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Psychologic stress, reduced NK cell activity, and cytokine dysregulation in women experiencing diagnostic breast biopsy

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Summary

The purpose of this study was to evaluate a woman's psychological and immunological response to breast biopsy before and after the procedure. Women were enrolled into the study when notified of the need for breast biopsy. Psychological and immunological assessments were made at enrollment, on the day of breast biopsy, as well as 1 month and 4 months after notification of biopsy results. Psychological assessments demonstrated that perceived stress, anxiety, and mood disturbance were heightened before biopsy and remained elevated after biopsy regardless of the diagnosis. Immunologically, the women exhibited reduced natural killer cell activity and INF γ production before biopsy with reductions significant one month after the procedure. In contrast, IL-4, IL-6, and IL-10 production were increased before and after the procedure with most significant increases prior to the procedure and continuing one month after the procedure. These results demonstrate that undergoing biopsy of the breast for cancer diagnosis is an emotional experience, characterized by increased perceived stress, anxiety, and mood disturbance. This emotional distress is accompanied by reduced NK cell activity and cytokine dysregulation. The psychological and immunological impact of breast biopsy is not transient, but persists well beyond the actual experience of the biopsy procedure. Noteworthy is the observation that women with benign or malignant biopsy results experienced similar psycho-immune consequences. Hence, these observations are of relevance not only to women diagnosed with malignancy, who face the challenges of cancer treatment and adaptation to illness, but also to women with benign biopsy findings.

Keywords

Breast biopsy; NK cell activity; Cytokines; Perceived stress; Anxiety; Mood disturbance

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1. Introduction

Breast cancer diagnosis is a time of considerable uncertainty, anxiety, and emotional distress (Northouse et al., 1995; Deane and Degner, 1998; Stark and House, 2000). This emotional experience often begins with the discovery of clinical or radiographic findings that indicate the need for biopsy of the breast (Benedict et al., 1994). Breast biopsy, either by surgical excision or by less invasive procedures, provides for the pathological evaluation and diagnosis of a suspicious breast abnormality. Despite knowing that the large majority of women undergoing breast biopsy have benign results (Ghosh et al., 2005), anticipating a breast biopsy and experiencing the concomitant uncertainty and fear of a cancer diagnosis, is emotionally disturbing (Nagabhushan et al., 2001; O'Mahony, 2001; Montgomery et al., 2003). Women express high levels of anxiety after the discovery of a breast lump (MacFarlane and Sony, 1992) and at the time of biopsy they report higher levels of stress compared to patients awaiting general surgery (Hughson et al., 1988). The impact of psychological stress and anxiety is not limited to central nervous system activation but has repercussions for numerous biological systems, including the immune system. Reciprocal neuro-chemical pathways and shared receptor systems connect the nervous, endocrine, and immune systems as a network (Madden and Felten, 1995; Weigent and Blalock, 1999; Charmandari et al., 2005). This intricate neuro-endocrine-immune network provides the link whereby perceived environmental stressors or demands, such as undergoing biopsy of the breast, may effect the immune system and influence health status (Andersen et al., 1994; Witek-Janusek, and Mathews, 2000; Glaser, 2005).

A large body of evidence supports the assertion that psychological stress can adversely affect the human immune response (Biondi, 2001; Glaser, 2005). Stress-induced immunosuppression accompanies a variety of acute and chronic life stressors such as bereavement (Irwin et al., 1987), depression (Weisse, 1992), marital conflict (Kiecolt-Glaser et al., 1993), academic exam stress (Kiecolt-Glaser et al., 1986), and care giving in chronic disease (Kiecolt-Glaser et al., 1987). A relationship between stress and natural killer cell activity (NKCA) has been suggested for some time (Trinchieri, 1989). Andersen *et al.* (1998) demonstrated stress-induced immune alterations in women newly diagnosed with invasive breast cancer (Stage II and III). These women were evaluated within four months of their breast surgery but prior to adjuvant therapy initiation. The results of that study showed that higher stress levels were predictive of lower NKCA, diminished natural killer (NK) cell response to interferon (IFN), and decreased lymphocyte proliferation (Andersen et al., 1998). It is possible that stress may influence cancer control. Although a direct relationship between NKCA and cancer has not been clearly established, patients with a variety of solid tumors (e.g., breast, cervix, endometrium, ovary, and lung) do exhibit reduced NKCA (Pross and Lotzova, 1993). More recent investigations with gene-depleted mice, provide evidence that NK cells mediate protection from tumors (Diefenbach and Raulet, 2002) including; initiation (van den Broek et al., 1996; Street et al., 2001), primary tumor growth (van den Broek et al., 1996), and tumor metastasis (Zhang et al., 1999; Street et al., 2001).

