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Title: MELANOMA TUMORS ALTER PROINFLAMMATORY CYTOKINE PRODUCTION AND MONOAMINE BRAIN FUNCTION, AND INDUCE DEPRESSIVE-LIKE BEHAVIOR IN MALE MICE

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ABSTRACT

Depression is a commonly observed disorder among cancer patients; however, the mechanisms underlying the relationship between these disorders are not well known. We used an animal model to study the effects of tumor development on depressive-like behavior manifestation, proinflammatory cytokine expression, and central monoaminergic activity. Male OF1 mice were inoculated with B16F10 melanoma tumor cells and subjected to a 21-day behavioral evaluation comprising the novel palatable food (NPF) test and tail suspension test (TST). The mRNA expression levels of proinflammatory cytokines, interleukin 1β and 6 (IL-1β) and (IL-6), and tumor necrosis factor-alpha (TNF-α), were measured in the hypothalamus and hippocampus and the levels of IL-6 and TNF-α were measured in the blood plasma. We similarly determined the monoamine turnover in various brain areas. The tumors produced an increase in immobility during the TST and an increase in the hippocampal expression of IL-6. These increases corresponded with a decrease in dopaminergic activity in the striatum and a decrease in serotonin turnover in the prefrontal cortex. Similarly, a high level of tumor development produced increases in the brain expression levels of IL-6 and TNF-α and plasma levels of IL-6. In summary, the results indicated that tumor development, especially at advanced stages, produced increases in the brain and plasma proinflammatory cytokine levels and alterations in monoaminergic functions that might be responsible for the manifestation of depressive-like behaviors.

Keywords: anhedonia, cytokines, inflammation, melanoma tumor, monoamines, tail suspension test.

1. INTRODUCTION

Depression is a commonly observed disorder among cancer patients [1]. The prevalence of depressive symptoms in patients with cancer exceeds that observed in the general population [2] and depression is associated with a poorer prognosis and increased treatment noncompliance [3]. A growing literature reveals that the increased risk for developing depression is not solely explained by the psychosocial stress associated with the cancer diagnosis, but with the chronic inflammatory processes associated as well [4].

According to the American Psychiatric Association (APA), the characteristic symptoms of major depression include anorexia, weight loss, fatigue, lethargy, sleep disorders, hyperalgesia, reduction of locomotor activity, and failure to concentrate. These characteristic symptoms of major depression evidence a considerable phenomenological similarities with *sickness behavior*. Sick individuals experience depressive-like behaviors such as anhedonia, fatigue, activity reduction, loss of interest in social activities, decreased appetite, increased sensitivity to pain, and sleep alterations, like a part of a natural homeostatic reaction the body uses to fight infection [5, 6].

Pathogen infections, tissue damage, neoplastic processes, and psychosocial stress all activate immune cells to secrete proinflammatory cytokines and thus coordinate local and systemic inflammatory responses. Cytokines such as interleukins 1 and 6 (IL-1 and IL-6) and tumor necrosis factor-alpha (TNF-α) act on the brain and exert effects on neurotransmission, neuroendocrine function, and behavior [7]. Different studies that have investigated the central or peripheral administration of IL-1, IL-6, and TNF-α [8, 9] or those that have studied molecules that promote the production of these cytokines, including endotoxins and lipopolysaccharides (LPS), have shown that these cytokines are directly or indirectly causative of sickness behavior [10, 11]. Other studies have provided evidence of increases in various proinflammatory cytokine levels in patients suffering from depression [12, 13]. These data have given rise to the inflammatory hypothesis of depression, in which inflammatory processes and brain-systemic immune interactions are implicated in the pathogenesis of major depression [14-18].

