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Cancer induces inflammation and depressive-like behavior in the mouse: Modulation by social housing

Donald M. Lamkin^{1,2}, Susan K. Lutgendorf^{1,3,4,5}, David Lubaroff^{4,5,6}, Anil K. Sood⁷, Terry G. Beltz¹, and Alan Kim Johnson^{1,8,9,10}

¹ Department of Psychology, University of Iowa

² Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles

³ Department of Obstetrics & Gynecology, University of Iowa

⁴ Department of Urology, University of Iowa

⁵ Holden Comprehensive Cancer Center, University of Iowa

⁶ Department of Microbiology, University of Iowa

⁷ Departments of Gynecologic Oncology and Cancer Biology, M.D. Anderson Cancer Center, University of Texas

⁸ Department of Pharmacology, University of Iowa

⁹ Department of Health & Human Physiology, University of Iowa

¹⁰ Cardiovascular Center, University of Iowa

Abstract

Considerable data demonstrate a high prevalence of depressive symptoms in cancer patients. This study introduces an experimental model to examine the effect of tumor on depressive-like behavior. Female C57BL/6 mice were injected i.p. with syngeneic ID8 ovarian carcinoma. Experiment 1 measured sucrose intake before and after tumor incubation to assess the effect of tumor on anhedonic depressive-like behavior. Experiment 2 examined effects of tumor and social housing on anhedonia and a second depressive-like behavior, tail suspension test (TST) immobility. Systemic proinflammatory and antiinflammatory cytokines were measured following each experiment. Additional behaviors assessed the specificity of tumor's effect on depressive-like behavior. Tumor caused a reduction in sucrose intake relative to baseline and control levels ($P < .05$). Moreover, individually-housed tumor-bearing mice exhibited a lower sucrose preference than group-housed tumor-bearing or control mice in either housing condition ($P < .05$). Although tumor-bearing mice exhibited less locomotion than controls ($P < .001$), there was no significant effect of tumor on TST immobility. Tumor caused higher levels of systemic proinflammatory and antiinflammatory cytokines and smaller body weight ($P < .05$), but appetite and motor capacity were not significantly affected. Statistical mediation analysis showed that circulating interleukin-6

Corresponding Author: Alan Kim Johnson, Ph.D. University of Iowa, E11 Seashore Hall, Iowa City, IA, 52242, alan-johnson@uiowa.edu, Phone: 319-335-2423, Fax: 319-335-0191.

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partially mediated the effect between tumor and home cage locomotion ($P < .01$) but not between tumor and sucrose intake. It is concluded that tumor elicits anhedonic depressive-like behavior in a murine model of ovarian cancer. This may have important implications for etiology of depression in the clinical cancer setting.

Keywords

ovarian cancer; inflammation; cytokines; anhedonia; tail suspension test; locomotion

1. Introduction

Considerable data demonstrate a high prevalence of depressive symptoms in cancer patients (Raison & Miller, 2003). The median prevalence for major depressive disorder (MDD) in cancer patients is estimated at 15%, while the median prevalence for all depressive disorders is 30% (Hotopf et al., 2002). These rates are substantially higher than those found in the general population for MDD, which ranges from 2-4% (Kessler & Wang, 2009).

Receiving a diagnosis of cancer may result in a cascade of responses such as shock, disbelief, anxiety, anger, and despair that could persist and develop into MDD (McDaniel et al., 1995). Psychosocial factors in cancer patients that may increase the risk for developing MDD after receiving a cancer diagnosis include socioeconomic pressures, pessimistic personality traits, and social isolation (Weisman & Worden, 1976). Social isolation, in particular, is a significant risk factor for MDD in the population at large. A series of studies in women showed that those who lacked a close confidante or had no outside employment were more likely than other women to become depressed after experiencing a major stressful life event (Brown, 1988; Brown & Harris, 1978).

The occurrence of a stressful life event like a cancer diagnosis, with or without concomitant social isolation, may lead one to conclude that cancer patients who develop MDD are manifesting a so-called “reactive depression” (Paykel, 2008). However, if the presence of cancer in the body is generating a systemic inflammatory response, then tumor-derived inflammatory products may also contribute to symptoms of MDD. Tumors have been shown to produce several proinflammatory cytokines (Aggarwal, 2004; Coussens & Werb, 2002; Weinberg, 2007), which are positively associated with the presence of MDD and/or depressive symptoms (Howren et al, 2009) and sickness behaviors (Dantzer et al, 2008). Systemic administration of the recombinant proinflammatory cytokine, interferon alpha (IFN- α), induces symptoms consistent with MDD in 30-50% of patients (Capuron & Miller, 2004).