The effects of stress upon the immune system extend not just to NK cells but also to the peripheral blood cell production of cytokines. Heightened levels of stress have been related to decreased synthesis of IFN γ (Kiecolt-Glaser et al., 1986) and a poorer NK response to IFN and IL-2 (Esterling et al., 1994; Esterling et al., 1996). Posttraumatic stress disorder (Maes et al., 1999) and academic exam stress (Marshall et al., 1998) are reported to lead to cytokine dysregulation. Moreover, interventions that reduce stress can modulate NKCA and cytokine responsiveness (Esterling et al., 1994), suggesting an interactive relationship between stress and NK function, that may be mediated by cytokines (Esterling et al., 1996). The cytokine response to stress can be triggered by changes in adrenal hormone production (McEwen et al., 1997; Elenkov and Chrousos, 2002), in that cortisol secreted during stress,

can switch a Th1 to a Th2 immune response (Daynes et al., 1990; Rook et al., 1994; Elenkov, 2004). Such a switch in cytokine balance, characterized by low levels of IFN γ , can depress NK cell function (Targan and Dorey, 1980; Reiter, 1993; Biron et al., 1999).

The purpose of this study was to evaluate the psychological impact of the breast biopsy experience and to determine whether this experience was accompanied by changes in NKCA and cytokine production. The experience of breast biopsy represents a naturalistic human paradigm known to be characterized by uncertainty, mood disturbance and emotional distress (Nagabhushan et al., 2001; O'Mahony, 2001; Montgomery et al., 2003). As such this paradigm can be used to understand potential links between a woman's psychological and immunological states. In this study, psychological stress and immune response were measured before and after breast biopsy and compared to a group of control women not undergoing breast biopsy.

2. Methods

2.1. Subjects and procedure

Women, over 21 years of age, who were recommended for breast biopsy were eligible subjects. Breast biopsy comprised any surgical biopsy or image-guided core-needle biopsy (including stereotactic or ultrasound guided) carried out on the breast for the purpose of diagnosis; women receiving fine needle aspirates of the breast were excluded. Other exclusionary criteria were: pregnancy, recent history of major psychiatric disorder, concurrent major immune-based disease, use of immune-altering medication, and active substance abuse. Women within 5 years of a cancer diagnosis were also excluded. A comparison group of control women, not undergoing breast biopsy were enrolled from the community. Similar exclusionary criteria were applied to these women. Data were collected from the non-biopsy control women at one time period only. This study was approved by the Loyola University Medical Center Institutional Review Board for the Study of Human Subjects. All procedures were carried out with the adequate understanding and written consent of the subjects. This informed consent was obtained at the initial data collection period.

Ninety-two women scheduled to undergo breast biopsy were enrolled into the study from the University Breast Care Center during a clinic visit. Of the 92 women, 70 had benign breast biopsy results, while the remaining 22 women were found to have breast cancer. A separate group of 29 women without cancer who did not undergo any breast biopsy were enrolled into the comparison group. Data were collected at 4 time points, 2 pre-biopsy (T1 and T2), and 2 post-biopsy (T3 and T4). T1 occurred during the clinic visit when women were informed by their physician of the need for a breast biopsy, T2 occurred on the day of breast biopsy prior to the procedure, T3 was the first post-biopsy time point and occurred approximately 1 month after notification of the biopsy results, and T4 was the second post-biopsy time point and occurred approximately 3 months after T3. Participants had their blood drawn (24 ml) by venipuncture and were given a packet of psychological measures as well as a health history questionnaire to be completed and returned by mail. The return rate for the women with benign results was 77%, 100%, 83%, and 97% for T1 through T4, respectively, while that for the women with malignant breast findings was 73%, 86%, 91% and 86%. Women in the comparison group had a 100% return rate. Blood samples were not able to be obtained from each woman at all 4 time points due to technical issues related to venipuncture, clinic logistics, and subject refusal. Also, sufficient blood volumes were not always available for every immune analysis. Blood samples were collected for T1, T3 and T4 between 1–5PM. Due to the constraints of the surgical and interventional radiology schedule the investigators were unable to control the time of day for T2 sampling and these samples were collected between 8AM–1PM.

2.2 Psychological measures

For the purposes of this study the psychological distress associated with breast biopsy was conceptualized as a state evoked by an event appraised as a threat to the individual's well-being, which, in turn, led to feelings of anxiety and mood disturbance. With this in mind, the psychological assessment of women experiencing breast biopsy included measurement of perceived stress (Perceived Stress Scale), mood state (Profile of Mood States) and anxiety (State-Trait Anxiety Inventory).

2.2.1. Perceived Stress Scale (PSS)—The PSS is a 10-item scale that provides a general appraisal index of stress (Cohen et al., 1983). It measures the degree to which experiences are appraised as uncontrollable. For the PSS women were instructed to rate their degree of perceived stress over the past month. Internal consistency is good with coefficient alphas ranging from 0.75–0.86. Test-retest reliability has been reported to be 0.85 (Cohen et al., 1983).