The presence of tumor cells generates a systemic inflammatory response that, together with inflammatory products generated by the tumor itself, can also contribute to symptoms of depression [19-23]. Using a rodent model of ovarian cancer, Lamkin et al. (2011) observed that the tumors produced high systemic levels of both proinflammatory and anti-inflammatory cytokines and elicited anhedonic behaviors [24]. Similarly, rats with mammary cancer exhibited increased proinflammatory cytokine levels in the hippocampus and the periphery as well as depression and anxiety-like behaviors [25]. Melanoma cells produce various proinflammatory cytokines such as IL-1β, IL-6, and TNF-α that act as autocrine growth factors [26-28]. These cytokines have been positively correlated with the presence of depressive symptoms [29] and sickness behavior [14]. The 45% of patients who receive exogenous chronic INF-α administration to treat malignant melanoma have been found to experience symptoms of depression [30]. Evidence suggests that a possible mechanism by which cytokines can induce depressive symptomatology is the ability to influence the monoamine metabolism [15, 31-33]. The effect of inflammatory cytokines on basal ganglia dopamine (DA) might be especially relevant to depression and fatigue as well as to psychomotor disturbances and the development of neurovegetative disorders [34]. Various animal studies have implicated a role for cytokines in serotonergic transmission [10, 35, 36]. Additionally, antidepressant drugs such as paroxetine have been observed to alleviate depressive symptoms, and this drug was reported to facilitate the regulatory feedback pathway of proinflammatory cytokine release and to elevate serotonergic system activity [37].

Previous studies completed in our lab have shown that the development of B16F10 melanoma tumors produces behavioral and neurochemical changes characteristic of sickness behavior [38]. Therefore, when confronted with a challenging social situation, the subjects implanted with these tumors manifested a decrease in diverse proactive behaviors that was accompanied by increases in the DA receptor D2 density in the striatum and DA and serotonin (5-HT) turnover in the hypothalamus [39, 40]. Similarly, we found that tumor development induces increased immune system activity [38]. These results have allowed us to hypothesize that the neurochemical and behavioral changes produced by B16F10 melanoma might result from the secretion of proinflammatory cytokines in various brain areas.

From this perspective, the objective of this study was to determine whether the development of B16F10 melanoma tumors promotes the manifestation of depressive behaviors by evaluating motor and hedonic abilities. We also studied how the manifestation of these behaviors correlated with increases in proinflammatory cytokine secretion and alterations in central monoaminergic activity.

2. MATERIALS AND METHODS

2.1. Subjects and husbandry

Six-week-old OF1 outbred male mice (Charles River, Oncins, France) were individually housed for 15 days in transparent plastic cages measuring 24.5 x 24.5 x 15 cm. Food and water were available *ad libitum*. The holding room was maintained at a constant temperature of 20 ºC with a reversed 12-h light/dark cycle (white lights on from 20:00 to 08:00 h) to enable the testing of these nocturnal animals during their active phase (1 h after the beginning of the dark cycle). All experimental procedures were conducted under dim red lighting in a room adjacent to the holding facility. All procedures involving mice were performed according to the European Directive (2010/63/EU) on the protection of animals used for scientific purposes (September 22, 2010). The procedures were approved by the Ethical Committee for Animal Welfare of the Basque Country University (CEBA).

2.2. Experimental design

The experiment began after a 15-day adaptation period. Individually housed animals were randomly allocated to 2 groups. After recording the body weights of all mice, 1 group was inoculated with B16F10 melanoma cells ($n = 34$) and the other with vehicle (tumor inoculation factor; $n = 9$). Fourteen days after inoculation, all inoculated and non-inoculated were subjected to the tail suspension test (TST). On day 20, all mice were subjected to the novel palatable food (NPF) test. The next day (day 21), all mice were sacrificed by cervical dislocation and the body weights were recorded. Blood was immediately collected from each

mouse via cardiac puncture and the isolated plasma samples were frozen at -80 °C prior to the determination of the IL-6 and TNF-α levels. The lungs were infused with formal calcium and conserved in Bouin's solution until the tumor area could be determined. Finally, the brain was quickly removed and the whole hypothalamus, frontal cortex, striatum and hippocampi were dissected. All dissections were performed under sterile conditions and stereomicroscopic observation with reference to the mouse brain atlas [41]. The samples were stored at -80 ºC prior to measuring IL-1ß, IL-6, and TNF-α mRNA expression in the hippocampus and hypothalamus, and dopaminergic and serotonergic activity in the hippocampus, striatum, and prefrontal cortex.

