Published in final edited form as:

Brain Behav Immun. 2008 January ; 22(1): 65–73. doi:10.1016/j.bbi.2007.06.005.

Positive psychosocial factors and NKT cells in ovarian cancer patients

Donald M. Lamkin^{a,*}, Susan K. Lutgendorf^{a,b,c,d}, Stephanie McGinn^a, Minh Dao^a, Heena Maiseri^a, Koen DeGeest^b, Anil K. Sood^e, and David M. Lubaroff^{c,d,f,g}

^aDepartment of Psychology, University of Iowa, USA

^bDepartment of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Iowa, USA

^cUniversity of Iowa Holden Comprehensive Cancer Center, USA

^dDepartment of Urology, University of Iowa, USA

^eDepartments of Gynecologic Oncology and Cancer Biology, University of Texas M.D. Anderson Cancer Center, USA

^fDepartment of Microbiology, University of Iowa, USA

^gVeterans Affairs Medical Center, Iowa City, Iowa, USA

Abstract

Psychosocial factors are known to be associated with properties of both NK cells and T cells in cancer patients. Less is known about the relationship between psychosocial factors and NKT cells, a rare group of lymphocytes that have known relevance for tumor control. We examined four psychosocial factors and percentage and number of CD3⁺CD56⁺ NKT cells, CD3⁺CD56⁺ NK cells, and CD3⁺CD56⁺ T cells in peripheral blood lymphocytes (PBL), ascites, and tumor of 35 ovarian cancer patients and 28 patients with benign pelvic masses. Patients awaiting surgery for a suspected cancerous mass completed questionnaires and gave a pre-surgical blood sample. Ascites and tumor were taken during surgery. After lymphocyte isolation, subpopulations were analyzed by flow cytometry. Benign and cancer patients did not differ on PBL subpopulations. Among cancer patients, NKT cell percentage was significantly higher in tumor and ascites than in PBL; T cell percentage was significantly higher in PBL than tumor. NKT, NK, and T cell number were significantly higher in peripheral blood than in ascites. Positive reframing was related to significantly higher NKT cell percentage and number in PBL. Social support was related to significantly higher NKT cell percentage in tumor. Vigor was related to significantly higher NKT cell percentage in PBL. Total mood disturbance was not related to NKT cell percentage or number. No significant relationships were found between psychosocial factors and NK cell percentage and number and T cell percentage and number. Given the anti-tumor activity of CD3⁺CD56⁺ cells, these relationships may have relevance for cancer control.

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*Corresponding Author: Donald M. Lamkin, University of Iowa, Department of Psychology, E11 Seashore Hall, Iowa City, IA 52242, donald-lamkin@uiowa.edu, Phone: 319 353 2031, Fax: 319 335 0191.

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Keywords

Social Support; Coping; Vigor; Distress; T Cells; NK Cells; NKT Cells; Psychoneuroimmunology

1. Introduction

Among cancer patients, psychosocial factors are known to be associated with the properties of NK cells and T cells, both of which are important for tumor control (Shibuya et al., 2002; Whiteside & Herberman, 1994). We have previously reported that ovarian cancer patients with depressed mood have poorer natural killer (NK) cell activity in lymphocytes isolated from the tumor (tumor infiltrating lymphocytes: TIL), whereas patients with higher levels of social support have higher NK cell activity in lymphocytes isolated from both peripheral blood and from the tumor (Lutgendorf et al., 2005a). Digestive tract cancer patients with depression, exhibited significantly lower numbers of CD56⁺ (NK) cells and total lymphocytes than did those who were not depressed (Nan et al., 2004). In a study of breast cancer patients, stress was found to negatively associate with both the lytic ability of NK cells and the blastogenic response of T cells to concanavalin A (Con A), phytohemagglutinin A (PHA), and the monoclonal antibody (mAb) to CD3 (Andersen et al., 1998). Moreover, breast cancer patients receiving a psychological intervention aimed at managing stress showed an improvement in the T-cell blastogenic response to CD3 mAb (McGregor et al., 2004). The relevance of stress factors to NK cell activity and the critical role of NK cells in tumor control has also been demonstrated in laboratory animals (Ben-Eliyahu, 2003).

