

## Social Support, Psychological Distress, and Natural Killer Cell Activity in Ovarian Cancer

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### A B S T R A C T

#### Purpose

Psychosocial stress has been related to impaired immunity in cancer patients. However, the extent to which these relationships exist in immune cells in the tumor microenvironment in humans has not been explored. We examined relationships among distress, social support, and natural killer (NK) cell activity in ovarian cancer patients in peripheral-blood mononuclear cells (PBMC), ascitic fluid, and tumor-infiltrating lymphocytes (TIL).

#### Patients and Methods

Patients awaiting surgery for a pelvic mass suspected of being ovarian cancer completed psychological questionnaires and gave a presurgical sample of peripheral blood. Samples of tumor and ascites were taken during surgery, lymphocytes were then isolated, and NK cytotoxicity and percentage were determined. The final sample, which was confirmed by surgical diagnosis, included 42 patients with epithelial ovarian cancer and 23 patients with benign masses.

#### Results

Peripheral NK cell activity was significantly lower among ovarian cancer patients than in patients with benign masses. Among ovarian cancer patients, NK cytotoxicity in TIL was significantly lower than in PBMC or ascitic fluid. Social support was related to higher NK cytotoxicity in PBMC and TIL, adjusting for stage. Distress was related to lower NK cytotoxicity in TIL. A multivariate model indicated independent associations of both distress and social support with NK cell activity in TIL.

#### Conclusion

Psychosocial factors, such as social support and distress, are associated with changes in the cellular immune response, not only in peripheral blood, but also at the tumor level. These relationships were more robust in TIL. These findings support the presence of stress influences in the tumor microenvironment.

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### INTRODUCTION

The severe emotional distress accompanying a diagnosis of cancer and its initial treatment has been extensively documented.<sup>1,2</sup> Because of the seriousness of the disease, women with ovarian cancer are at particular risk for depression and anxiety around the time of diagnosis.<sup>3,4</sup> The adverse effects of stress and distress (negative mood states such as anxiety, dysphoria, and anger) on the immune response in cancer have been

well documented.<sup>5-8</sup> In clinical populations, these effects include impaired natural killer (NK) cell activity, poorer NK cell response to stimulation by interferon gamma, and decreased T-cell proliferative response to mitogens.<sup>5,6,9</sup> Experimental stressors have been associated with diminished NK cell cytotoxicity (NKCC) and increased tumor progression in animal models of cancer.<sup>7,10</sup>

In contrast, social support, defined as perceived satisfaction with social relationships, is thought of as a psychological

resource, particularly during stressful circumstances such as cancer diagnosis and impending surgery.<sup>11</sup> High levels of social support have been consistently associated with positive health outcomes.<sup>12</sup> A substantial amount of literature supports a reliable positive association between social support and the immune response,<sup>13-15</sup> including more robust NKCC and longer recurrence-free survival among breast cancer patients,<sup>14,16</sup> although not all findings have been consistent.<sup>17</sup> One model proposes that social support does not directly influence the immune response but, instead, functions by decreasing distress, which has subsequent effects on the immune response.<sup>13</sup>

NK cells are thought to play a significant role in inhibition and surveillance of tumor metastases,<sup>18</sup> and impairments in NK activity have been associated with ovarian cancer progression.<sup>19</sup> In ovarian cancer, immune cells are found in the ascitic fluid surrounding the tumor, in tumor-infiltrating lymphocytes (TIL), and in peripheral-blood mononuclear cells (PBMC).<sup>20</sup> Significantly lower NKCC has been observed in PBMC of advanced ovarian cancer patients compared with healthy controls,<sup>21,22</sup> with further decrements observed in ascites<sup>22,23</sup> and TIL.<sup>24</sup> A longitudinal study of advanced ovarian cancer patients found significantly lower peripheral NKCC before initial surgery among patients whose disease ultimately progressed compared with patients without progression.<sup>19</sup> In the patients who did have disease progression, further reductions in NKCC were observed at the time of disease recurrence, suggesting the potential prognostic importance of NKCC in ovarian cancer.<sup>19</sup> In another study, *in vivo* augmentation of NKCC using a virus-modified allogeneic tumor cell extract in ovarian cancer patients with both malignant ascites and palpable abdominal tumor masses resulted in profound increases of NKCC in ascites and regression of malignant ascites, suggesting the importance of NK cells in defense against ovarian tumors.<sup>22,23</sup>

Psychosocial-immune research in clinical populations has generally examined relationships of psychosocial factors with the immune response in peripheral blood. However, tumor cells downregulate many aspects of the immune response, including alterations of signal transduction.<sup>24</sup> Altered expression and activity of signaling molecules in NK cells derived from ascites of ovarian patients and concomitant impairments in cellular immunity and cytokine expression have been reported in ovarian cancer.<sup>24,25</sup> Cellular immunity in lymphocytes isolated from the tumor microenvironment is often profoundly suppressed.<sup>22,26</sup> Thus, examination of the peripheral immune response may provide limited information about the integrity of the cellular immune response in the tumor microenvironment. A computerized Medline search between 1966 and 2005 revealed no human clinical studies that had examined relationships of psychosocial factors with functional activity of immune cells isolated from the vicinity of the tumor. Ovarian carci-

noma was selected because the majority of women with epithelial ovarian cancer are diagnosed with advanced-stage disease and their 5-year survival rate is approximately 29%.<sup>27</sup> Thus, identification of factors contributing to compromised or enhanced host resistance at the earliest possible stage may have implications for survival.