In ovarian cancer patients, tumor-induced inflammatory mediators (e.g., IL-6) have been associated with depressive symptoms (Lutgendorf et al., 2008). To begin examining a potential pathway from tumor to depressive symptoms via tumor-induced inflammation, the present study introduces a syngeneic mouse model of ovarian cancer to experimentally test for an effect of tumor on measures of systemic inflammation and depressive-like behaviors. The animal behaviors employed were previously validated with antidepressants (Weiss & Kilts, 1995).

In Experiment 1, it was predicted that tumor-bearing animals would exhibit a significant increase in anhedonic depressive-like behavior as assessed by decreases in sucrose intake relative to baseline and controls. Additional behaviors were examined to assess the specificity of the effect of tumor on anhedonic behavior.

Experiment 2 examined effects of ovarian tumor and social housing on the outcomes listed above and on the depressive-like behavior of tail suspension test (TST) immobility. Consistent with the increased risk for MDD in persons who are socially isolated (see above), social isolation in female animals has been shown to increase depressive-like behaviors (Grippe et al., 2007; Martin & Brown, 2010). Thus, it was predicted that tumor-bearing individually-housed mice would show more depressive-like behavior than tumor-bearing group-housed mice.

Finally, it was predicted that tumor-induced cytokines would mediate the effect of tumor on behavior.

2. Methods

2.1 Protocols

2.1.1 Experiment 1—Mice habituated to their new environment for one week. During the second week, in addition to food and water, mice were given free continuous access to a 3% sucrose solution for five days followed by a two-day withdrawal period. Daily vaginal cytology for determination of estrous phase was begun at the midpoint of week 2. During the third week, baseline measures of estrous cycle phase, body weight, locomotion, food intake, and sucrose intake were obtained on three days over the course of the week with two days between each measurement day. After baseline measurement, mice were randomly assigned to tumor-bearing ($n = 34$) or control ($n = 18$) conditions and given the injection appropriate for their condition. After tumor became palpable or ascites became visible (8-12 weeks following injection, median = 9), mice underwent post-injection measurement as described above. Rotarod performance was measured on the first day of the post-injection measurement week. Mice were euthanized via rapid decapitation for collection of peripheral trunk blood. Tumor and/or ascites were removed from the peritoneum. See Figure 1 for graphical timeline.

2.1.2 Experiment 2—Mice were allowed to habituate to their housing condition (individual vs. group) for two weeks. Baseline body weight was then measured. The mice in each housing condition were randomly assigned to ovarian carcinoma vs. vehicle injection, resulting in four experimental groups: group-housed tumor-bearing mice ($n = 14$), group-housed control mice ($n = 15$), individually-housed tumor-bearing mice ($n = 23$), individually-housed control mice ($n = 21$). All mice in a particular group-housed cage received the same treatment (all were either tumor-bearing or controls). After tumor became palpable or ascites became visible (6-12 weeks following injection, median = 9), the mice underwent post-injection measurement of estrous cycle phase, body weight, locomotion, rotarod performance, TST immobility, food intake, sucrose intake, and water intake. The procedures followed in Experiment 1 were used to collect samples. Graphical timeline for Experiment 2 appears in Figure 1.

2.2 Animals

Female C57BL/6 mice (Harlan, Indianapolis, IN) 11-12 weeks of age were maintained at a temperature of 22-24°C and a 12/12-hour diurnal light/dark cycle with continuously available food and water. All procedures were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Iowa Institutional Animal Care and Use Committee. Experiment 1 used 52 mice housed individually in cages with bedding. Experiment 2 employed 73 mice randomly assigned to be housed individually or in groups of 4-5.

2.3 Estrous Cycle

Because estrous cycles can affect behaviors (e.g., food intake and locomotion) (Asarian & Geary, 2006; Pilorz et al., 2009), estrous phase was monitored by microscopic examination of the number and proportion of cell types in vaginal lavage smears (Marcondes et al., 2002). Cells were collected 1 hour before the dark period.

2.4 ID8 Ovarian Carcinoma

ID8 murine ovarian carcinoma cells were kindly provided by Dr. Katherine Roby (University of Kansas, Kansas City, KS) (Roby et al., 2000). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), insulin (5 $\mu\text{g/mL}$), transferrin (5 $\mu\text{g/mL}$), and sodium selenite (5 ng/mL). Mice randomly assigned to the tumor group received an i.p. injection of 5×10^6 viable cancer cells in 200 μL of vehicle (unsupplemented DMEM). Control group mice received a 200 μL i.p. injection of vehicle only. After tumor became palpable or ascites became visible, post-injection measurements were taken, after which tumor was excised from the peritoneum, weighed, flash frozen in a cryovial or in optimal cutting (OCT) media, and stored at -80°C . Similarly, ascites in the peritoneum was aspirated, weighed, and flash frozen before storage at -80°C . Mice that did not exhibit tumor at the conclusion of an experiment were excluded from the analysis ($n = 5$).