Less is known about the relationship between psychosocial factors and NKT cells, a rarer group of lymphocytes having cell markers characteristic of both NK cells and T cells. When first discovered in 1986, these cells were characterized as CD3⁺CD56⁺ in humans (Lanier et al., 1986; Schmidt et al., 1986), but in mice they came to be more specifically defined as having the NK cell marker NK1.1 and a particular invariant T cell receptor (TCR) in the CD3-TCR complex (Godfrey et al., 2004). In the years that have followed since their discovery, a substantial amount of research with these lymphocytes has been performed in relation to cancer. NKT cells of various phenotypes in both humans and mice have been examined in the cancer context with inconsistent results. For example, in a mouse model where 15-12RM fibrosarcoma tumor cells were injected subcutaneously to model a pattern of cancer growth, regression, and recurrence, NK1.1⁺ NKT cells were found to downregulate cytotoxic T lymphocyte (CTL) activity against the tumor (Terabe et al., 2000). Likewise, in a mouse model of ultraviolet (UV) radiation-induced skin cancer, NKT cells with the NK cell marker DX5 suppressed rejection of the UV-induced regressor tumor, UV-2240, after the NKT cells had been activated by UV light (Moodycliffe et al., 2000).

In contrast, many studies have shown NKT cells to inhibit tumor growth. NKT cells in a mouse model of methylcholanthrene-induced sarcoma were found to be directly cytotoxic to tumor cells, lysing them in much the same way as a conventional CTL or NK cell (Smyth et al., 2000). Similar results have been found with CD3⁺CD56⁺ NKT cells from humans exhibiting melanoma, breast cancer, ovarian cancer, pancreatic cancer, lung cancer, and colorectal cancer (Gritzapis et al., 2002).

Despite the potential relevance for cancer control, there is a paucity of research examining associations between NKT cells and psychosocial factors in humans. In the studies that do exist, CD3⁺CD56⁺ cells have generally been used. One investigation found that extraversion and social support were associated with higher numbers of NKT cells in an elderly population (Bouhuys et al., 2004). Another study of healthy community elderly found a positive association between distress and percentage of NKT cells (Lutgendorf et al., 2005b). In

contrast, an investigation of psychosocial factors and NKT cells in a population of breast and prostate cancer patients found no relationship between NKT cells and distress or other psychosocial factors. Moreover, little change was seen in NKT cell number or percentage over the course of a stress reduction intervention, and thus, there was no association observed between intervention associated NKT cell changes and changes in psychosocial factors (Carlson et al., 2003).

Other than the Carlson study, relationships between psychosocial measures and NKT cells in cancer patients have not been examined. To address this knowledge gap, we examined relationships between a panel of psychosocial factors and NKT cells in an ovarian cancer population before any pharmacological or surgical intervention that might serve as a confound. The psychosocial factors examined had previously been associated with either quality of life or immune function in clinical populations. For example, we have previously reported the importance of coping by positive reframing in association with functional, emotional, and physical well-being in gynecologic cancer patients (Lutgendorf et al., 2002). Thus, because of its importance in quality of life, it was hypothesized that positive reframing would be associated with higher levels of NKT cell percentage and number. Also, because of previous findings from this lab and others about the relationships of social support with NK cell function (Lutgendorf et al., 2005a; Levy et al., 1990) and NKT cells (Bouhuys et al., 2004), we hypothesized that social support would be positively associated with NKT cell percentage and number. Finally, as mood has been shown to be a relevant factor in the larger study from which these ovarian cancer patients were drawn (Lutgendorf et al., 2005a), it was hypothesized that positive mood would be associated with higher levels of NKT cell percentage and number and negative mood would be associated with lower levels. NK cells (CD3⁻CD56⁺) and T cells (CD3⁺CD56⁻) were examined for comparison.

2. Materials and methods

2.1. Participants

2.1.1. Inclusion and exclusion criteria—Women were approached for the study if they were over 18 years of age and had received a new diagnosis (no prior cancer) of a pelvic or abdominal mass suspected to be ovarian cancer. These patients were then included in the study if histologic diagnosis confirmed they had a primary invasive epithelial ovarian, primary papillary peritoneal, or primary fallopian tube malignant tumor. Patients found to have benign ovarian neoplasms at surgery were also included in the study as a comparison group. Patients were excluded for primary cancer of another organ site, a nonepithelial ovarian tumor, an ovarian tumor of low malignant potential, a history of systemic corticosteroid medication use in the last 4 months, or comorbidities known to alter the immune response such as an immunomodulatory or inflammatory disease. This research was approved by the University of Iowa Institutional Review Board.

