict endocrine and immune responses to acute psychological stress. J Pers Soc Psychol 1995;69:736–43. [PubMed: 7473028]
32. Whiteside TL, Herberman RB. Role of human natural killer cells in health and disease. Clin Diagn Lab Immunol 1994;1:125–33. [PubMed: 7496932]
33. Levy SM, Herberman RB, Maluish AM, Schlien B, Lippman M. Prognostic risk assessment in primary breast cancer by behavioral and immunological parameters. Health Psychol 1985;4:99–113. [PubMed: 4018006]
34. Cannon GB, Dean JH, Herbermann RB, Perlin E, Reid J, Miller C, et al. Association of depressed postoperative lymphoproliferative responses to alloantigens with poor prognosis in patients with stage 1 lung cancer. Int J Cancer 1980;25:9–17. [PubMed: 6447114]
35. Han T, Takita H. Depression of T lymphocyte response by non-T suppressor cells in lung cancer patients: a possible prognostic value of suppressor cell activity. Cancer 1979;44:2090–8. [PubMed: 228835]
36. Ludwig CU, Hartmann D, Landmann R, Wesp M, Rosenfelder G, Stucki D, et al. Unaltered immunocompetence in patients with non-disseminated breast cancer at the time of diagnosis. Cancer 1985;55:1673–8. [PubMed: 3872158]
37. Baron RS, Cutrona CE, Hicklin D, Russell DW, Lubaroff DM. Social support and immune function among spouses of cancer patients. J Pers Soc Psychol 1990;59:344–52. [PubMed: 2213497]
38. Bartrop RW, Luckhurst E, Lazarus L, Kiloh LG, Penny R. Depressed lymphocyte function after bereavement. Lancet 1977;1:834–6. [PubMed: 67339]
39. Locke SE, Kraus L, Leserman J, Hurst MW, Heisel JS, Williams RM. Life change stress, psychiatric symptoms, and natural killer cell activity. Psychosom Med 1984;46:441–53. [PubMed: 6494387]
40. Kiecolt-Glaser JK, Glaser R, Williger D, Stout J, Messick G, Sheppard S, et al. Psychosocial enhancement of immunocompetence in a geriatric population. Health Psychol 1985;4:25–41. [PubMed: 2990890]
41. Fawzy FI, Kemeny ME, Fawzy NW, Elashoff R, Morton D, Cousins N, et al. A structured psychiatric intervention for cancer patients. II. Changes over time in immunological measures. Arch Gen Psychiatry 1990;47:729–35. [PubMed: 2143062]

Table 1

Results of regression analyses for predicting natural killer (NK) cell lysis across six effector-to-target cell (E:T) ratios

	Dependent variable: NK cell lysis at E:T ratios					
	100:1	**50:1**	**25:1**	**12.5:1**	**6.25:1**	**3.125:1**
Model A, R^2_A *	.005	.007	.012	.015	.020	.023
Model AA, R^2_{AA} †	.085	.148	.185	.233	.250	.241
Model B‡						
R^2_B	.135	.212	.238	.268	.275	.253
$R^2_{B–AA}$ §	.050	.064	.053	.035	.025	.012
β_{Stress} //	−.234	−.265	−.240	−.194	−.165	−.115
$t(df = 110)$ ¶	−2.462	−2.921	−2.672	−2.223	−1.892	−1.280
P	.016	.004	.008	.028	.062	.204
Model C#						
R^2_C	.067	.091	.084	.066	.056	.032
$t(df = 110)$ ¶	−2.826	−3.338	−3.199	−2.811	−2.558	−1.867
P	.006	.002	.002	.006	.012	.066

*Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, NK cell lysis. The R^2_A is the total variance in NK cell lysis explained by these three predictors.

†Model AA includes model A variables plus the control predictor percentage of NK cells for the immune outcome, NK cell lysis. The R^2_{AA} is the total variance in NK cell lysis explained by these four predictors

‡Model B includes model AA control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, NK cell lysis. The R^2_B is the total variance in NK cell lysis explained by the four control predictors and the stress predictor.

§$R^2_{B–AA}$ is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the NK cell lysis outcome.

//β_{Stress} is the standardized regression beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

¶*df* refers to the degrees of freedom in model B.

#Model C includes stress as the only predictor of the immune outcome, NK cell lysis. The R^2_C is the total variance in NK cell lysis explained by stress; this model provides the simple association between psychologic stress and immune function.