2.2.2. Profile of Mood States (POMS)—The POMS is a 65-item measure designed to identify and assess general distress/mood. A total mood score and six sub-scores for mood were obtained. The sub scores were tension, depression, anger, vigor, fatigue, and confusion. Women were asked to indicate the extent to which each of the scale's 65 adjectives of mood describe the way they had been feeling during the past week, on a scale ranging from 0 (not at all) to 4 (extremely). A total mood disturbance score is derived by summing each of the six subscales, with vigor weighted negatively. The possible range of scores is –40 through 192. Internal consistency alphas range from 0.87–0.95, and stability coefficients (test-retest) are 0.65–0.74 (McNair et al., 1992).

2.2.3. Spielberger State-Trait Anxiety Inventory (Anxiety)—The State-Trait Anxiety Inventory is a 40-item self-report measure of state and trait anxiety. Only the state anxiety inventory was used in this study. The State-Trait Anxiety Inventory has alpha reliability coefficients of 0.83–0.92 and convergent validity with other anxiety tools (alpha = 0.75–0.80) (Spielberger et al., 1970). The State-Trait Anxiety Inventory has been used to discriminate anxiety in women with benign versus malignant breast biopsy findings (Sachs et al., 1995).

2.2.4 Psychosocial Assessment Tool—The Psychosocial Assessment Tool, a self-administered cancer screening tool developed by Barg et al., 1994, was used to obtain demographic information (ethnicity, education, employment, income) and to determine availability of support systems. This tool has been used in the clinical assessment of risk of cancer patients for psychosocial problems.

2.3. Immune measures

2.3.1. Isolation of peripheral blood mononuclear cells—Blood was collected in sterile heparinized tubes and processed immediately. Heparinized peripheral blood was overlaid onto Ficoll/Hypaque and centrifuged at 1000×g for 20 min. The peripheral blood mononuclear cells (PBMC) at the interface were washed twice with Hank's Balanced Salt Solution prior to assessment of NKCA, phenotypic analysis, or cytokine production. Phenotypic analysis was as described previously (Nagabhushan et al., 2001). Briefly, isolated PBMCs were analyzed with specific fluorochrome conjugated antibodies in order to identify specific subsets of PBMCs including: CD3 for T lymphocytes, CD16 and/or CD56 for NK cells; CD4 for helper lymphocytes; CD8 for cytotoxic lymphocytes, and CD16 single positive cells were identified as circulating monocytes. Semi-quantitative analysis of the phenotypic expression of relevant leukocyte surface molecules was determined by immunofluorescence using a FACS Star Plus System. Antibodies reactive with surface

markers were obtained as direct conjugates from BD PharMingen. Interassay variability ranges for laboratory PBMC values ranged from 1.5 – 7.9%. The numbers of subject analyses for each group were; Control=29, Benign at T1= 46, Benign at T2= 60, Benign at T3= 55, Benign at T4= 57, Malignant at T1= 13, Malignant at T2= 22, Malignant at T3= 20, Malignant at T4= 19.

2.3.2. Natural killer cell activity—K562 tumor cells, obtained from the American Type Culture Collection, Rockville, MD, were radioactively labeled with 100 uCi of [⁵¹Cr] (New England Nuclear, Boston, MA). Radiolabeled K562 cells were incubated for 4 hr with PBMC. Following incubation the supernatants were removed using a Skatron harvesting press (Skatron Inc., Sterling, VA) and the associated radioactivity was determined. E:T ratios for NKCA were 100, 50, 25 and 12.5:1.

Results are expressed as % cytotoxicity and calculated by the formula:

$$\% \text{Cytotoxicity} = \frac{(\text{experimental DPM}^*) - (\text{minimum DPM})}{(\text{maximum DPM}) - (\text{minimum DPM})} \times 100.$$

All experimental means were calculated from triplicate values. Lytic units (LU) were calculated by a program written by David Coggins, FCRC, Frederick, MD and represents the number of cells per 10⁷ effectors required to achieve 20% lysis of the targets.

*DPM=disintegrations per minute.

2.3.3. Evaluation of PBMC for cytokine production—Cytokines were measured under optimal conditions in bulk PBMC culture supernatant fluids as described previously (Witek-Janusek and Mathews, 1999). Briefly, PBMC (1 × 10⁶ cells/ml) were cultured with and without PMA/PHA (PMA @ 20 ng/well; PHA @ 0.05%/well) in 24 well plates for 48 hr at 37° C. Aliquots of the culture supernatants were stored at –80° C for subsequent cytokine analysis.

2.3.4. Cytokine measurement (ELISA)—All cytokines were measured using quantitative sandwich enzyme immunoassay techniques (Quantikine kits, R & D Systems). Sensitivities for cytokines were; IL-2 < 7 pg/ml, IL-6 < 0.7 pg/ml, IFN γ < 3 pg/ml, IL-10 < 2 pg/ml, and IL-4 < 4.1 pg/ml. Coefficient of variation ranged between 2.6 – 4.9% for the individually assessed cytokines.

2.4. Statistical methods

Data are expressed as means with the standard error of the mean (SE) or standard deviation (SD) as noted. To determine differences in study outcomes among the three groups (benign, malignant, and control) a one-way ANOVA was used. Post hoc analysis was determined using Tukey's (HSD) test. Missing data were treated as such and no imputation techniques were used. A two-sided alpha of 0.05 was set for statistical significance. The Statistical Package for Social Sciences (SPSS: version 9.0) was used for data analysis.