2.1.2. Sample characteristics—Of 152 potentially eligible patients approached for study participation, 128 (84.2%) agreed to participate. Thirty-seven of the 128 patients were later excluded from the study because they failed to meet the inclusion criteria. Eleven patients withdrew from the study before surgery because of time constraints or emotional distress. Ten patients could not be included because of cancellation or rescheduling of surgery, surgery not conducted at study site, surgery preceded by chemotherapy, or difficulty with venous access. Seven were excluded because peripheral NKT cell, NK cell, and T cell data was not obtained for them. Thus, the final sample included 35 ovarian cancer patients and 28 benign patients. The majority of ovarian cancer patients (85.8%) in this final sample had advanced-stage disease (stages III and IV) with predominantly high-grade tumors (grade 3, 65.8%). Cells for flow cytometry were isolated from ascites in 22 patients and from tumor in 14 patients.

2.2. Procedure

Patients were recruited before surgery at their clinic visit and completed psychosocial questionnaires before surgery. On the morning of surgery, before administration of preoperative medication or general anesthesia, patients gave a 40-mL sample of peripheral venous blood in heparinized vacutainer tubes (Becton Dickinson Co., Rutherford, NJ). To control for potential circadian variation, peripheral blood samples were taken between 6:00 AM and 12:00 PM. Samples of ascites and tumor were obtained from surgery and were immediately processed as described below.

2.3. Measures

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

2.3.2. Coping—The Brief COPE is a 28-item self-report scale used to assess a broad range of coping responses to stress (Carver, 1997). This inventory consists of 14 scales representing distinct coping behaviors. Patients completed a modified version of the inventory and were instructed to rate their coping behaviors in response to the stress of their illness. In this study the positive reframing scale was used because of demonstrated relevance in previous research (Lutgendorf et al., 2002). Coefficient alpha for this subscale in the present study was .78.

2.3.3. Perceived social support—The Social Provisions Scale is a 24-item self-report scale measuring the degree to which an individual perceives their social relationships as supportive. Facets of total social support examined by the scale include one's social attachment, social integration, reassurance of worth, reliable alliance, guidance, and opportunity for nurturance. The scale has demonstrated adequate reliability and validity in a number of studies with different populations (Cutrona, 1984; Cutrona & Russell, 1987; Russell et al., 1984). In this study, the patient's total social support score was used. Coefficient alpha for the total scale in the present study was .92.

2.3.4. Mood—The Profile of Mood States Short Form (POMS-SF) 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, fatigue, confusion, and vigor. A composite score of distress, called total mood disturbance, is created by summing all factors except vigor and subtracting vigor from the total score (Curran et al., 1995; Shacham, 1983). In this study, the patient's total mood disturbance and vigor scores were used as measures of negative mood and positive mood respectively. In the present study, coefficient alpha for total mood disturbance was .96 and for vigor was .88.

2.4. Immunologic methods

2.4.1. Lymphocyte isolation in peripheral blood, ascites, and tumor—Methods for isolating and labeling lymphocytes in this sample of patients have been described elsewhere (Lutgendorf et al., 2005a). Briefly, peripheral blood lymphocytes (PBL) were isolated from whole blood using a Ficoll gradient and centrifugation at $650 \times g$ at 4°C for 10 minutes. After washing three times with medium, the cells were resuspended in CTL media and counted in 10% acetic acid.

After ascites was filtered through sterile mesh filters, this compartment was processed in the same manner as described above. These lymphocytes were then further separated from tumor cells using anti-CD45 human microbeads (Miltenyi Biotec, Auburn, CA) before counting.

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 above.

2.4.2. Labeling of CD3/56 cells—Cell suspensions from patients were prepared in CTL medium at a concentration of 5×10^6 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⁺CD56⁺, CD3⁺CD56[−], or CD3[−]CD56[−] cells. Total cell number for each subpopulation of lymphocytes in a given compartment was calculated by multiplying that subpopulation percentage by the ratio of the total number of lymphocytes isolated to the total volume or mass of the compartment from which the lymphocytes were obtained. Thus, total cell number is expressed as cells/mL of blood, cells/mL of ascites, or cells/gram of tumor.

2.5. Statistical analyses

All distributions were examined for outliers and non-normality. Square root transformations were applied to normalize immune data. Two-sided *t* tests were used to examine differences between ovarian cancer patients and benign patients on demographic variables, biophysical variables, and medication use. ANCOVAs, adjusting for age, were used to examine differences in cell percentage and total cell number/mL between ovarian cancer patients and benign patients in peripheral blood. Among ovarian cancer patients, paired *t* tests were used to examine differences in cell percentage and number in peripheral blood, ascites, and tumor. Pearson product moment correlations were calculated to assess relationships between disease stage and cell percentage and number in each compartment among cancer patients. To examine relationships of psychosocial variables with cell percentage and number, hierarchical linear regressions were performed within each compartment, adjusting for stage of disease. Level of significance was set at $p < .05$ for all analyses.