2.3. Experimental tumor induction

Tumors were induced by the inoculation of B16F10 melanoma murine cells. The B16F10 cells were maintained *in vitro* by subculturing the tumor cells at 37 ºC in a humidified atmosphere of 5% CO_2 and at a concentration of 10^5 cells/ml in 75-cm^2 cell culture flasks (Corning Inc., Corning, NY, USA) in RPMI-1640 culture medium (Sigma-Aldrich, Madrid, Spain) supplemented with 10% fetal calf serum (Gibco, Life Technologies, Carlsbad, CA, USA), 25 mM HEPES, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, and 2 g/l of sodium bicarbonate (Sigma-Aldrich, Madrid, Spain). Adherent B16F10 cells were detached by exposure to 0.02% EDTA for 5 min and were subsequently washed 3 times in RPMI-1640 medium. Mice that had been pre-anesthetized via intraperitoneal (ip) Nembutal (sodium pentobarbital; 60 mg/kg) administration were inoculated with 5×10^4 viable B16F10 cells in 0.1 ml of medium into the lateral tail veins using a 30 ½ gauge needle after previously heating the tails with a thermal pillow. To ensure the success of the tumor inoculation, all subjects that did not receive the complete 0.1-ml doses during the first injection were eliminated (n = 3).

2.4. Novel palatable food (NPF) test

Mice were habituated to a NPF (a peanut) in their home cages for 1 h/day (5:00–6:00 p.m.) for 3 days before testing (days 17–19 after inoculation). On day 20, when the palatable food

ingestion latency was expected to have decreased because of habituation to the NPF [42, 43], the peanut was presented (1 h) in a normal housing cage from which the bedding had removed. Consumption (grams) was measured by weighing the peanut before and after the test session. As consumption depends on the body weight, this variable was expressed as a percentage of body weight by calculating the ratio between the amount of consumed peanut and the body weight × 100, as described in Frenois et al. (2007) [44].

2.4.2. Tail suspension test (TST)

The TST was conducted in a manner similar to that described by Steru et al. (1985) [45]. Mice were individually suspended by their tails from a horizontal ring-stand bar placed 60 cm above the floor. The tails were secured with adhesive tape that was placed approximately 2 cm from the tips of the tails. The test was performed for 6 min and was recorded with video cameras (GZ-MG773; JVC, Yokohama, Japan) for subsequent behavioral assessments with the ANY-maze© version 4.96 video-tracking software (Stoelting Europe, Dublin, Ireland). As described in Lamkin et al., 2011, the immobility time was defined as the average immobile bout time (s/bout) for each mouse; this was calculated by dividing the immobility time by the number of immobile-mobile cycles [24]. Tail climbing behavior not was observed.

2.5. Physiological determinations

2.5.1. Determination of the pulmonary tumor area

After several days in Bouin's solution, the 5 lobes of the lung were separated to determine the tumor areas and digital images were obtained. Each photograph was captured with the same exposure conditions and included a standard-sized reference circle (9.62 mm^2). The tumor area of each mouse was determined with the public domain ImageJ software program developed at the U.S. National Institutes of Health (ImageJ, U.S. NIH, Bethesda, MD, USA; available at http://rsbweb.nih.gov/ij/).

2.5.2. Determination of the IL-6 and TNF-α plasma levels

The blood collected via cardiac puncture after sacrifice was collected in heparinized containers and centrifuged at 1800 × g for 15 min at 4 ºC. The resulting plasma was collected and stored at -80ºC until the cytokine assays were performed. The IL-6 and TNF-α plasma levels were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D Systems Europe, Ltd. (Abingdon, UK). According to the manufacturer's instructions, the ELISA plates for both cytokines were read at 450 nm on a Synergy HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The detection limits were 1.6 pg/ml (with intra- and inter-assay variation coefficients of 3.5–6.7% and 6.2–8.8%, respectively) and 1.88 pg/ml (variation coefficients of 3.1–3.8% and 5.8–7.7%, respectively) for the IL-6 and TNF-α assays, respectively.

2.5.3. Real time RT-PCR measurements of IL-1β, IL-6, and TNF-α mRNA expression in the hypothalamus and hippocampus

Whole hypothalamus and hippocampus tissues were homogenized in Trizol reagent (Life Technologies S.A., Madrid, Spain), and total RNA was isolated according to a standard phenol: chloroform extraction method [46]. A UV spectrophotometric analysis was performed at 260 nm to determine the nucleic acid concentrations, while the 260:280 absorbance ratio was utilized to assess the nucleic acid purity. The samples were DNase-treated (DNase I; Invitrogen, Madrid, Spain) to remove contaminating DNA prior to cDNA synthesis. The total RNA was then reverse-transcribed with the PrimeScript ™ RT reagent kit (Takara Bio Inc., Madrid, Spain) and the resulting cDNA levels were quantified by SybrGreen-based (SYBR® Premix Ex Taq™, Takara Bio Inc., Madrid, Spain) real-time PCR. PCR product formation was monitored in real time with the Applied Biosystems 7500 Real Time PCR System (Applied Biosystems, Madrid, Spain). The cDNA sequences were obtained from GenBank at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov). Glyceraldehyde-6-phosphate dehydrogenase (GAPDH) served as a housekeeping gene. All primer sequences (Table 1) were designed with the Primer Express Software v3.0 package (Applied Biosystems) and were obtained from Applied Biosystems.