The primary focus of this study was to examine relationships among the psychosocial factors of distress, depressed mood, and social support and the innate immune response in mononuclear cells derived from three compartments (PBMC, ascitic fluid, and TIL) among ovarian cancer patients. Comparisons with a reference group of benign patients who underwent surgery for suspected ovarian cancer were performed for assistance in interpretation of findings, given the common surgical stressor. It was hypothesized that social support would be associated with higher levels of NKCC in each compartment and that distress or depressed mood would be associated with impaired NKCC in each compartment. It was further hypothesized that the association of social support with lower distress would account for any observed relationships between social support and NKCC.

## PATIENT AND METHODS

### Patients

**Inclusion criteria.** This study was approved by the University of Iowa Institutional Review Board. Women over 18 years of age with a new diagnosis of a pelvic or abdominal mass suspected for ovarian cancer were eligible for the study. Inclusion in the study as an ovarian cancer patient was confirmed after histologic diagnosis of a primary invasive epithelial ovarian, primary papillary peritoneal, or fallopian tube malignant tumor. Patients with a cancer history, a primary cancer of another organ, nonepithelial ovarian malignant tumors or low malignant potential tumors, history of systemic corticosteroid medication use in the last 4 months, or comorbidities known to alter the immune response (eg, HIV, multiple sclerosis, or lupus) were excluded. Patients who were found to have benign ovarian neoplasms at surgery and who did not have inflammatory conditions, such as endometriosis, were included as a comparison group.

**Sample characteristics.** Of 152 potentially eligible patients with a newly diagnosed pelvic or abdominal mass suspicious for an ovarian malignancy who were approached for study participation, 128 (84.2%) agreed to participate. A total of 47 patients were excluded for reasons such as cancellation or rescheduling of surgery, surgery not conducted at study site, surgery preceded by chemotherapy, nonovarian or low malignant pathology, inflammatory disease, or difficulty with venous access. Eleven patients withdrew from the study before surgery; reasons for withdrawal included time constraints or emotional distress before surgery. Peripheral NKCC data was not available for five assays because of technical reasons (three benign patients and two cancer patients) such as poor viability of target cells. Patients were included in the final sample if they had valid NKCC available in peripheral blood. Thus, the final sample included 42 ovarian cancer patients and 23 patients with benign pelvic masses. Adequate cells to perform an NKCC assay were isolated from ascites in 27 patients and from tumor cells in 20 patients. The majority

of ovarian cancer patients (83.4%) had advanced-stage disease (stages III and IV) with predominantly high-grade tumors.

### Procedure

Patients were recruited before surgery at their initial clinic visit. Eligibility for study participation was confirmed by histologic examination at the time of surgery. Patients completed psychosocial questionnaires and gave a 40-mL sample of peripheral venous blood in heparinized vacutainer tubes (Becton Dickinson Co, Rutherford, NJ) on the morning of surgery before administration of preoperative medication or general anesthesia. Peripheral-blood samples were taken between 6:00 AM and 12:00 PM to control for potential circadian variation. Samples of ascites and tumor were obtained from surgery and were immediately processed as described in the following sections.

### Immunologic Measures

**Cytolytic activity of NK cells.** Fresh mononuclear cells isolated from peripheral blood, ascites, and tumor were tested for cytolytic activity against the NK-sensitive K562 cells and the NK-resistant, lymphokine-activated killer cell-sensitive ZKBL target cells in a standard 4-hour separated blood <sup>51</sup>chromium-release assay, as used previously in this laboratory.<sup>28</sup> Results are reported as percent specific lysis using the following formula: percent lysis = (experimental CPM – spontaneous CPM/maximal CPM – spontaneous CPM) × 100, where CPM equals gamma counts per minute. Because of generally low values of NKCC at the lower effector to target (E:T) ratios, particularly in TIL, NK cell activity data were expressed at the maximum specific lysis, which in all cases occurred at the 100:1 E:T ratio. In secondary analyses, NK cell activity was expressed as area under the curve (AUC) across all E:T ratios (calculated using the trapezoidal method). This estimation has previously been used in NK analyses and is thought to be more reliable than measures based on a single E:T ratio or on lytic units.<sup>29</sup> The correlation between AUC and specific lysis at the 100:1 ratio is  $r = 0.98$  for PBMC,  $r = 0.96$  for ascites, and  $r = 0.99$  for TIL. In the data presented in Results, NKCC refers to percent specific lysis at the E:T of 100:1. A cohort of normal controls from laboratory personnel was used to control for day-to-day assay variability. Assays were deemed invalid when the laboratory control value was more than 2.0 standard deviations (SDs) from the mean of their repeated assays.