2.5 Body Weight

Weight was measured gravimetrically. The amount of weight from tumor and/or ascites in this model can be substantial (Hagemann et al., 2007), and higher body weight of tumor-bearing mice (compared to controls) has been used as a marker of advanced disease status (Bak et al., 2007). Total disease weight (i.e., the sum of tumor weight and ascites weight) was subtracted from post-injection body weight. However, for examination of appetite (food intake per body weight) and anhedonic depressive-like behavior (sucrose intake per body weight), only ascites weight was subtracted because, whereas ascites is mostly non-metabolic exudate (i.e., water), tumor tissue is highly metabolic and thus contributes to ingestive behavior.

2.6 Sucrose Intake and Sucrose Preference

In Experiment 1, after a 5-day period of continuous access to a 3% sucrose solution followed by a 2-day period of withdrawal, the baseline sucrose intake of each mouse was determined by measuring the change in volume of the sucrose solution over 1 hour after the dark phase had begun and following measurement of home cage locomotion (i.e., approximately 1-4 hours into the dark phase). A glass graduated cylinder with fitted sipper-tube held the sucrose solution in the home cage. The recorded volume of solution was then divided by the mouse's body weight minus ascites weight and reported as mL/100g of body weight (BW). After tumor incubation, 1-hour measurements were again recorded. Note that water intake was not measured at this time because the mice no longer drank any of the water that was available to them. After being exposed to a palatable sucrose solution, the majority of C57BL/6 mice stop drinking plain water and prefer to drink only the sucrose solution (cf. Elizalde et al., 2008).

In Experiment 2, in order to control for any differences in thirst among animals, we exposed the mice to sucrose solution only once at the end of tumor incubation to ensure measurement of both sucrose solution consumption and plain water consumption for determination of sucrose preference. Sucrose preference was determined for a subgroup of mice ($n = 37$) by measuring sucrose intake in relation to water intake and calculated according to the following formula: % preference = (sucrose solution intake) / (sucrose solution intake +

water intake). Group-housed mice were placed into individual cages for the duration of sucrose intake and water intake measurement. Measurement took place over three hours following all other behavioral measures at the onset of the dark phase.

2.7 Food Intake

Chow was weighed at the beginning of the day and 24 hours later. Care was taken to include chow that had fallen through the hopper onto the cage bottom. The recorded weight change of food was then divided by the mouse's body weight minus ascites weight. Food intake is reported as mg/g-BW. Individual food intake could not be measured in group-housed mice in Experiment 2 due to the shared food source.

2.8 Locomotion

Home cage locomotion was recorded using an EthoVision® Video Tracking System (Noldus Information Technology, Leesburg, VA). The software tracked the distance in cm that the mouse traveled during a 6-minute period. Each recording began after replacing the metal-grid/food-hopper lid with a vented Plexiglas® cover. All recordings took place within the first 4 hours of the dark phase under red light illumination.

2.9 Rotarod Performance

Post-injection motor capacity was determined by measuring performance in the rotarod test. Each mouse was placed on a cylinder (diameter: 3.5 cm; height: 27 cm) rotating at a speed of 4 rpm and then accelerating uniformly to 17 rpm over 1 minute. Three trials were conducted separated by a 30-second rest. The time (seconds) that each mouse stayed on the rotating cylinder was used as the rotarod score. A mouse remaining on the rotating cylinder for 90 seconds on a given trial was removed from the cylinder, and scored as 90 seconds. The three trial times for a given mouse were averaged to derive the rotarod time.

2.10 TST Immobility

In Experiment 2, the TST was utilized with a modification to prevent tail climbing behavior, which occurs substantially more in C57BL/6 mice compared to other strains (Mayorga & Lucki, 2001). Specifically, an adhesive piece of duct tape was applied to a steady vertical metal rod (1.3 cm diameter), and the tail was then fastened between the tape and the vertical rod such that the base of the tail was also flush with the end of the rod making it impossible to climb neither the tail nor the rod. Mice were then video recorded for six minutes. The number of immobile-mobile cycles was counted by a trained observer blind to experimental condition. Immobility time was the average immobile bout time (s/ bout) for each mouse, which was calculated by dividing immobility time by the number of immobile-mobile cycles.