3. Results

3.1. Descriptive statistics

3.1.1. Demographic characteristics—The mean age of participants was 56.3 years (range, 29 – 79 years). Benign patients were significantly younger (age $M = 51.86$, $SD = 11.22$) than cancer patients (age $M = 59.86$, $SD = 10.20$; $t(61) = -2.96$, $p = .004$); thus, age was included as a covariate in all between group analyses for primary outcome variables. There were no significant differences between groups in alcohol use, hours of sleep over the last week or over the last night, exercise frequency, coffee consumption, or cigarette use (all p 's $> .12$). Additionally, there were no significant differences between groups in the use of beta-blockers

($p > .13$), corticosteroids ($p > .24$), hormone replacement therapy ($p > .56$), or antidepressants ($p > .12$). See Table 1.

Cancer stage was not significantly associated with NKT cell percentage or number (all p 's $> .17$), NK cell percentage or number (all p 's $> .09$), or T cell percentage or number (all p 's $> .12$) in any compartment.

3.1.2. NKT, NK, and T cells in benign and ovarian cancer patients—There was no significant difference between cancer patients and benign patients in peripheral blood NKT cell percentage ($p = .72$) or number ($p = .98$), NK cell percentage ($p = .93$) or number ($p = .90$), or T cell percentage ($p = .44$) or number ($p = .58$), adjusting for age.

As seen in Figure 1, NKT cell percentage was significantly higher in tumor ($t(11) = -2.69$, $p = .02$) and ascites ($t(21) = -2.95$, $p = .008$) than in peripheral blood. NKT cell percentage was not significantly different between ascites and tumor ($p = .29$). There were no significant differences among compartments for NK cell percentage in cancer patients (all p 's $> .10$). However, as seen in Figure 2, T cell percentage was significantly higher in peripheral blood than in tumor ($t(11) = 2.65$, $p = .02$). T cell percentage was not significantly different between ascites and peripheral blood ($p = .87$) and was marginally different between ascites and tumor ($t(7) = 2.10$, $p = .07$).

As seen in Table 2 there were significantly higher numbers of NKT, NK, and T cells/mL in PBL as compared to ascites (all p 's < 0.001). NKT, NK and T cells/gram of tumor are also reported in Table 2.

3.2. Regression of NKT, NK, and T cells in ovarian cancer patients

Hierarchical regression analyses for immune variables were performed separately for each psychosocial variable in each compartment. All analyses were adjusted for stage of disease.

As seen in Table 3, positive reframing was related to significantly higher NKT cell percentage ($\beta = .54$, $p = .004$) and NKT cell number ($\beta = .55$, $p = .003$) in peripheral blood and was marginally related to NKT cell percentage in tumor ($\beta = .56$, $p = .06$). No relationships were found between positive reframing and NKT cell number in tumor ($p = .13$) or ascites ($p = .18$), or with NKT cell percentage in ascites ($p = .82$).

As seen in Table 4, greater social support was related to significantly higher NKT cell percentages ($\beta = .73$, $p = .03$) but not to NKT cell number ($p = .78$) in tumor. Social support was not related to NKT percentage ($p = .97$) or NKT cell number ($p = .80$) in PBL. Social support was marginally related to lower NKT cell percentage in ascites ($\beta = -.39$, $p = .09$) but showed no relationship to NKT cell number in ascites ($p = .61$).

As seen in Table 5, vigor was significantly related to higher NKT cell percentage in peripheral blood ($\beta = .38$, $p = .04$) and marginally related to higher NKT cell number in peripheral blood ($\beta = .36$, $p = .06$). Vigor was not related to NKT cell percentage or NKT cell number in any other compartment (all p 's $> .30$).

There was no relationship between total mood disturbance and NKT cell percentage or number in any compartment (all p 's $> .22$).

There were no significant relationships between the psychosocial variables examined and NK cell percentage or number in any compartment (for peripheral blood all p 's $> .26$; for ascites all p 's $> .15$; for tumor all p 's $> .49$), although there was a marginal relationship between lower NK cell number and social support in tumor ($\beta = -.64$, $p = .07$).