**Separation of tumor cells and lymphocytes in ascites and tumor.** Ascites were filtered through sterile mesh filters before centrifugation at  $650 \times g$  at 4°C for 10 minutes. Cells were isolated using a Ficoll gradient. After washing three times with medium, the cells were resuspended in CTL media and counted in 10% acetic acid. Lymphocytes were then separated from tumor cells using anti-CD45 human microbeads (Miltenyi Biotec, Auburn, CA).

Tumor cell samples were collected in saline solution and immediately processed. The tumor was minced and then digested in a mixture of 4 mg/mL each of collagenase (Sigma, St Louis, MO) and hyaluronidase (Sigma) and 400 µg/mL DNase (Sigma) dissolved in Hanks' BSS (Gibco, Grand Island, NY). All materials were transferred to a sterile digesting container with a magnetic stir bar for approximately 3 to 6 hours at room temperature. After digestion, cells were filtered through sterile mesh filters using CTL media and were collected in sterile conical tubes. Cell counts and separation into tumor- and lymphocyte-enriched fractions were performed by methods described earlier.

**Labeling of CD3/CD56 NK cells.** Cell suspensions from patients were prepared in CTL medium at a concentration of  $5 \times 10^6$  cells/mL. Working antibody sets (BD Pharmingen, San Diego,

CA) suspended in human serum staining buffer included the following: (experimental) well 1: 5 µL of fluorescein isothiocyanate anti-CD3 antibody plus 2.5 µL of phycoerythrin anti-CD56 antibody; and isotype control well 2: 1 µL of fluorescein isothiocyanate-immunoglobulin G1 plus 1 µL of immunoglobulin G1-phycoerythrin. Cells were stored at 4°C until analysis on a flow cytometer (FACScan; Becton Dickinson, Franklin Lakes, NJ). Data were analyzed using FlowJo Software version 4.4 (Tree Star, Ashland, OR) and expressed as percent CD3<sup>+</sup>CD56<sup>+</sup> cells. Flow cytometry data for two cancer patients were judged invalid and not used in analyses.

### Psychosocial Measures

**Perceived social support.** The Social Provisions Scale<sup>30</sup> is a 24-item self-report scale measuring the degree to which an individual perceives their social relationships as supportive. The scale has demonstrated adequate reliability and validity in a number of studies with different populations.<sup>31,32</sup> The facet of social support of interest in this study was attachment, which assesses perceived emotional bonds with others and has been shown to be relevant in a number of stressed populations.<sup>13,30</sup>

**Distress.** The Profile of Mood States Short Form<sup>33,34</sup> is a scale that lists 37 mood-related adjectives to which subjects respond according to their mood over the past week. These are rated on a 5-point scale from 0 (not at all) to 4 (extremely), and scores are summed to create subscales of anxiety, depression, anger, vigor, fatigue, and confusion. A distress composite (total mood disturbance) is created by summing all factors except vigor and subtracting vigor from the total score. This composite was used in this study to provide a comprehensive examination of patient distress. Depressed mood was analyzed as a second facet of mood disturbance to provide more specific data on dysphoric symptoms.

**Demographic and biophysical information.** Biophysical factors, such as hours of sleep and intake of alcohol, coffee, and cigarettes, within the last 7 days before the blood sampling were assessed to control for possible immune confounds. Demographic information was collected, and clinical and histopathologic information was obtained from medical records.

### Statistical Analyses

All distributions were examined for outliers and non-normality. A square root transformation was used to normalize NKCC and NK AUC data, which was positively skewed. All analyses were performed first on NKCC at the 100:1 E:T ratio, and secondary analyses were performed on the NK AUC. All analysis of variance (ANOVA) models used two-sided tests, and  $P < .05$  was considered significant. ANOVAs were used to compare cancer patients and benign controls on age and biophysical variables. Analyses of covariance, adjusting for age, were used to compare psychosocial and immune variables between ovarian cancer and benign patients. The  $\chi^2$  test was used to compare categorical demographic variables between groups. Pearson correlations were computed to test relationships between psychological and immune variables. A repeated-measures multivariate ANOVA was used to compare the immune variables between the different compartments to help control the overall type I error rate. Because of the varying sample sizes among the compartments, paired  $t$  tests were used as follow-up tests to allow inclusion of all available data for each pair-wise comparison. Regressions were used within each compartment of ovarian cancer patients to examine relationships of psychosocial variables with NK cell activity. First, univariate relationships between psychosocial variables and NKCC or NK

AUC were modeled within each compartment. Subsequently, multivariate relationships were examined within each compartment, examining both distress and social support simultaneously. For these regressions, as a conservative measure, stage was included as a covariate to control for possible influence of disease extent on NK activity.