2.11 Systemic Cytokines

Peripheral trunk blood was collected and centrifuged at 2000 g for 10 minutes. Plasma was stored at -80° C. Samples were processed using a 7-plex multiplex kit for the simultaneous measurement of IL-6, IL-1 β , TNF- α , IFN- γ , IL-17, IL-4, IL-10 (Millipore, St. Charles, MO, #MPXMCYTO-70K), and a singleplex kit for measurement of TGF- β (#TGFB-64K-01). Prepared kits were analyzed using a Luminex® 200™ dual laser detection system. Bio-Plex Manager™ software was used to extrapolate sample concentrations from sample fluorescence intensities in the assay. Samples for which the fluorescence intensity of a given cytokine was very low and could not be converted to an observed concentration (22% of samples) were assigned the lowest detectable concentration in the assay for that cytokine. Square root transformations were applied to all cytokine distributions to normalize the data.

2.12 Statistical Analyses

2.12.1 Experiment 1—The effect of the estrous cycle on sucrose intake, food intake, and locomotion at baseline was examined with a repeated measures ANOVA. This same effect was examined in post-injection measurements with a 2×2 mixed model ANOVA where the between-subjects factor was tumor condition (control mice vs. tumor-bearing mice) and the within-subjects factor was phase (estrus vs. non-estrus). Estrous cycle was treated as a covariate when found to have a significant effect. Sucrose intake, food intake, body weight, and locomotion were each examined with a 2×2 mixed model ANOVA where the between-subjects factor was tumor condition and the within-subjects factor was time (average baseline vs. average post-injection). Significant interactions were followed with an independent groups *t* test to examine the simple effect at post-injection time. Rotarod performance and cytokines were each examined with an independent groups *t* test.

2.12.2 Experiment 2—The effect of estrous cycle on TST average immobile bout time, locomotion, sucrose preference, sucrose intake, water intake, and food intake was examined with an independent groups *t* test (estrus phase vs. non-estrus phase). TST average immobile bout time, locomotion, rotarod performance, sucrose preference, sucrose intake, water intake, body weight, and cytokines were each examined with a 2×2 univariate ANOVA where the first factor was housing condition (group-housed mice vs. individually-housed mice) and the second factor was tumor condition. Follow up tests to examine simple effects were completed with Tukey's HSD test for multiple comparisons. Food intake in individually-housed mice was examined with an independent groups *t* test.

2.12.3 Mediation Analysis—Locomotion data and sucrose intake data from both Experiment 1 and Experiment 2 (individually-housed subjects only) were pooled together to test for mediation of the effect of tumor on these behaviors via cytokines. Sucrose intake data were converted into *z* scores before pooling. Cytokines were selected for mediation analysis if there was evidence that tumor had an effect on it and it correlated with the behavior of interest. The multiple mediator model developed by Preacher and Hayes (2008) was used for this analysis. This approach provides the advantage of comparing the relative strength of multiple potential mediators without the parameter bias inherent in a single mediator model due to omission of other mediators. The Sobel test was used to determine the significance of potential cytokine mediators.

SPSS software (Chicago, IL) was used for all statistical procedures. Group parameters are reported as mean $\pm$ standard error of the mean (SEM). *P* values $< .05$ were considered statistically significant. Effect sizes are noted using Cohen's *d*.

3. Results

3.1 Experiment 1

3.1.1 ID8 Ovarian Tumor—Of the 34 mice injected with ID8 ovarian carcinoma, 29 mice exhibited visible tumor and/or ascites at necropsy. Mean tumor weight was 0.7 ± 0.17 g, and mean ascites weight was 2.7 ± 0.99 g. Control mice exhibited no sign of disease in the peritoneum.

3.1.2 Estrous Cycle—As mice in estrus phase moved significantly more than mice in non-estrus phases ($P < .01$, $d = .86$) (data not shown), baseline estrous cycle was treated as a covariate for analysis of locomotion. There was no significant effect of estrous cycle on baseline measures of food intake ($P = .38$, $d = .25$) or sucrose intake ($P = .11$, $d = .47$). Post-injection examination of estrous cycle showed no significant effect on any of these measures (P values $> .32$).

3.1.3 Sucrose Intake, Food Intake, and Body Weight—As presented in Figure 2A, tumor-bearing mice consumed significantly less sucrose solution than control mice at the end of the experiment ($P < .05$, $d = .76$). Figure 2B shows that both groups significantly decreased food intake over time, but there was no significant difference between groups at the end of the experiment ($P = .48$, $d = .21$). Control mice exhibited significantly more body weight than tumor-bearing mice at the end of the experiment ($P < .05$, $d = .65$) (Figure 2C).

3.1.4 Locomotion and Motor Capacity—Tumor-bearing mice moved significantly less in the home cage than control mice at the end of the experiment, adjusting for baseline estrous cycle phase ($P < .001$, $d = 1.17$) (data not shown). In contrast, rotarod time for tumor-bearing mice was not significantly different from control mice ($P = .39$, $d = .26$).