There were no significant relationships between the psychosocial variables and T cell percentage or number in any compartment (for peripheral blood all p 's > .33; for ascites all p 's > .11; for tumor all p 's > .18), although there was a marginal relationship between higher T cell percentage and positive reframing in ascites ($\beta = .42, p = .09$).

4. Discussion

The three positive psychosocial factors examined in this study, positive reframing, social support, and vigor, were related to higher levels of NKT cells in ovarian cancer patients. Specifically, patients who coped with their illness by reframing it in a positive way (e.g. they "looked for something good in what was happening to them") had significantly higher percentages and numbers of NKT cells in peripheral blood, with marginally higher percentages in tumor. Similarly, patients who reported more perceived social support had significantly higher percentages of NKT cells in tumor. Patients who reported more vigor (e.g. describing themselves as "energetic," "cheerful," "full of pep") had significantly higher NKT cell percentages and marginally higher total numbers of NKT cells in peripheral blood. Negative mood (total mood disturbance) was not significantly related to NKT cell percentage or total NKT cell number. Additionally, no significant relationships were found between NK cell percentage or number or T cell percentage or number and any of the psychosocial variables examined. Among the three compartments examined in cancer patients, NKT cell percentages were significantly higher in tumor and ascites than in peripheral blood whereas T cell percentages were significantly higher in peripheral blood than tumor, but NK cell percentage did not significantly differ among the compartments. Cell number for each subpopulation was significantly higher in peripheral blood than in ascites. To the best of our knowledge, this is the first study to demonstrate significant relationships between positive psychosocial factors and NKT cells in the peripheral blood and tumor of ovarian cancer patients.

These findings are consistent with previous work relating social support with more robust cellular immune profiles in breast cancer patients (Levy et al., 1990) and ovarian cancer patients (Lutgendorf et al., 2005a). Furthermore, as social support (Goodwin et al., 1987; Maunsell et al., 1995; Reynolds & Kaplan, 1990; Waxler-Morrison et al., 1991) and vigor (Teshima et al., 1991) have been related to positive outcomes in cancer, the present findings suggest an additional mechanism (NKT cells) by which these relationships may be mediated. Although positive mood, as assessed by POMS vigor, was associated with higher NKT cells in peripheral blood, negative mood, as assessed by POMS total mood disturbance, had no association with NKT cells in any compartment. This is consistent with findings by Carlson and colleagues (2003) who failed to find an association between POMS total mood disturbance and NKT cell number or percentage in cancer patients.

The CD3⁺CD56⁺ NKT cell has been shown to mediate anti-tumor results in an MHC-independent manner. Despite this capability, NKT cells have been researched less since their more recent discovery in comparison to NK or T cells. This may be due to their relatively low levels in peripheral blood (about 1–5% of all peripheral blood mononuclear cells) and to the initial finding that their cytotoxicity was less potent than regular NK cells (Lanier et al., 1986; Schmidt et al., 1986). However, more recent studies have reported superior killing ability of CD3⁺CD56⁺ cells after modulation *in vitro*, killing not only more K562 cells and LAK-sensitive Daudi cells but also more autologous tumor cells than regular CD3⁺CD56⁺ NK cells (Gritzapis et al., 2002). Furthermore, although studies of the clinical significance of CD3⁺CD56⁺ percentage *in vivo* are lacking, percentage of CD3⁺ cells has been shown to be significantly lower in cancer populations than in healthy populations, and this reduction is related to significantly lower function of these cells (Agarwal et al., 2006; van der Pompe et al., 1996). Thus, a higher percentage of CD3⁺CD56⁺ cells in peripheral blood or tumor may have potential relevance for cancer control.

The mechanisms by which positive reframing, social support, and vigor are related to CD3⁺CD56⁺ NKT cells in cancer patients are unclear. Such mechanisms may be similar to those known to mediate the relationship between stress and other lymphocyte populations. Although lymphocytes, especially CD56⁺ cells, have been shown to increase in the periphery after short-duration stress or acute administration of β -adrenergic receptor agonists (Benshop et al., 1994; 1996), all major lymphocyte subpopulations in peripheral blood are downregulated in response to chronic stress, and this seems to be the result of a complex process resulting from glucocorticoid-induced and catecholamine-induced alterations in both cell survival and cell trafficking (Engler et al., 2004a). In this regard, B cells have been shown to accumulate in the spleen after chronic stress (Engler et al., 2004b), and CD3⁺ cells have been shown to predominately traffic into the bone marrow (Stefanski et al., 2003). The CD3⁺CD56⁺ cells examined in the present study may also be subject to these neuroendocrine stress factors.