## RESULTS

### Patient Characteristics

The mean age of participants was 56.5 years (range, 29 to 79 years). Participants were predominantly white. Patients with benign neoplasms were significantly younger (mean age, 51.17 years; SD = 11.26 years) than cancer patients (mean age, 59.21 years; SD = 10.07 years;  $P = .004$ ); thus, age was included as a covariate in all between-group ANOVAs. There were no significant differences between groups in demographics, alcohol use, hours of sleep over the last week or over the last night, exercise frequency, coffee consumption, or percentage of current smokers (all  $P > .22$ ; Table 1). There were no significant associations of any demographic or biophysical variable with NKCC in any compartment (all  $P > .19$ ). Additionally, there were no significant differences in peripheral NKCC associated with the use of beta-blockers ( $P > .75$ ), hormone replacement therapy ( $P > .18$ ), and antidepressants ( $P > .23$ ), and these factors were not related to NKCC in ascites or TIL (all  $P > .25$ ). There were no significant differences in peripheral NKCC ( $P > .14$ ) or percent ( $CD3^+CD56^+$ ) NK cells ( $P > .60$ ) in PBMC according to stage.

### Psychosocial and Immune Factors in Patients With Ovarian Cancer and Benign Masses

As seen in Table 2, there were no significant differences in distress ( $P = .90$ ), depressed mood ( $P = .81$ ), or social support ( $P = .08$ ) between groups, adjusting for age. However, adjusting for age, patients with benign masses had significantly higher mean peripheral NKCC than ovarian cancer patients, both at the 100:1 E:T ratio ( $F_{1,62} = 5.24$ ,  $P = .025$ ) and for NK AUC ( $F_{1,62} = 4.23$ ,  $P = .044$ ). Percentage of NK cells in PBMC was elevated (although not significantly) in ovarian cancer patients compared with patients with benign neoplasms ( $F_{1,48} = 2.60$ ,  $P = .11$ ).

### NK Cell Activity and Percentage in Three Compartments Among Ovarian Cancer Patients

An omnibus multivariate ANOVA indicated that, among ovarian cancer patients, there was a significant overall difference in the mean NKCC in the three compartments ( $F_{2,28} = 9.80$ ,  $P = .001$ ). As seen in Figure 1, NKCC in PBMC was significantly higher than NKCC in TIL ( $t = 4.66$ ,  $P < .001$ ) and marginally higher than NKCC in ascites ( $t = 1.95$ ,  $P = .06$ ). NKCC in ascites was significantly higher than NKCC in TIL ( $t = 2.65$ ,  $P = .019$ ). For NK AUC, the overall test was significant as well ( $P = .001$ ). NK AUC in PBMC was significantly

Characteristic	% of Patients	
	Cancer Patients (n = 42)	Benign Patients (n = 23)
Age, years		
Mean	59.4	51.2
Standard deviation	10.1	11.3
Education*		
Some high school	10.3	10.0
High school graduate	33.3	20.0
Trade school/some college	35.9	45.0
College graduate	12.8	20.0
Postgraduate	7.7	5.0
Marital status*		
Single	10.0	10.0
Divorced	12.5	20.0
Widowed	5.0	10.0
Married/living with partner	72.5	60.0
Stage		
I	7.1	
II	9.5	
III	61.9	
IV	21.5	
Grade		
1	7.1	
2	28.6	
3	64.3	
Tumor histology		
Serous	76.19	
Endometrioid	7.14	
Mucinous	7.14	
Other	9.53	
Residual disease		
Yes	64.3	
No	35.7	

\*Educational and marital status information was missing for three benign patients and two cancer patients; an additional cancer patient was also missing educational information.

cantly higher than NK AUC in TIL ( $t = 4.49$ ,  $P < .001$ ) and in ascites ( $t = 2.34$ ,  $P = .027$ ), and NK AUC in ascites was significantly higher than NK AUC in TIL ( $t = 2.42$ ,  $P = .030$ ). Mean percentages of NK cells did not significantly differ between compartments (overall ANOVA,  $P > .35$ ).