3.1.5 Systemic Cytokines—As presented in Figure 3, mice bearing ovarian tumors exhibited higher systemic levels of proinflammatory and antiinflammatory cytokines than controls. These levels were significant for IL-6 ($P < .001$, $d = 1.31$), TNF- α ($P < .05$, $d = .71$), and IL-10 ($P < .001$, $d = 1.28$), and marginal for IL-4 ($P = .09$, $d = .52$). Mean differences for other cytokines were not significant (P values $> .11$, $d = .32$ to $.49$).

3.2 Experiment 2

3.2.1 ID8 Ovarian Tumor—All 37 mice injected with ID8 ovarian carcinoma exhibited visible tumor and/or ascites in the peritoneum at necropsy. There was no significant difference in tumor weight between individually-housed (0.7 ± 0.13 g) and group-housed mice (1.0 ± 0.39 g) ($P = .34$, $d = .33$). Similarly, there was no significant difference in ascites weight between individually-housed mice (3.8 ± 1.21 g) and group-housed mice (2.8 ± 1.15 g) ($P = .58$, $d = .19$). Control mice exhibited no evidence of disease in the peritoneum.

3.2.2 Estrous Cycle—There was no significant effect of estrous cycle on behaviors in Experiment 2 (all P values $> .22$).

3.2.3 Sucrose Preference, Food Intake, and Body Weight—As presented in Figure 4A, individually-housed tumor-bearing mice exhibited a significantly lower sucrose preference than group-housed tumor-bearing mice and control mice in both housing conditions ($P < .05$, $d = .76$). This effect on sucrose preference occurred in conjunction with a significant main effect of tumor ($P < .05$, $d = .67$) and housing condition ($P < .05$, $d = .71$) on sucrose intake, as tumor-bearing mice consumed less sucrose solution than control mice (Figure 4B) and individually-housed mice consumed less sucrose solution than group-housed mice (Figure 4C). In contrast, neither tumor ($P = .84$, $d = .07$) nor housing condition ($P = .51$, $d = .23$) had a significant main effect on water intake. Interaction effects between tumor condition and housing condition on sucrose intake and water intake were not significant (all P values $> .26$).

Consistent with Experiment 1, there was no significant difference in food intake per body weight between tumor-bearing mice and control mice ($P = .63$, $d = .15$) (Note that food intake was measured only in individually-housed mice in Experiment 2). Tumor-bearing mice weighed less than control mice ($P < .01$, $d = .75$) (data not shown). Neither the main effect of housing condition ($P = .14$, $d = .36$) nor the interaction effect between tumor condition and housing condition ($P = .11$, $d = .39$) on body weight was significant.

3.2.4 TST Immobility, Locomotion, and Motor Capacity—There was no significant main effect of tumor condition on average immobile bout time ($P = .54$, $d = .08$) (Figure 5A). In contrast, Figure 5B shows that tumor-bearing mice regardless of housing condition

moved significantly less than control mice in the home cage ($P < .001$, $d = 1.26$) as in Experiment 1. Rotarod time was again not significantly different between tumor-bearing mice and controls ($P = .22$, $d = .29$).

The average TST immobile bout time was significantly longer for individually-housed mice than group-housed mice ($P < .05$, $d = .54$) (data not shown). Individually-housed mice exhibited significantly more home cage locomotion than group-housed mice ($P < .05$, $d = .47$) (data not shown), but no significant difference in rotarod time ($P = .23$, $d = .29$). Interaction effects between housing condition and tumor condition on TST immobility, locomotion, and rotarod time were not significant (all P values $> .14$).

3.2.5 Systemic Cytokines—As presented in Figure 6, systemic proinflammatory and antiinflammatory cytokine levels in Experiment 2 followed a similar pattern to that found in Experiment 1. Tumor-bearing mice had significantly higher levels of IL-6 ($P < .001$, $d = 1.86$), IL-17 ($P < .05$, $d = .64$), and IL-10 ($P < .001$, $d = 1.54$) compared to controls. Main effects of tumor on other cytokines were not significant (P values $> .16$, $d = .03$ to $.39$). The main effects of housing condition on cytokines and the interaction between tumor condition and housing condition on each of the cytokines were not significant (all P values $> .16$).

3.3 Mediation Analysis

Given the consistent effects of ovarian tumor on locomotion and sucrose intake in Experiments 1 and 2, data on these measures in individually-housed subjects were pooled together for mediation analysis. Of the five cytokines presented above in which tumor had a significant or marginal effect in Experiment 1 or 2, three of these cytokines, IL-6, TNF- α , and IL-10, showed evidence of a significant zero-order correlation with locomotion (Table 1). Thus, these cytokines were entered into a multiple mediator model with tumor condition as the independent variable and locomotion as the dependent variable to test for a mediation effect (Figure 7A). The indirect effect of tumor on locomotion through IL-6 was significant ($P < .01$, $d = .57$). TNF- α showed evidence of a marginal effect ($P = .07$, $d = .40$). IL-10 was not a significant mediator ($P = .71$, $d = .08$).