Some findings were unanticipated. There was a trend towards an inverse relationship between social support and NKT cell percentage in ascites as well as NK cell number in tumor. The reasons for this are unclear, and future work will be needed to determine whether this is a spurious finding or whether it is supported in a larger sample. Also, no significant relationships were observed between psychosocial factors and NK cell percentage in any compartment. Although the larger study from which these ovarian cancer patients were drawn observed a significant positive relationship between social support and NK cell activity in tumor (Lutgendorf et al., 2005a), recent stress research has shown that NK cell number and NK cell cytotoxicity can be significantly modulated independently of each other by different stress hormones (Tseng et al., 2005). Furthermore, tumor infiltrating NK cells show substantial modulation in cytolytic and proliferative capacity that make them characteristically different from normal NK cells found in the periphery (Lai et al., 1996). Thus, one may speculate that the correlation between NK cell percentage and NK cell activity normally seen in peripheral NK cells of healthy persons can become altered in the tumor microenvironment of ovarian cancer patients. However, this question remains to be fully investigated.

The presence of significantly higher NKT cell percentages in ascites and tumor than in peripheral blood was not observed for NK cell percentages, which were relatively consistent among the 3 compartments, or T cell percentages, which were higher in peripheral blood than tumor. It is possible, although speculative at this point, that these differences between NKT and NK and T cells could be due to CD3⁺CD56⁺ NKT cells having a greater avidity or total binding capacity for malignant cells. It is also possible that the NKT cell is better equipped than the NK cell or T cell at tumor surveillance. Thus, the higher proportion of NKT cells in tumor and ascites where tumor cells are concentrated would represent better tumor surveillance on the part of the NKT cell. It is also possible that cell differences observed among the compartments have to do with the relative isolation of the ascites and tumor from exposure to glucocorticoids and catecholamines. These and other hypotheses about the trafficking patterns of NKT cells are important questions for future research.

4.1. Limitations

The findings reported in this study are correlational, and, therefore, direction of causality cannot be determined from them. Further experimental work is needed to determine causality. Additionally, the sample size was small, particularly for tumor infiltrating lymphocytes, and limited power may have precluded the detection of some relationships. Thus, findings reported in this study need replication. Future studies that attempt to replicate these findings would benefit from use of a control group of healthy matched participants not anticipating surgery. Such a comparison would provide indication of whether psychological stress in cancer patients awaiting surgery affects the PBL indices examined in this study and may suggest additional benefit from pre-surgical interventions for stress-management.

In this study, tumor size was measured in units of mass rather than volume. Because peripheral blood and ascites were measured in mL while tumor was measured in grams, comparisons of cell number could not be made among all three compartments. Thus, future studies may wish to ascertain the volume of the tumor in order to facilitate comparison of absolute cell numbers among all three compartments in cancer patients. Also, although we strove to control potential circadian effects on peripheral immune cells by collecting such cells only in the morning, the timeframe for such collection (6 AM – 12 PM) was broad and was necessitated by constraints of surgical schedules.

Although previous studies have reported anti-tumor responses for CD3⁺CD56⁺ NKT cells, the functional implications of the present findings for anti-tumor activity are unclear. The intensity of the NKT cell response is known to be variable under the influence of cytokines, other exogenous factors (e.g., *in vitro* culturing with multiple reagents), and the presence and functional capacity of adhesion molecules (Gritzapis et al., 2002). Moreover, it is possible that variability in the effectiveness of NKT cells against tumor may be accounted for by variability in the ratio of inhibitory to activating NK receptors on the cell. It has been pointed out that the cytotoxic response of CD3⁺ cells against melanoma tumor can be less effective or abrogated when the CD3⁺ cell also expresses particular inhibitory receptors characteristic of a CD56⁺ NK cell (Casado et al., 2005; Tarazona et al., 2004). Killer cell inhibitory receptors (KIRs) as well as inhibitory and activating subtypes of CD94/NKG2 from the C-type lectin family have been investigated. Ligation of KIRs on T cells by melanoma cells has been shown to reduce lysis of those cells (Bakker et al., 1998), and CD94/NKG2 expression on T cells can be modulated by cytokines (Mingari et al., 2000; Vetter et al., 2000). Thus, to summarize, the anti-tumor capacity of the CD3⁺CD56⁺ NKT cell in the present study may be variable for reasons such as these. Therefore, future studies should involve additional characterization of surface molecules on the NKT cell being examined as well as *in vivo* assessment of cytokines, hormones, and other cofactors that may be influencing cell number and function.