3. Results

3.1. Demographic Characteristics of Study Participants

Table 1 summarizes the demographic characteristics of all study women. As expected women with malignant findings were somewhat older (60.3 ± 2.2 years, X ± SE) compared to women with benign biopsy results (50.1 ± 1.5 years). Likely due to their older age, fewer of the women with malignancy were married or employed at study entry. Respectively,

85%, 89%, and 93% of the Benign, Malignant, and Comparison (Control) group women were Caucasian. The only Hispanic women (3%) who participated in the study were in the Benign biopsy group; while 11% of the Malignant group, 12% of the Benign group, and 7% of the Comparison group were African American. All women reported high levels of available social support systems. Women in the benign biopsy group report a higher family history of cancer. Only 5% of women in the Malignant group reported use of hormone replacement therapy at the time of study entry; whereas, women in the Benign and Comparison groups reported this usage as 20% and 23%, respectively.

3.2. Psychological Assessments

The results ($X \pm SE$) of psychological measures for each of the 3 study groups (Benign, Malignant, and Control) are illustrated in Figures 1 and 2. Figure 1 illustrates the mean perceived stress (PSS), anxiety (Spielberger's State Anxiety Inventory), and total mood disturbance (Profile of Mood State, POMS-TMD) for women pre biopsy (T1 and T2) and post biopsy (T3 and T4), as well as for a comparison group of non-biopsied women (Control).

The results indicate the period surrounding breast biopsy to be a time of heightened perceived stress, as measured by the PSS (Figure 1). One way ANOVA revealed significant differences in PSS among groups at all times evaluated ($F[2, 99]=10.8$; $p<0.001$; $F[2, 118]=6.2$; $p<0.003$; $F[2, 106]=3.9$; $p<0.02$; $F[2, 113]=5.0$; $p<0.009$), respectively for T1 through T4). Post hoc analysis (Tukey's test) showed that at each time (T1–T4), the women who had benign results, reported elevations in perceived stress that were significantly greater than that of the non-biopsied Control group of women; whereas, the levels of PSS for women in the malignant group were significantly greater than that of Control women at T2 only. No differences in PSS between Benign and Malignant groups were demonstrated at any time. Like the PSS, significant elevations in state anxiety were observed at each time evaluated ($F[2, 100]=17.0$; $p<0.001$; $F[2, 120]=22.0$; $p<0.001$; $F[2, 106]=3.7$; $p<0.3$; $F[2, 115]=3.3$; $p<0.4$), respectively for T1 through T4 (Figure 1). The women in the Benign group reported higher levels of anxiety than Control women at T1, T2 and T4; whereas, anxiety scores for women in the Malignant group were significantly greater than the Control group at T2 and T3 only (Tukey's post hoc test). No differences in anxiety were found between women in the benign versus malignant group. Total mood disturbance (TMD), as measured by the POMS, is also shown in Figure 1. Significant elevations in mood disturbance were found at all times evaluated ($F[2, 98]=15.6$; $p<0.001$; $F[2, 118]=14.8$; $p<0.001$; $F[2, 107]=4.5$; $p<0.4.5$; $F[2, 114]=5.3$; $p<0.006$) respectively for T1 through T4. Examination of these differences with post hoc testing showed that POMS-TMD was significantly elevated in the Benign group of women compared to Control women at each time point, indicating that mood disturbance remained high despite knowing that the biopsy findings were benign (T3 and T4). Women in the Malignant group reported significantly less mood disturbance than the women in the Benign group at T1 (pre-biopsy) and the level of mood disturbance, although numerically higher than that of Control women at all times was only significantly elevated at T2 and T3.

The Profile of Mood State (POMS) is comprised of six subscales that measure Tension, Depression, Anger, Vigor, Fatigue, and Confusion. One-way ANOVA revealed significant differences in all POMS subscale values for the three groups of women (F values ranged from 20.1 to 2.9, with corresponding p values ranging from $p<0.001$ to $p<0.05$). Figure 2 indicates the source of significant differences as revealed by post hoc analysis. In general, the results demonstrate that the period prior to biopsy (T1 and T2) was a time of elevated tension, depression, anger, fatigue, and confusion, as well as reduced vigor. For the Benign group of women at T1; tension, depression, anger, fatigue, and confusion were significantly increased relative to these measures for the Control group; while vigor scores were

significantly lower. The levels of tension, anger and confusion in the Benign group of women were significantly greater than that reported by women in the Malignant group at T1. In general the POMS subscales for the Malignant group of women were numerically greater than that of the Control group of women at T1, while vigor scores were reduced; however, none of these differences attained significance. On the day of biopsy (T2) both the Benign and Malignant groups of women reported significantly elevated tension, depression, and anger as well as significantly reduced vigor, when compared to the Control group of women. At T2 women in both the Benign and Malignant groups had higher fatigue scores than Control women but this was only significant for the Malignant group of women. After biopsy, numerical reductions in tension, depression, anger, fatigue, and confusion were reported by both the Benign and Malignant groups of women. However, despite reductions in mood disturbance from pre to post biopsy, the levels of tension, depression, anger and confusion reported by women in the Benign group at T4 remained significantly greater than that of the Control women.