IL-6, TNF- α , and IL-10 also showed evidence of a correlation with sucrose intake (see Table 1). Thus, these cytokines were entered into a multiple mediator model with tumor condition as the independent variable and sucrose intake as the dependent variable to test for a mediation effect as shown in Figure 7B. In contrast to locomotion, neither IL-6 ($P = .98$, $d = .01$), TNF- α ($P = .33$, $d = .24$), nor IL-10 ($P = .52$, $d = .16$) emerged as significant mediators of sucrose intake.

4. Discussion

4.1 Anhedonia

Ovarian tumor caused a significant reduction in sucrose intake, a validated measure of anhedonia in animal models of depression (Weiss & Kils, 1995). Despite tumor-bearing mice weighing significantly less than controls at the end of the experiments, there was no significant difference in appetite between tumor-bearing mice and controls. Thus, the difference in sucrose consumption appeared to be a specifically anhedonic effect. This is consistent with the previous finding that mammary tumors caused a decrease in sucrose intake in rats (Pyter et al., 2009) and extends that finding into a mouse model of cancer.

4.2 Modulation by Social Housing

Group-housed mice consumed significantly more sucrose solution than individually-housed mice resulting in a significant interaction effect in sucrose preference such that individually-

housed tumor-bearing mice exhibited a lower preference for sucrose solution than group-housed tumor-bearing mice or controls in either housing condition. Thus, the present study provides new experimental evidence suggesting that social factors may contribute to the relationship of tumor to depression in a clinical setting. Social isolation is a significant risk factor for MDD in cancer patients (Weisman & Worden, 1976). The results suggest that social isolation does not simply contribute to a so-called “reactive depression” (Paykel, 2008) but may sensitize the mechanisms by which the tumor itself contributes to depression, at least in the case of anhedonia.

4.3 TST Immobility

Despite the fact that tumor-bearing mice moved significantly less during measures of home cage locomotion, ovarian tumor did not cause a significant difference in TST immobility compared to controls, suggesting a functional dissociation between the mechanisms that mediate TST immobility and those that mediate spontaneous motor activity in ovarian tumor-induced behavioral change. There is evidence that changes in spontaneous locomotion and TST immobility can occur independently following a systemic inflammatory response (Frenois et al., 2007; Godbout et al., 2008; O'Connor et al., 2009). It has been suggested that inflammation-induced changes in locomotor activity and TST immobility are mediated by partially different brain systems (Frenois et al., 2007).

4.4 Systemic Inflammation

The findings that systemic levels of proinflammatory and antiinflammatory cytokines are elevated in the ID8 ovarian cancer model compared to controls are consistent with previous reports that IL-6 and TNF- α are found in high levels in the ascites of mice in this model (Hagemann et al., 2007; Said et al., 2007). The findings are also consistent with studies that report elevated systemic levels of proinflammatory (e.g., IL-6, IL-8, and VEGF) (Gorelik et al., 2005) and antiinflammatory cytokines (e.g., IL-10) (Santin et al., 2001) in ovarian cancer patients compared to healthy control patients.

That ovarian tumor would cause a systemic inflammatory response, an increase in anhedonia, and a psychomotor change as exhibited by a decrease in spontaneous home cage locomotion but no decrease in mobility during the TST may seem paradoxical. However, there is some indication that the level of inflammation in the periphery may need to be higher to affect TST performance than that which is needed to affect anhedonia (cf. Dunn & Swiergiel, 2005; Swiergiel & Dunn, 2002).

This heterogeneity of results in animal models of inflammation-induced behavioral change is similar to the heterogeneity of depressive symptoms found in the human clinical literature. Investigation of inflammation-induced MDD with the proinflammatory cytokine IFN- α has shown that neurovegetative and somatic symptoms occur early during cytokine treatment while depressive, anxious, and cognitive symptoms occur later (Capuron et al., 2002). Furthermore, the specific neurovegetative features of fatigue and appetite loss are not remedied by treatment with an SSRI, suggesting that the effects of proinflammatory cytokines on different MDD symptom dimensions are mediated by different mechanisms (Musselman et al., 2001).

The results of the present study are also similar to the finding in ovarian cancer patients that proinflammatory cytokines do not significantly correlate with all symptom dimensions of MDD. Specifically, IL-6 levels in ascites and systemic circulation significantly correlated only with the vegetative symptom subscale of the Center for Epidemiological Studies-Depression (CES-D) scale (Lutgendorf et al., 2008). The other three subscales (depressed

affect symptoms, positive affect symptoms, interpersonal discord symptoms) were not significantly correlated with IL-6.