### Associations Among Psychosocial Variables and NK Cell Activity in Ovarian Cancer Patients

As seen in Table 3, univariate regressions indicated that patients with higher levels of social support had significantly higher NKCC (100:1 E:T ratio) in both PBMC ( $\beta = .39$ ,  $P = .017$ ) and TIL ( $\beta = .48$ ,  $P = .045$ ). In contrast, patients with greater distress showed significantly poorer NKCC in TIL ( $\beta = -.58$ ,  $P = .02$ ). The pattern was similar in PBMC but did not reach significance ( $\beta = -.22$ ,  $P = .21$ ). These findings are particularly noteworthy given the effect sizes in TIL, which were 0.29 for social support and 0.47 for distress. According to Cohen,<sup>35</sup> these would be

**Table 2.** Age-Adjusted Means of Psychosocial and Immune Measures

Measure	No. of Benign Patients	No. of Cancer Patients	Benign Patients		Ovarian Cancer Patients	
			Mean	95% CI	Mean	95% CI
Total distress, score	19	33	30.86	7.23 to 44.96	29.72	19.58 to 39.85
Depressed mood, score	19	33	7.91	4.06 to 11.74	8.51	5.66 to 11.37
Social support, score	20	38	13.63	12.68 to 14.58	14.69	14.02 to 15.37
NK cytotoxicity, PBMC, % specific lysis at 100:1	23	42	39.67	31.83 to 47.51	27.30*	21.55 to 33.07
NK cytotoxicity AUC, PBMC	23	42	2,765.18	2,149.90 to 3,380.47	2,008.12†	1,561.32 to 2,454.92
% NK (CD3 <sup>-</sup> CD56 <sup>+</sup> ) cells in PBMC	18	34	9.62	6.54 to 12.70	12.73	10.48 to 14.98
% NK (CD3 <sup>-</sup> CD56 <sup>+</sup> ) cells in ascites‡	0	22			17.54	11.95 to 23.13
% NK (CD3 <sup>-</sup> CD56 <sup>+</sup> ) cells in TIL‡	0	12			14.94	9.58 to 20.30

NOTE. For psychosocial questionnaires, number of patients shown represents individuals who completed the questionnaire. Abbreviations: NK, natural killer; PBMC, peripheral-blood mononuclear cells; AUC, area under the natural killer cytotoxic curve; TIL, tumor-infiltrating lymphocytes.

\**P* = .015.

†*P* < .05.

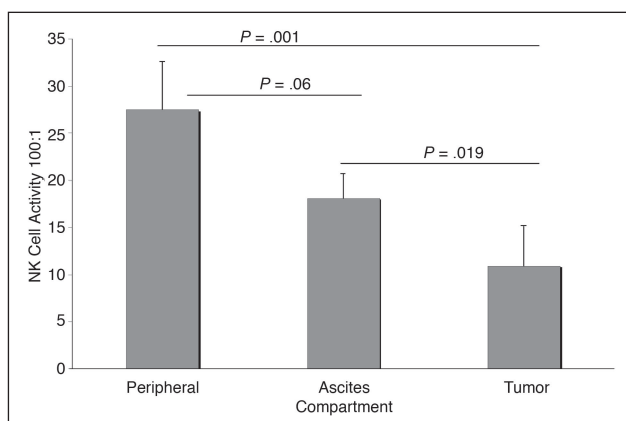
‡Means and CIs for percent NK cells in ascites and TIL are not age adjusted because they were not computed within an analysis of variance model.

considered large effect sizes. Parallel regression models using NK AUC showed a similar pattern of findings, and these findings are listed in Table 3. To account for possible effects of stage, we also examined relationships of social support and distress with NKCC in PBMC and TIL after adjusting for tumor stage (Figs 2 and 3).

In a multivariate model including stage, social support, and distress, both social support and distress were independent predictors of NKCC at the 100:1 E:T ratio in TIL, with greater social support associated with greater NKCC ( $\beta = .51, P = .03$ ) and greater distress associated with more impaired NKCC ( $\beta = -.51, P = .018$ ). In the multivariate model in PBMC, although greater social support was associated with higher NKCC, this relationship did not reach significance. There were no significant relationships of psy-

chosocial variables with NKCC in ascites in either univariate or multivariate models. Parallel regression models testing these relationships with NK AUC as the outcome variable demonstrated similar relationships (Table 3). When these analyses were repeated examining depression rather than distress, similar patterns to those just described were found in all compartments. Univariate analyses found depressed mood to be a significant predictor of NKCC in TIL, and multivariate analyses indicated that both social support and depressed mood were independent predictors of NKCC (100:1 E:T ratio) in TIL (*P* = .013 for each variable). As with distress, there was no relationship of depressed mood with NKCC in PBMC or ascites. Similar patterns were seen using NK AUC in TIL as the outcome variable (multivariate analyses: social support, *P* = .015; depression, *P* = .024). These psychosocial variables were not significantly associated with percentage of NK cells in any compartment.