3.3. Immunological Assessments

NKCA and the production of IFN γ by PBMC derived from women before and after breast biopsy are illustrated in Figure 3. Significant differences in NKCA were observed at each time evaluated ($F[2, 90]=6.9$; $p<0.002$; $F(2, 121)=3.3$; $p<0.04$; $F[2, 111]=11.9$; $p<0.001$; $F[2, 108]=6.9$; $p<0.001$), respectively for T1 through T4. Post hoc analysis demonstrated that both women with benign and malignant biopsy results had reductions in NKCA at T1 (pre biopsy) relative to that of the Control group. At T2 (day of biopsy) this reduction in NKCA persisted for both the Benign and Malignant groups but was only significantly reduced for the Malignant group of women. Post biopsy (T3) NKCA was significantly less in both the Benign and Malignant women compared to the Control women. By T4, NKCA increased for the Benign group of women so that their lytic activity was no longer different from that of the Control group; whereas, for the Malignant group of women, NKCA remained significantly less than that measured in the Control women and was also significantly less than that of the Benign women. IFN γ production was numerically less than that of Control group pre biopsy (T1 and T2) for both the Benign and Malignant groups; whereas, post biopsy (T3) the reduction in IFN γ was significantly less ($F[2, 97]=5.8$; $p<0.004$) than that of the Control group. By T4, IFN γ production increased for both the Benign and Malignant groups of women so that it was no longer different from the IFN γ production observed in the Control group.

IL-6, IL-4 and IL-10 production by PBMC of women before and after biopsy is shown in Figure 4. For both the Benign and Malignant groups, at all time points, the numerical amount of each cytokine produced was greater than the amount produced by the PBMC of the Control group. Statistically significant increases above the levels of Control women were most dramatic for IL-4 ($F[2, 87]=4.6$; $p<0.01$; $F[2, 106]=4.6$; $p<0.01$; $F[2, 103]=6.6$; $p<0.002$; $F[2, 101]=5.1$; $p<0.007$) for T1 through T4, respectively. Based on post hoc analysis, the amount of IL-4 produced was significantly greater than the Control group for both Benign and Malignant groups at all time points. The exception was T1, at which the Benign group was not statistically greater than the Control group. For IL-6 production, statistically significant increases were observed for the Malignant group at T2 and T4 ($F[2, 102]=3.5$; $p<0.03$; $F[2, 97]=3.1$; $p<0.05$, respectively). IL-10 production was consistently greater in the biopsied women compared to the Control women but these increases were statistically significant only for the Benign group at T1 and T3 ($F[2, 90]=3.4$; $p<0.04$; $F[2, 102]=3.2$; $P<0.5$, respectively). Production of IL-2 was also assessed for the Benign, Malignant, and Control groups but no numerical or statistically significant differences were noted. Data are not shown. None of these cytokines were detected in the serum of any biopsied or control subject. Data are not shown. Phenotypic analysis of circulating NK cells

and other PBMC subsets are shown in Figure 5, in which results for the biopsied women are presented. No difference between the Control group of women and the Biopsied women at any time point was observed. No difference was observed between the Benign and Malignant groups of women. For simplicity the results of the biopsied women are solely presented.

4. Discussion

Suspicious breast findings that require evaluation by biopsy herald the onset of an emotional experience that is often accompanied by uncertainty and fear of a cancer diagnosis (Benedict et al., 1994; Northouse et al., 1995; Deane and Degner, 1998). For most women, the unfolding of this emotional scenario occurs over a period of several weeks, from the time of discovery of questionable breast findings, through the scheduling and conduct of the biopsy, and finally to the time that the biopsy results are learned. Unlike paradigms of acute mental stress, the lasting nature of the emotional experience of breast biopsy permits an evaluation of the psycho-immune response to a threatening event with an extended period of endurance. The data show that the anticipation of breast biopsy and the possibility of a cancer diagnosis lead to a situation marked by heightened perceived stress, elevated state anxiety, and considerable mood disturbance that extends from weeks to months. Sources of anticipatory distress prior to breast surgery include worry, uncertainty and fear about the impending surgical diagnosis as well as concern about the risk of undergoing the procedure itself (Montgomery et al., 2003). Others have previously described the emotional distress that accompanies breast biopsy. However, some of those studies either used a retrospective approach and/or did not assess the dynamics over time of the breast biopsy experience. None of those studies investigated the long-term impact of this experience on immune function (Hughson et al., 1988; Benedict et al., 1994; Northouse et al., 1995; Deane and Degner, 1998; Andrykowski et al., 2002; Lebel et al., 2003; Drageset and Lindstrom, 2003).