4.2. Conclusion

This study has revealed intriguing relationships between positive psychosocial factors and CD3⁺CD56⁺ NKT cells in ovarian cancer patients. These associations may be relevant for cancer control in light of the known but less studied anti-tumor capacity of CD3⁺CD56⁺ cells.

Acknowledgments

We thank Andrew Misfeldt, Joshua Lukenbill, Hannah Chang, Daniel Pederson, and Elizabeth King for assistance with the immunologic assays, and Anna Hoffman for assistance in patient recruitment.

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Fig. 1.

NKT cell % was significantly different between peripheral blood ($M = 4.82$, $SE = .81$) and ascites ($M = 10.59$, $SE = 1.95$), $t(21) = -2.954$, $p = .008$, and between peripheral blood ($M = 4.82$, $SE = .81$) and tumor ($M = 8.94$, $SE = 1.55$), $t(11) = -2.688$, $p = .02$. NKT % was not significantly different between ascites and tumor, $p = .29$. Means are for all available samples.

Fig. 2.

T cell % was significantly different between peripheral blood ($M = 52.72$, $SE = 3.95$) and tumor ($M = 42.6$, $SE = 6.22$), $t(11) = 2.65$, $p = .02$. There was a marginal difference between ascites ($M = 48.39$, $SE = 5.06$) and tumor ($M = 42.6$, $SE = 6.22$), $t(7) = 2.10$, $p = .07$. T cell % was not significantly different between ascites and peripheral blood, $p = .87$. Means are for all available samples.

TABLE 1

Demographic Characteristics of Sample

Characteristic	Cancer Patients (n = 35)	Benign Patients (n = 28)
Age, years		
Mean	59.9	51.9
Standard Deviation	10.2	11.2
	<i>% of Patients</i>	
Education *		
Some high school	6.3	11.5
High school graduate	37.5	23.1
Trade school/some college	34.4	42.3
College graduate	12.5	19.2
Postgraduate	9.4	3.8
Marital Status *		
Single	8.8	18.5
Divorced	14.7	18.5
Widowed	5.9	3.7
Married/living with partner	70.6	59.3
Stage		
I	8.6	
II	5.7	
III	62.9	
IV	22.9	
Grade		
1	8.6	
2	25.7	
3	65.8	
Tumor histology		
Serous	77.2	
Endometrioid	8.6	
Mucinous	5.7	
Other	8.6	
Residual disease		
Yes	74.3	
No	25.7	

* Education information was missing for 2 benign and 3 cancer patients; marital status was missing for 1 benign and 1 cancer patient.

TABLE 2
NKT, NK, and T Cell Levels in 3 Compartments of Ovarian Cancer Patients

Cells	Peripheral Blood		Ascites		Tumor	
	M	SE	M	SE	M	SE
NKT %	4.82	.81	10.59**	1.95	8.94*	1.55
NK %	13.32	1.36	17.60	2.59	15.78	2.92
T %	52.72	3.95	48.39	5.06	42.60*	6.22
NKT #	23.08	2.64	10.15***	2.38	11.64	2.93
NK #	40.51	3.97	12.39***	1.56	17.59	4.31
T #	69.90	7.27	21.68***	3.93	63.33	22.87

M = Mean; SE = Standard Error of the Mean;

* Different from peripheral blood at $p < .05$

** Different from peripheral blood at $p < .01$

*** Different from peripheral blood at $p < .001$

Note:

In peripheral blood and ascites, cell number (#) is $\times 10^4$ per mL.

In tumor, cell number (#) is $\times 10^4$ per gram.

No comparisons between tumor cell number and peripheral blood or ascites numbers.

TABLE 3
Association of Positive Reframing and NKT Cells in 3 Compartments of Ovarian Cancer Patients

Compartment	R	ΔR^2	Standardized β	t	p
<i>NKT Percentage</i>					
Peripheral Blood ^a (n = 31)	.19	.00	-.37	-2.208	.036
Step 1. Stage					
Step 2. Positive Reframing	.54	.24	.54	3.183	.004
Ascites ^b (n = 20)	.02	-.06	.01	.030	.976
Step 1. Stage					
Step 2. Positive Reframing	.06	-.11	.06	.227	.823
Tumor ^c (n = 12)	.24	-.03	-.28	-1.067	.314
Step 1. Stage					
Step 2. Positive Reframing	.61	.23	.56	2.099	.065
<i>NKT Number</i>					
Peripheral Blood ^d (n = 31)	.14	-.02	-.32	-1.889	.069
Step 1. Stage					
Step 2. Positive Reframing	.53	.23	.55	3.204	.003
Ascites ^e (n = 17)	.15	-.04	-.24	-.957	.355
Step 1. Stage					
Step 2. Positive Reframing	.38	.02	.36	1.415	.179
Tumor ^f (n = 12)	.08	-.09	.04	.146	.887
Step 1. Stage					
Step 2. Positive Reframing	.49	.07	.48	1.652	.133

ΔR^2 : Adjusted R Square.