It should be noted that some of the regression analyses, particularly the multivariate models, were performed with relatively low power and, thus, may underestimate the relationships involved. For example, posthoc power analyses indicated that, although the univariate relationship was significant, power for testing the relationship between social support and peripheral NKCC was 0.71 (*n* = 48 for 0.80 power), suggesting that, with a larger sample, the multivariate relationship may have been significant. In contrast, the relationship between distress and peripheral NKCC would have required 176 patients to reach 0.80 power, and the relationship between social support and ascites NKCC would have required 2,175 patients to attain 0.80 power. This suggests that there is minimal relationship between these variables. The relationship between distress and ascites NKCC would have required 82 patients for 0.80 power.



**Fig 1.** Means and SEs for natural killer (NK) cell activity (100:1) in three compartments in invasive epithelial ovarian carcinoma patients. NK cell cytotoxicity (NKCC) in peripheral-blood mononuclear cells (PBMC) was significantly higher than in tumor-infiltrating lymphocytes (TIL; *P* < .001) and marginally higher than in ascites (*P* = .06). NKCC in ascites was significantly higher than in TIL (*P* = .019). Means are for all available samples (TIL, *n* = 20; ascites, *n* = 27; PBMC, *n* = 42).

**Table 3.** Univariate and Multivariate Models Showing Associations of Social Support, Distress, and Natural Killer Cell Cytotoxicity in Three Compartments in Ovarian Cancer Patients

Compartment and Variable	Standardized Univariate $\beta^*$	<i>t</i>	<i>P</i>	Standardized Multivariate $\beta^\dagger$	<i>t</i>	<i>P</i>
Peripheral blood, 100:1 E:T ratio						
Stage	.12	0.80	.43	.07	0.37	.71
Social support	.39	2.50	.017	.30	1.65	.11
Distress	-.21	-1.81	.25	-.18	-1.00	.33
Peripheral blood, area under the curve						
Stage	.19	1.21	.23	.14	0.78	.44
Social support	.39	2.56	.015	.29	1.64	.11
Distress	-.22	-1.27	.21	-.21	-1.20	.23
Ascites, 100:1 E:T ratio						
Stage	.23	1.17	.25	.28	1.19	.25
Social support	.06	0.29	.78	-.12	-0.48	.64
Distress	-.30	-1.33	.20	-.32	-1.32	.21
Ascites, area under the curve						
Stage	.28	1.43	.16	.39	1.76	.10
Social support	.029	0.14	.89	-.18	-0.51	.62
Distress	-.31	-1.39	.18	-.34	-1.46	.17
TIL, 100:1 E:T ratio						
Stage	.36	1.62	.12	.27	1.53	.15
Social support	.48	2.18	.045	.51	2.89	.013
Distress	-.58	-2.69	.018	-.51	-2.90	.013
TIL, area under the curve						
Stage	.41	1.88	.076	.27	1.49	.16
Social support	.48	2.17	.045	.48	2.65	.02
Distress	-.57	-2.61	.021	-.50	-2.77	.018

Abbreviations: E:T ratio, effector to target ratio; TIL, tumor-infiltrating lymphocytes; PBMC, peripheral-blood mononuclear cells.

\*Results from univariate models with stage or psychosocial variables in which each variable was entered separately. PBMC: stage, *n* = 41; social support, *n* = 37; distress, *n* = 32; ascites: stage, *n* = 26; social support, *n* = 24; distress, *n* = 19; TIL: stage, *n* = 19; social support, *n* = 17; distress, *n* = 15.

†Results from multivariate models including the psychosocial variables simultaneously, adjusting for stage. PBMC: *n* = 32; ascites: *n* = 19; TIL: *n* = 15.

In TIL, conditions for a test of mediation were not met<sup>36</sup> because social support and distress were not significantly correlated ( $r = -0.12, P = .50$ ), and entering distress into the regression equation on the second step before social support did not eliminate the unique significant effect of social support in this model. This indicates that the relationship of social support with NKCC was not a result of distress.