The marked elevations in perceived stress, state anxiety, and mood disturbance reported by women before biopsy underscore the emotional intensity that an impending breast biopsy evokes. The sustained nature of this response is evident in that perceived stress, anxiety, and mood disturbance were not only significantly increased before biopsy, but also remained elevated post biopsy. Even though each of these psychological measures decreased after biopsy, women continued to report higher levels of perceived stress, anxiety, and mood disturbance post biopsy relative to the group of women not undergoing breast biopsy. This enduring psychological disturbance was observed for women in the Benign group despite the knowledge that their breast biopsy did not reveal any malignant breast disease. Such prolonged psychological disturbance observed in women with benign breast findings was unexpected and was well beyond the acute biopsy experience. Although the design of this study does not permit an understanding of the impetus for the increased duration of this psychological distress, the results suggest that these women have not completely resolved the psychological impact of the biopsy experience and may harbor feelings of uncertainty and unrealistic fear regarding their risk for developing breast cancer in the future. It is highly likely that these women were advised by their physicians to have more frequent breast screening (i.e., early mammographic recall) and this may have triggered enduring distress and uncertainty. Other studies document prolonged psychologic disturbance in women with benign breast biopsy results. Those studies found that women who had benign breast biopsies (both surgical and fine needle aspirate biopsies) continued to experience greater psychological consequences 1 month, 5 months, and 11 months later, when compared to women who had a clear result (no abnormalities) following their initial mammogram screening (Ong et al., 1997; Brett et al., 1998; Brett, 1999). For some of those women, this psychological distress was observed at 1 month prior to their routine breast screening three years later (Brett and Austoker, 2001). It is likely that the approaching date for breast

screening triggered the re-emergence of this distress for these women. Interestingly, women who have experienced false-positive mammograms also report long-lasting psychological distress. For example, elevations in anxiety in response to an abnormal mammogram continued for months beyond the knowledge that their mammogram was indeed falsely positive (Brett et al., 1998; Brett, 1999; Barton et al., 2004). These women typically undergo additional testing, such as breast ultrasound or biopsy of their breast. Indeed, women undergoing more intensive follow-up (i.e., biopsy or fine-needle aspiration) after their abnormal mammogram, had the greatest levels of distress (Barton et al., 2004). Collectively, the findings of these studies demonstrate that a benign breast biopsy is not always “emotionally benign” and emphasize the need for health care providers to consider the extended psychological costs of breast screening by mammography and/or biopsy, even if these results in the end are benign. Of course the group of women given a diagnosis of cancer, undergo a distinct and prolonged pattern of emotional distress that is magnified by the burden of cancer treatment (Stark and House, 2000). It is especially noteworthy that stress-induced immune dysregulation in this group of women may jeopardize their cancer control (Anderson, 1994; Albertsson et al., 2003).

The psychological distress observed in response to breast biopsy was accompanied by alterations in immune function. NKCA was reduced in anticipation of biopsy (T1 and T2) and this reduction in NKCA continued after biopsy (T3). The time period marked by the T3 (10–14 days after breast surgery) measurement in this study is similar to the time when Anderson *et al.* (1998), who measured NKCA in women diagnosed with breast cancer. That study found reductions in NKCA in women with breast cancer evaluated after surgery but prior to adjuvant therapy (i.e., radiation or chemotherapy). Our results at T3 are consistent and show that these reductions in NKCA extend to women with benign biopsy results. This observation suggests that the reduction in NKCA is due to the psychological experience and not to the malignancy, per se. Given that these reductions in NKCA in the benign group of women were not accompanied by changes in the number of circulating NK cells, and since these women did not experience cancer treatment, it is likely that the psychological experience of breast biopsy affected the functional activity of the circulating NK cells. It is also important to note that measures of NKCA at T3 occurred prior to any radiation, chemotherapy, or tamoxifen; hence, eliminating the influence of these treatment factors. In contrast, the results obtained at T4 from the Malignant group of women may be confounded by cancer treatment (55% underwent radiotherapy, 56% underwent chemotherapy, and 31% were taking tamoxifen at T4). Furthermore, it is also possible that the malignancy itself may independently impact the immune system and consideration of results obtained with the Malignant group of women at all time points (T1–T4) must include this possible confound.

It has become evident that the effect of stress upon the immune system can vary markedly depending upon whether stress is acute or sustained (Mataika, 2003). A large number of studies have shown that psychological stress for prolonged periods can lead to reductions in NKCA (Kiecolt-Glaser et al., 1986; Irwin et al., 1987; Kiecolt-Glaser et al., 1987; Trinchieri, 1989; Weisse, 1992; Kiecolt-Glaser et al., 1993; Andersen et al., 1998). These reductions in NKCA were a consequence of chronic stress and are in direct contrast to changes in NKCA associated with the experience of acute stress wherein NKCA is increased as a consequence of a rapid increase in circulating NK cells (Benschop et al., 1996; Segerstrom and Miller, 2004; Viswanathan and Dhabhar, 2005). Mobilization of NK cells into the circulation is a reproducible cellular immunological response to physical and psychological stressors in humans (Maisel et al., 1990; Naliboff et al., 1991; Brosschot et al., 1992; Gabriel et al., 1992; Murray et al., 1992; Schedlowski et al., 1993) and is a result of changes in the CD56dim subpopulation of NK cells (Bosch et al., 2005). Human NK cells comprise a finite percentage of all peripheral blood lymphocytes and are composed of two distinct subsets identified by cell surface density of CD56 (Robertson and Ritz, 1990). The