^aFinal Model $F(2,28) = 5.78, p = .008$.

^bFinal Model $F(2,17) = .03, p = .97$.

^cFinal Model $F(2,9) = 2.63, p = .13$.

^dFinal Model $F(2,28) = 5.49, p = .01$.

^eFinal Model $F(2,14) = 1.19, p = .33$.

^fFinal Model $F(2,9) = 1.40, p = .30$.

TABLE 4
Association of Social Support and NKT Cells in 3 Compartments of Ovarian Cancer Patients

Compartment	R	ΔR^2	Standardized β	t	p
<i>NKT Percentage</i>					
Peripheral Blood ^a (n = 32)	.22	.02	-.22	-1.091	.284
Step 1. Stage					
Step 2. Social Support	.22	.02	-.01	-.035	.972
Ascites ^b (n = 21)	.04	-.05	-.04	-.199	.845
Step 1. Stage					
Step 2. Social Support	.39	.06	-.39	-1.795	.089
Tumor ^c (n = 11)	.07	-.10	-.34	-1.229	.254
Step 1. Stage					
Step 2. Social Support	.68	.33	.73	2.602	.032
<i>NKT Number</i>					
Peripheral Blood ^d (n = 32)	.18	.00	-.16	-.788	.437
Step 1. Stage					
Step 2. Social Support	.19	-.03	-.05	-.249	.805
Ascites ^e (n = 18)	.19	-.02	-.20	-.800	.436
Step 1. Stage					
Step 2. Social Support	.23	-.07	-.13	-.521	.610
Tumor ^f (n = 11)	.28	-.02	.24	.672	.521
Step 1. Stage					
Step 2. Social Support	.30	-.14	.11	.294	.776

ΔR^2 : Adjusted R Square.

^aFinal Model $F(2,29) = .74, p = .48$.

^bFinal Model $F(2,18) = 1.62, p = .22$.

^cFinal Model $F(2,8) = 3.43, p = .08$.

^dFinal Model $F(2,29) = .52, p = .60$.

^eFinal Model $F(2,15) = .42, p = .66$.

^fFinal Model $F(2,8) = .40, p = .68$.

TABLE 5
Association of Vigor and NKT Cells in 3 Compartments of Ovarian Cancer Patients

Compartment	R	ΔR^2	Standardized β	t	p
<i>NKT Percentage</i>					
Peripheral Blood ^a (n = 29)	Step 1. Stage .24	.02	-.16	-.895	.379
	Step 2. Vigor .44	.13	.38	2.106	.045
Ascites ^b (n = 18)	Step 1. Stage .18	-.03	.10	.381	.710
	Step 2. Vigor .32	-.02	-.28	-1.080	.300
Tumor ^c (n = 10)	Step 1. Stage .08	-.12	-.04	-.108	.917
	Step 2. Vigor .10	-.27	-.07	-.180	.862
<i>NKT Number</i>					
Peripheral Blood ^d (n = 29)	Step 1. Stage .17	-.01	-.09	-.499	.622
	Step 2. Vigor .39	.08	.36	1.936	.064
Ascites ^e (n = 15)	Step 1. Stage .19	-.04	-.14	-.463	.652
	Step 2. Vigor .26	-.09	.18	.635	.537
Tumor ^f (n = 10)	Step 1. Stage .28	-.04	.26	.643	.540
	Step 2. Vigor .29	-.18	.07	.176	.865

ΔR^2 : Adjusted R Square.

^aFinal Model $F(2,26) = 3.14, p = .06$.

^bFinal Model $F(2,15) = .85, p = .45$.

^cFinal Model $F(2,7) = .04, p = .96$.

^dFinal Model = $F(2,26) = 2.29, p = .12$.

^eFinal Model = $F(2,12) = .43, p = .66$.

^fFinal Model = $F(2,7) = .32, p = .74$.