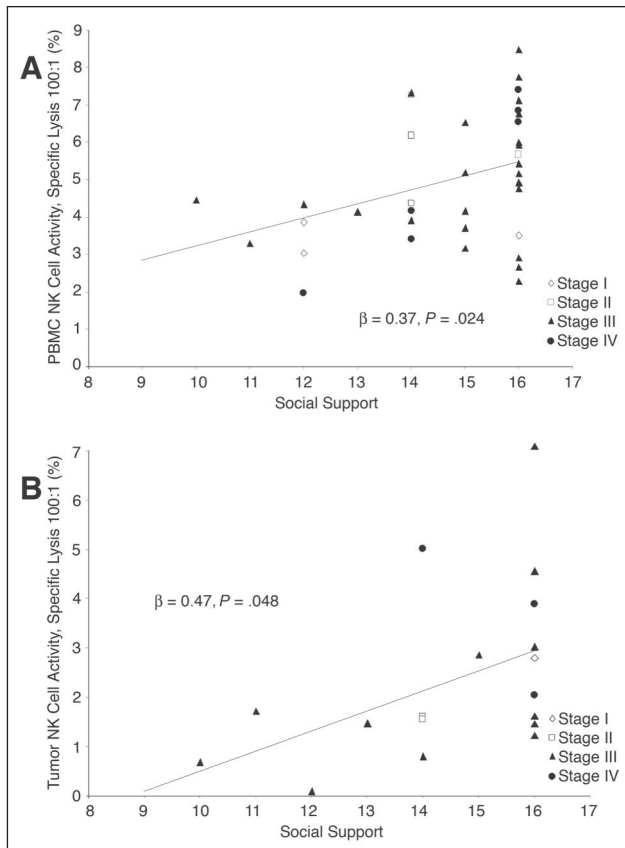
## DISCUSSION

The present findings extend existing literature by demonstrating that, not only is there an association between psychosocial factors and a measure of the cellular immune response (NK cell activity) in PBMC in ovarian cancer patients, but also that psychosocial factors are associated with NK cell activity in TIL as well. Specifically, patients with greater social support had higher levels of NK cell activity both in PBMC and TIL, whereas patients with greater distress had more impaired NK cell activity in TIL. A multivariate model indicated that both social support and distress were independently associated with NK cell activity

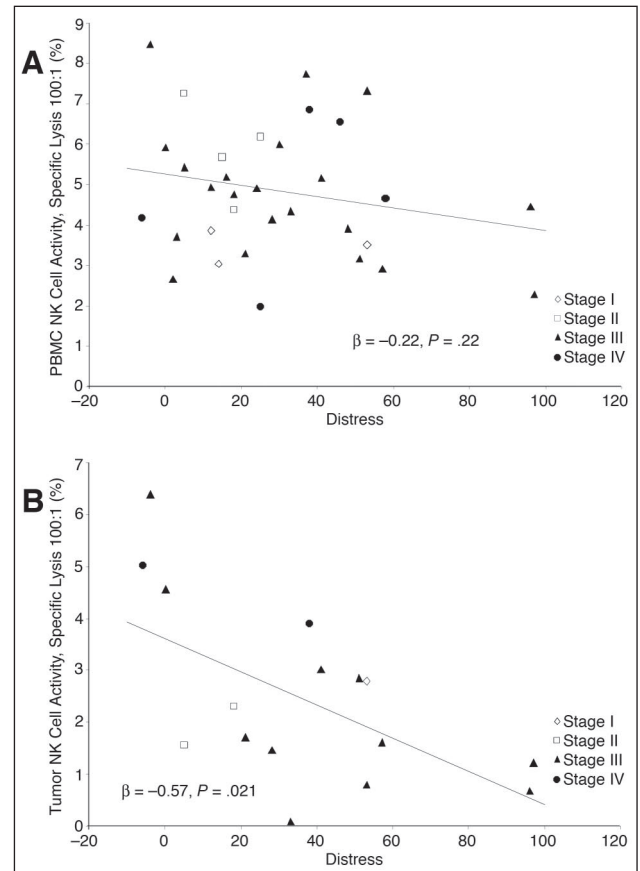
in TIL, adjusting for cancer stage. Two measures of NK activity (specific lysis at the 100:1 E:T ratio and AUC) showed similar results. To the best of our knowledge, this is the first study to demonstrate a relationship between a psychosocial factor and a functional cellular immune parameter in immune cells isolated from a human tumor.

NK cell activity was significantly diminished in ovarian cancer patients compared with patients with benign neoplasms. All patients were uniformly counseled before surgery regarding a likelihood of ovarian cancer. Because patients with benign neoplasms demonstrated equivalent levels of presurgical distress to those reported by ovarian cancer patients, it is unlikely that the immune decrements reported here were merely secondary to greater distress among ovarian cancer patients or were related to differing presurgical conditions between patient groups. The observed differences in lytic activity of NK cells suggest a downregulation in cytotoxicity, even of peripheral NK cells, in ovarian cancer patients, with a further diminution of NKCC in TIL.

Some controversy exists about the efficacy and relevance of the immune response, particularly that in PBMC,



**Fig 2.** Social support and natural killer (NK) cell activity (100:1) in (A) peripheral-blood mononuclear cells (PBMC;  $\beta = .37$ ,  $P = .024$ ) and (B) tumor-infiltrating lymphocytes ( $\beta = .47$ ,  $P = .048$ ) in patients with invasive epithelial ovarian carcinoma. All analyses were adjusted for cancer stage. Regression line is representative of all stages of disease.