majority (approximately 90%) of human NK cells are CD56dim and express high levels of CD16. Whereas a minority (approximately 10%) are CD56bright and CD16dim (Lanier et al., 1986; Cooper et al., 2001). Resting CD56dim NK cells are more cytotoxic against NK-sensitive targets than CD56bright NK cells (Nagler et al., 1989; Cooper et al., 2001). CD56bright NK cells are the primary population responsible for NK cell cytokine production in response to stimulation (Cooper et al., 2001). Our results indicate that reduced NKCA is not due to changes in the numbers of CD56dim or CD56+16+ NK cells, but rather changes in cellular NKCA. Chronic stressors (in this case the experience of breast biopsy) appear to decrease NKCA, not as a result of changes in the number of circulating NK cells but rather as a result of changes in functional activity.

These results may have implications for overall health and quality of life of individuals experiencing chronic stress. Although not definitive, evidence supports a role for NK cells in cancer control. In general, patients with lower immune function appear to have a more prolonged disease course and to suffer more severe symptoms than patients whose immune function remains normal (Cannon et al., 1980; Whiteside and Herberman, 1994). Chronically stressed, but healthy individuals with decrements in the cellular immune response (including NKCA) have a higher incidence of infection (Kiecolt-Glaser et al., 1991). Hence, individuals who experience high levels of stress and concomitant reductions in T lymphocyte responsiveness and decreased NK cell function may be at greater risk for infectious illnesses (Biron, 1999; Lanier, 2001). NK cells release cytokines and chemokines that mediate inflammatory responses that induce hematopoiesis and modulate subsequent adaptive immune responses (Trinchieri, 1989; Biron et al., 1999). These lymphocytes (which are considered part of the innate immune system) are fast-acting frontline defenders that have the capacity to swiftly migrate in and out of tissues, and are among the first immune cells to arrive at sites of tissue damage and infection (Allavena et al., 1996; Somersalo, 1996). NK cells have the ability to immediately destroy their targets (e.g., infected cells) and to attract and activate other immune cells. Hence, stress may diminish resistance to infection following breast biopsy and may be disadvantageous to cancer control.

To our knowledge, no other study has used a psychoneuroimmunologic framework to evaluate the psychological effect of the experience of breast biopsy on NKCA and cytokine production. Th1, Th2, and proinflammatory cytokine production fell into three distinct categories for the women in the breast biopsy group. Production either remained unchanged (IL-2), showed an increase compared to control subjects (IL-4, IL-6, and IL-10), or alternatively showed a reduction when compared to control subjects (IFN γ). IL-2 is known to increase NKCA and NK cell proliferation (London et al., 1986) and IFN γ is known to enhance NK cell cytotoxicity (Vose et al., 1983; Reiter, 1993; Biron et al., 1999). It is clear from the data that NKCA is decreased in the women undergoing breast biopsy, while IL-2 production remained the same as that observed in control women, suggesting that the subjects had the capacity to produce IL-2 but this capacity did not impact NKCA. The production of IFN γ is decreased in these women and may relate to the observed reductions in NKCA. Stress can down-regulate NKCA and modulate IFN γ and IL-2 synthesis (Levy et al., 1987). Heightened levels of stress are related to decreased synthesis of IFN γ by lymphocytes from healthy subjects (Kiecolt-Glaser et al., 1986). A poorer NK response to IFN γ and/or IL-2 was observed in stressed individuals compared to non-stressed individuals (Fawzy et al., 1990; Esterling et al., 1994; Esterling et al., 1996; Andersen et al., 1998). Stress reduction interventions modulate NKCA and cytokine synthesis (Esterling et al., 1994) suggesting that the link between stress and NK function may be mediated, in part, by cytokines.