Interestingly, administration of IL-6 directly into the brain (intracerebral-ventricular [i.c.v.] injection) in animal models of inflammation-induced behavioral change does not cause increases in sickness behavior. For example, one study showed that i.c.v. administration of rat recombinant IL-6 did not decrease social investigatory patterns or general locomotor activity in rats (Lenczowski et al., 1999). In contrast, i.c.v. administration of IL-1 β along with IL-6 in that study did bring about decrements in these behaviors. Consistent with this direct role for IL-1 β in the brain during sickness behavior, a separate study showed that while i.c.v. administration of TNF- α or IL-1 β can each cause a decrease in social investigatory behavior, the effect of TNF- α is inhibited when an IL-1 receptor antagonist is co-administered into the brain (Bluthe et al., 1994). Complementing these brain cytokine sickness behavior studies are a series of experiments that show an opposite pattern of effects when the cytokines are examined in peripheral circulation. For example, peripheral administration of neutralizing binding protein to TNF following peripheral lipopolysaccharide (LPS) injection did not attenuate the decrease in locomotor activity that followed, even though it did block the significant increase of circulating TNF that followed (Tollner et al., 2000). Similarly, another study showed that peripherally administered antibodies to TNF- α and IL-1 β were unable to attenuate the decrease in locomotor activity that followed peripheral LPS injection (Harden et al., 2006). In contrast, this same study was able to attenuate decreased locomotor activity with antibodies to IL-6, suggesting that endogenous systemic IL-6 from an inflammatory response is a *peripheral* mediator of inflammation-induced sickness behavior while IL-1 β and TNF- α are more *central* mediators (Harden et al., 2006). Thus, the finding in the current study that circulating IL-6 was a significant mediator of the effect between tumor and locomotor activity but that systemic TNF- α only weakly mediated the effect is consistent with studies that examine sickness behavior and proinflammatory cytokines in the periphery.

That being said, there was no significant statistical mediation by any peripheral cytokine in the effect between tumor and the more specifically depressive-like behavior of sucrose intake in the current study. This suggests at least two possibilities: 1) aside from the peripheral cytokines examined in this study, there is a more directly acting peripheral factor(s) of mediation in the causal pathway from tumor to lower sucrose intake; 2) the effect of ovarian tumor-generated inflammatory cytokines on anhedonia is mediated in a non-linear dynamic manner. Although further investigations are necessary to evaluate each of these, a recently developed model of chronic inflammation-induced depressive-like behavior (cf. Moreau et al., 2008) has shown that systemic levels of IL-6 in mice exhibit a steady decrease in the weeks following inoculation with *Bacillus Calmette-Guerin* but that there is an appearance of lower sucrose preference by 3 weeks post-injection in the absence of lower body weight. At this time, a different factor in the periphery becomes significantly elevated when the anhedonia appears. This factor is kynurenine, which is produced in greater levels as a result of elevated indoleamine 2,3 dioxygenase (IDO) activity by macrophages and dendritic cells during an inflammatory response (O'Connor et al., 2009; Takikawa et al., 1999; Popov et al., 2006; Fujigaki et al., 2006). Kynurenine easily crosses the blood-brain barrier via the large neutral amino acid transporter system (Fukui et al., 1999), and metabolites of kynurenine, i.e., quinolinic acid and kynurenic acid, will agonize and antagonize glutamatergic N-methyl-D-aspartic acid (NMDA) receptors, respectively. Thus, such factors may represent more proximate mechanisms of inflammation-induced depression that are downstream of peripheral cytokines generated during a systemic inflammatory response (Dantzer et al., 2008).

4.5 Limitations

The primary goal of our studies was to show that ovarian cancer can induce behavioral changes in the mouse that are deemed uniquely representative of depressive disorders in humans based on previous demonstrations of construct, predictive, and face validity. However, animal models of depression are necessarily limited in their inference because depressive disorders are a heterogeneous cluster of symptoms diagnosable only in humans. Given the fact that only one of the two specifically depressive-like behaviors used in the study was altered by tumor (i.e., sucrose intake), further investigation of other depressive-like behaviors, such as immobility in the forced swim test and decreased responding for brain stimulation reward, is needed to provide convergent evidence for the effect found in the current study.

A second goal was to assess whether peripheral cytokines act as one of the first mediators in the effect between tumor and depressive-like behavior. Although we did not measure cytokines right after initial tumor injection, it is unlikely that the initial tumor treatment itself is responsible for the peripheral inflammation observed given the duration of time between these two events. Because we did not measure brain cytokines, our study does not show that changes in peripheral cytokines necessarily caused a change in brain cytokines that mediated the observed behavioral changes. Furthermore, our investigation of peripheral cytokine mediation was a correlational investigation. Thus, the finding that systemic IL-6 was a significant mediator in the effect between tumor and spontaneous locomotor activity needs further confirmation with peripheral IL-6 blocking experiments.