**Fig 3.** Distress and natural killer (NK) cell activity (100:1) in (A) peripheral-blood mononuclear cells (PBMC;  $\beta = -.22$ ,  $P = .22$ ) and (B) tumor-infiltrating lymphocytes ( $\beta = -.57$ ,  $P = .021$ ) in patients with invasive epithelial ovarian carcinoma. All analyses were adjusted for cancer stage. Regression line is representative of all stages of disease.

for tumor control.<sup>37,38</sup> After surgical removal of the primary tumor, an intact cell-mediated immune response is thought to be important for elimination of residual disease and micrometastases.<sup>10,39,40</sup> NK cells seem to have a significant role in control of metastases,<sup>18</sup> and the intactness of the perioperative NK cell response is thought to be involved in tumor control.<sup>40</sup> The present observations highlight the possible contribution of psychosocial factors to local tumor control during the perioperative period.

Our findings are consistent with previous work in breast cancer patients documenting impairments in NKCC in peripheral blood of distressed patients,<sup>6,41</sup> positive associations between social support and NKCC,<sup>14,16</sup> and modulation of NKCC by psychosocial interventions.<sup>42,43</sup> Possible mechanisms underlying our findings include well-characterized pathways between the brain, cells of the immune system, and lymphoid organs, whereby events in the CNS can modulate aspects of the immune response.<sup>44,45</sup> Stress-related alterations in NKCC are thought to be primarily driven by adrenergic mechanisms<sup>7,46</sup> and occur by a variety of pathways, including impaired binding of NK cells to target cells, disruption of

cytokine secretion, and inhibited activation of lytic mechanisms in NK cells.<sup>47</sup>

It is also possible that stress-related alterations in NKCC may occur by indirect pathways. For example, neuroendocrine stress hormones are known to modulate the production of interleukin-10 and transforming growth factor beta (TGF- $\beta$ ),<sup>48</sup> both of which have downstream inhibitory effects on the activity of NK cells.<sup>49</sup> TGF- $\beta$  is also produced by tumor cells.<sup>50</sup> Although it is not known whether neuroendocrine hormones impact tumor cell production of TGF- $\beta$ , norepinephrine and epinephrine have been shown to upregulate ovarian tumor cell production of other cytokines such as vascular endothelial growth factor,<sup>51</sup> which has downstream interactions with NK cells.<sup>52</sup> Future work is needed to determine neuroendocrine modulation of tumor-produced TGF- $\beta$ . Taken together, these findings suggest that both direct (via beta-adrenergic effects on NK cells) and indirect (tumor-induced downregulation of NK cell activity) pathways may be involved in effects of neuroendocrine stress hormones on NK cells; it is possible that these effects may be additive or interactive. This is a fertile area for future research.

It is noteworthy that the highest correlations of psychosocial variables with NKCC occurred in TIL. There are direct connections between the ovary and the CNS via the sympathetic nervous system,<sup>53</sup> and beta-adrenergic receptors exist on normal ovarian tissue.<sup>53</sup> These connections may provide a direct pathway by which psychological states could modulate ovarian catecholamines and thereby affect the local immune response within the ovary. Furthermore, ovarian tumors are hypoxic environments that tend to be acidic.<sup>54-56</sup> A low pH is frequently used for preservation of catecholamines.<sup>57,58</sup> Although speculative, it is possible that the low pH of ovarian tumors serves to preserve catecholamines in tumor tissue, thereby amplifying any existing relationships involving adrenergically mediated psychosocial factors.

It is not clear why there were no associations between psychological factors and NKCC in ascites. Because there is neither direct sympathetic innervation nor direct blood supply to the ascites, it is possible that neuroendocrine mediators may have less access to immune cells in this compartment. Understanding the mechanisms behind these findings is the focus of ongoing work in our laboratory.

The dimension of social support investigated in this study was social attachment. At the time of cancer diagnosis, a sense of connection with others can reduce feelings of isolation and increase perceptions of control. We have previously found a positive relationship between social support at diagnosis and clinical status 1 year later in gynecologic cancer patients.<sup>59</sup> Furthermore, there is a substantial body of research relating social support to lower morbidity and mortality from a variety of diseases.<sup>12</sup> Thus, these findings may

ultimately have relevance for disease outcomes. The relationship of social support with NKCC was not a result of decreased distress or depression among these patients. Instead, social support and distress seemed to operate independently. This is consistent with other reports indicating that decreased distress does not seem to be a major pathway by which social support influences physiological functions.<sup>13,15</sup>

It should be noted that direction of causality cannot be assumed from these correlational findings, and future experimental work will be necessary for determination of causality. Moreover, longitudinal research will be necessary to understand disease implications of these findings. The findings of this study are limited by small sample size; multivariate analyses in PBMC may have been significant with a larger sample. Nevertheless, the magnitude of relationships between social support, distress, and NKCC, particularly in TIL, was quite large and suggests that psychosocial factors may contribute to the robustness of the innate immune response in the tumor microenvironment.

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### Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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