An alternative explanation for the cytokine data is possible. Lymphocytes and the cytokines they produce can be placed into naïve, central memory, or effector memory subsets (Tussey et al., 2000; Martin-Fontecha et al., 2004; Sallusto et al., 2004). Naïve and central memory lymphocytes primarily produce IL-2 upon short-term stimulation (e.g. the 48 hr period employed in this study). In contrast, and under similar conditions, effector memory lymphocytes quickly produce IFN γ , IL-4, and IL-10. It is possible that the effect of the perceived stress, anxiety, and mood disturbance of breast biopsy is primarily upon lymphocytes that are capable of immediate production of these effector cytokines. Specifically, the capacity of effector memory lymphocytes to elaborate their cytokines may be markedly affected by the experience of breast biopsy. This interpretation is consistent with the data presented herein. With regard to IL-6, this cytokine is produced by a variety of cell populations and is associated with inflammatory events. The data described herein show that IL-6 is markedly increased in its production during the assessed time periods and is consistent with the previously demonstrated elevation in this cytokine during periods of stress (Maes et al., 1999; Chrousos, 2000). Much like IL-6, IL-4 and IL-10 were significantly increased in the biopsied women compared to the non-biopsied control women. This increase in IL-4 and IL-10 is similar to other reports that have demonstrated stress-associated shifts in Th1/Th2 cytokine balance toward a Th2 type of response (Marshall et al., 1998). The most dominant factor in the regulation of the Th1/Th2 cytokine balance is the cytokine environment of the immune response. IFN γ promotes the development of Th1 responses and inhibits Th2 responses (Parronchi et al., 1992; Manetti et al., 1993). In contrast, IL-4 promotes the development of a Th2 response and IL-4 and IL-10 inhibit the development of a Th1 response (Parronchi et al., 1992; Manetti et al., 1993; Rennick et al., 1995). Our data are consistent with the concept that in the absence of a Th1 response, conditions could permit the increased production of Th2 cytokines, which could result in the inhibition of Th1 responses and possibly NKCA (Agarwal and Marshall Jr., 2001).

These results provide evidence that the experience of breast biopsy for cancer diagnosis, leads to prolonged periods of stress, anxiety, and mood disturbance that appear to be associated with depressed NKCA and an altered pattern of cytokine production. Importantly, it appears that stress-induced alterations in the immune system are not transient but persist beyond the acute experience of breast biopsy. This may be of particular relevance to women diagnosed with malignancy since they face additional stressors related to cancer treatment and adaptation to illness.

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Figure 1.

Psychological measure of perceived stress, anxiety, and mood disturbance are separately depicted for women who ultimately had Benign or Malignant findings and for a non-biopsied, comparison group of Control women. Perceived stress was measured using Cohen's Perceived Stressor Scale (PSS). Anxiety was measured using Spielberger's State Anxiety Inventory (STATE ANXIETY). Mood state was measured using the Profile of Mood States (POMS) and the total mood disturbance (POMS-TMD) is depicted. Bars represent the mean values $\pm$ S.E. and are indicated for pre biopsy time points (T1 and T2) and post biopsy time points (T3 and T4). T1 was the time of notification of a need for breast biopsy, T2 was day of breast biopsy immediately before the procedure, T3 was the first post-

biopsy time point (approximately 1- month after notification of results), and T4 was the second post-biopsy time point (approximately 2–3 months after T3). Statistical comparisons (ANOVA followed by Tukey's HSD post hoc test) were between experimental mean and Control as well as between Benign and Malignant at each time period. Bars represent the mean values $\pm$ S.E.

Figure 2.

The subscales of the POMS are depicted for women with Benign and Malignant findings and for a non-biopsied, comparison group of Control women. Results are presented as in Figure 1. Bars represent the mean values $\pm$ S.E.

Figure 3.

NKCA, expressed as lytic units at 20%, and IFN γ production by PBMC of women with Benign and Malignant findings and for a non-biopsied, comparison group of Control women are illustrated. NKCA for PBMC was measured using K562 tumor cells as the target. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. Results are presented as in Figure 1. Bars represent the mean values $\pm$ S.E.

Figure 4.

IL-6, IL-4, and IL-10 production by PBMC of women with Benign and Malignant findings and for a non-biopsied, comparison group of Control women are illustrated. PBMC were activated with PMA/PHA and culture supernatants were collected at 48 hr. Cytokine concentration was determined by ELISA. Results are presented as in Figure 1. Bars represent the mean values $\pm$ S.E.

Figure 5.
Phenotypic analysis of PBMC for women who were biopsied. Bars represent the mean values $\pm$ S.E.

Table 1

Demographic Characteristics of the Study Groups.

Characteristics	Women in the Comparison Group (Control) N=29			Women Diagnosed With Breast Cancer (Malignant) N=22			Women With Benign Biopsy Findings (Benign) N=70		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Age (years)	54.9	9.1	2.4	60.3	12.3	2.2	50.1	13.0	1.5
Weight (lbs.)	159.6	22.7	4.6	171.0	41.3	7.8	168.6	37.1	4.2
Body Mass Index (Kg/m ²)	28.4	4.1	0.8	28.7	7.2	1.7	29.1	6.5	0.7
Education (Total Years)	18.0	3.7	0.7	13.5	2.6	0.7	14.4	2.6	0.3
Marital Status									
Married	78%				58%			72.4%	
Single	22%				42%			27.6%	
Employment									
Employed	84%				38%			71%	
Unemployed	16%				62%			29%	
Religious Affiliation									
Yes	85%				100%			97%	
Race									
Caucasian	93%				89%			85%	
African American	7%				11%			12%	
Hispanic	0%				0%			3%	
Support Systems									
Multiple	100%				100%			96%	
None	0%				0%			4%	
Familial Cancer History									
Yes	66%				68%			83%	
No	34%				32%			17%	
Hormone Replacement Therapy Upon Study Entry									
Yes	20%				5%			23%	