4.6 Conclusions

These experiments support the hypothesis that ovarian tumors generate a systemic inflammatory response in the body and cause anhedonia and psychomotor slowing. Furthermore, social isolation may sensitize the mechanisms by which tumor-induced factors contribute to anhedonia. Together, the evidence supports a biopsychosocial position that tumors induce inflammation and contribute to the symptoms of depression in ovarian cancer patients.

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Figure 1. Diagram for Experiments 1 and 2

Figure 2. Sucrose intake, food intake, and body weight between groups in Experiment 1

(A) To test for an effect of ovarian tumor on anhedonia, 1-hour sucrose intake was measured for control and tumor-bearing mice at baseline and 8-12 weeks post-injection. There was a significant interaction between tumor condition and time as tumor-bearing mice (diamond) ($n = 29$) consumed significantly less sucrose solution than control mice (triangle) ($n = 18$) at the end of the experiment, $*P < .05$. BW: body weight

(B) To further examine the specificity of the effect of ovarian tumor on sucrose intake, 24-hour food intake and body weight were measured to test for an effect of ovarian tumor on appetite. Both experimental groups ate significantly less chow per body weight at the end of the experiment than at baseline ($P < .001$), but there was no significant difference (n.s.) between groups at the end of the experiment ($P = .25$ for interaction).

(C) The interaction effect between time and tumor condition on body weight was significant as the increase in body weight over time was greater in control mice than in tumor-bearing mice, $*P < .05$.

Figure 3. Systemic proinflammatory and antiinflammatory cytokine levels in mice from Experiment 1

Tumor-bearing mice ($n = 29$) exhibited significantly higher levels of systemic IL-6, TNF- α , and IL-10 than control mice ($n = 17$) at 8-12 weeks post-injection, $*P < .05$, $***P < .001$. Difference between groups was marginal for IL-4 ($P = .09$). §: Units are ng/mL. SQRT: square root transformed.

Figure 4. Sucrose preference and sucrose intake between groups in Experiment 2

(A) To control for any differences in thirst among animals, mice were exposed to sucrose solution only once at the end of tumor incubation to ensure measurement of both sucrose solution consumption and plain water consumption for determination of sucrose preference [Sucrose preference = (sucrose solution intake) / (sucrose solution intake + water intake)]. Individually-housed tumor-bearing mice ($n = 10$) exhibited a significantly lower sucrose preference in comparison to group-housed tumor-bearing mice ($n = 8$) and control mice that were individually-housed ($n = 9$) or group-housed ($n = 10$), $*P < .05$.

(B) Tumor-bearing mice exhibited significantly less sucrose intake than control mice, $*P < .05$.

(C) Individually-housed mice exhibited significantly less sucrose intake than group-housed mice, $*P < .05$.

Figure 5. TST immobility and locomotion between tumor-bearing mice and control mice in Experiment 2

(A) Tumor-bearing mice ($n = 37$) exhibited no significant difference (n.s.) from control mice ($n = 36$) in average immobile bout time ($P = .73$).

(B) Tumor-bearing mice exhibited significantly less home cage locomotion than control mice, *** $P < .001$.

Figure 6. Systemic proinflammatory and antiinflammatory cytokine levels in mice from Experiment 2

Tumor-bearing mice in both housing conditions ($n = 30$) exhibited significantly higher levels of systemic IL-6, IL-17, and IL-10 than control mice in both housing conditions ($n = 27$) at 6-12 weeks post-injection, $*P < .05$, $***P < .001$. §: Units are ng/mL. SQRT: square root transformed.

Figure 7. Cytokine mediation models

(A) Regression coefficients and significance values are noted for each path from tumor to cytokines and, after adjustment, from cytokines to locomotion in individually-housed mice from Experiments 1 and 2 ($n = 82$). Total effect of tumor and cytokines on locomotion is delineated by the c coefficient. Direct effect between tumor and locomotion, adjusting for cytokine mediators in model, is delineated by c' coefficient.

(B) Sucrose intake model ($n = 66$).

Table 1
Systemic Cytokine Correlations with Locomotion and Sucrose Intake

Cytokine	Locomotion		Sucrose Intake	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
IL-6	-.55	<.001	-.32	.009
TNF- α	-.38	<.001	-.26	.03
IL-17	-.09	.44	-.02	.84
IL-4	-.17	.12	-.13	.28
IL-10	-.41	<.001	-.35	.004

correlations in bold are significant