Table 2

Results of regression analyses for predicting natural killer (NK) cell response to recombinant interferon gamma (rIFN γ) across five effector-to-target cell (E:T) ratios

	Dependent variable: NK cell response to rIFN γ at E:T ratios				
	50:1	**25:1**	**12.5:1**	**6.25:1**	**3.125:1**
Model A, R^2_A *	.025	.097	.080	.138	.124
Model B †					
R^2_B	.041	.151	.197	.257	.208
R^2_{B-A} ‡	.016	.054	.117	.119	.084
β_{Stress} §	−.128	−.244	−.358	−.358	−.301
t	−1.104	−2.190	−3.203	−3.084	−2.083
df //	82	81	74	65	46
P	.274	.032	.002	.004	.044
Model C ¶					
R^2_C	.015	.077	.149	.149	.088
t	−1.128	−2.586	−3.581	−3.343	−2.080
df //	82	81	74	65	46
P	.264	.012	.002	.002	.044

* Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, NK cell response. The R^2_A is the total variance in NK cell response explained by these three predictors.

† Model B includes model A control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, NK cell response. The R^2_B is the totla variance in NK cell response explained by the three control predictors and the stress predictor.

‡ R^2_{B-A} is the increment in variance due to stress only (i.e., variance beyond that explainde by the control predictors) in predicting the NK cell response.

§ β_{Stress} is the standardized regression beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress onthe immune outcome.

// *df* refers to the degrees of freedom in model B.

¶ Model C includes stress as the only predictor of the immune outcome, NK cell response. The R^2_C is the total variance in NK cell response explained by stress; this model provides the simple association between psychologic stress and immune function.

Tabic 3

Results of regression analyses for predicting the blastogenic response to concanavalin A (Con A) and phytohemagglutinin A (PHA) across three concentrations each

	Dependent variable: blastogenic response of mitogen					
	Con A			PHA		
	10 μg/mL	5 μg/mL	2.5 μg/mL	10 μg/mL	5 μg/mL	2.5 μg/mL
Model A, R^2_A *	.035	.043	.054	.022	.024	.033
Model AA, R^2_{AA} †	.105	.125	.115	.023	.024	.033
Model B‡						
R^2_B	.166	.174	.147	.083	.074	.080
R^2_{B-AA} §	.061	.049	.032	.060	.050	.047
β_{Stress} //	−.255	−.229	−.187	−.256	−.234	−.229
$t(df = 103)$ ¶	−2.668	−2.401	−1.927	−2.521	−2.299	−2.254
P	.010	.018	.058	.014	.024	.026
Model C#						
R^2_C	.053	.065	.053	.070	.054	.052
$t(df = 108)$ ¶	−2.443	−2.724	−2.443	−2.857	−2.489	−2.441
P	.016	.008	.016	.006	.014	.016

*Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, blastogenesis. The R^2_A is the totla variance in blastogenesis explained by these three predictors.

†Model AA includes model A variables plus the control predictor of number of T cells for the immune outcome, blastogenesis. The R^2_{AA} is the total variance in blastogenesis explained by these four predictors.

‡Model B includes model AA control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, blastogenesis. The R^2_B is the total variance in blastogenesis explained by the four control predictors and the stress predictor.

§R^2_{B-AA} is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the blastogenesis outcome.

//β_{Stress} is the standardized regression beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

¶*df* refers to the degrees of freedom in model B.

[#] Model C includes stress as the only predictor of the immune outcome, blastogenesis. The R^2C is the total variance in blastogenesis explained by stress; this model provides the simple association between psychologic stress and immune function.

Table 4

Results of regression analyses for predicting proliferative response of peripheral blood leukocytes to a monoclonal antibody to T-cell receptor (T3) across three dilutions

	Dependent variable: proliferative response at dilutions		
	128:1	**64:1**	**32:1**
Model A, R^2_A *	.026	.052	.064
Model AA, R^2_{AA} †	.088	.104	.143
Model B‡			
R^2_B	.155	.160	.200
$R^2_{B–AA}$ §	.067	.056	.057
β_{Stress} //	−.273	−.249	−.252
$t(df = 101)$ ¶	−2.747	−2.514	−2.604
P	.008	.014	.012
Model C#			
R^2_C	.102	.092	.094
$t(df = 101)$ ¶	−3.452	−3.255	−3.307
P	.002	.002	.002

* Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, proliferative response. The R^2_A is the total variance in proliferation explained by these three predictors.

† Model AA includes model A variables plus the control predictor of number of T cells for the immune outcome, proliferation. The R^2_{AA} is the total variance in proliferation explained by these four predictors.

‡ Model B includes model AA control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, proliferation. The R^2_B is the total variance in proliferation explained by the four control predictors and the stress predictor.

§ $R^2_{B–AA}$ is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the proliferation outcome.

// β_{Stress} is the standardized beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

¶ *df* refers to the degrees of freedom in model B.

Model C includes stress as the only predictor of the immune outcome, proliferation. The R^2_C is the total variance in proliferation explained by stress; this model provides the simple association between psychologic stress and immune function.