Primer specificity was verified in a melt curve analysis. The relative gene expression was determined according to the $2^{-\Delta\Delta t}$ method [47].

2.5.4. Determination of central monoamines in prefrontal cortex, hippocampus and striatum

To determine the dopaminergic and serotonergic activity in the prefrontal cortex, hippocampus, and striatum, the levels of these monoamines and their metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were analyzed via high-performance liquid chromatography (HPLC). The HPLC equipment comprised an Agilent 1200 LC system (Agilent Technologies, Madrid, Spain) equipped with a vacuum degasser, quaternary pump, cooled autosampler, thermostatted column compartment, and fluorescence detector. Chromatographic separation was performed on a Poroshell 120 EC-C18 column (100 x 4.6 mm, 2.7 µm) protected by a cartridge guard column that contained the same material as the analytical column (Agilent Technologies). The mobile phase comprised 0.05% trifluoroacetic acid (solvent A) and acetonitrile (solvent B), which were supplied by Acrōs Organics (Geel, Belgium). The flow rate was maintained at a constant 0.5 ml/min. The gradient elution program was as follows: from 0–8 min, 6% solvent B (v/v); from 8–15 min, 10% solvent B (v/v); from 15–22 min, 20% solvent B (v/v); and from 22–25 min, 2% solvent B (v/v). The column was maintained at 25 °C during the analysis and the samples were held at 4 °C in an autosampler unit. The effluent was monitored with the fluorescence detector at excitation wavelengths of 283 nm for DA, DOPAC, and 5-HIAA and 229 nm for 5-HT. For all analytes, the emission wavelength was 320 nm. The total sample analysis time was 22 min. The mobile phase was prepared daily and filtered through a 0.22-µm Durapore filter (Millipore, Madrid, Spain).

Prior to sample preparation, the frozen tissues were weighed on AG204 analytical scales (Mettler-Toledo International, Inc., Columbus, OH, USA). The tissues were homogenized and deproteinized in 60 µl of homogenization solution (1% formic acid in acetonitrile) for 1 min in a sonicator (LABSONIC P; B. Braun Biotech International GmBH, Melsungen, Germany). The aforementioned treatment denatured the protein molecules and thus the protein levels were virtually impossible to detect. The homogenates were immediately

vortexed for 5 min (Vortex Genie-2; Scientific Industries, Bohemia, NY, USA) and then centrifuged for 15 min at 15,000 × g and 4 ºC (Microfuge 22R Centrifuge; Beckman Coulter, Madrid, Spain). The supernatants were dried for 30 min with compressed air to concentrate the samples and were then reconstituted with 30 µl of 0.05% trifluoroacetic acid. Given the impossibility of filtering such small volumes, the samples were centrifuged for 15 min at 15,000 × g and 4 ºC. Ultimately, 20 µl of each supernatant was injected into the HPLC system for analysis.

Data processing was performed with the Agilent ChemStation software program (Agilent Technologies); this program was used to quantify all compounds by comparing the areas under the peaks with the areas of the reference standards. All standards were purchased from Sigma-Aldrich and were dissolved in a stock 0.1 N hydrochloric acid solution. The calibration samples were prepared by adding appropriate amounts of standard working solutions to chromatographic grade water obtained from a Millipore water purification system (Millipore, Madrid, Spain).

2.6. Statistical analysis

All statistical analyses were performed with the SPSS 17.0 for Windows software package (SPSS Inc., Chicago, IL, USA), and the level of significance was set at $p < 0.05$. The behavioral and physiological variables were analyzed with a 1-way or 2-way ANOVA. The body weight-derived data were analyzed with a 1-way ANOVA for repeated measures. When appropriate, specific comparisons were made with *post hoc* Bonferroni tests.

3. RESULTS

The subjects inoculated with the experimental tumors showed large variability in the development of pulmonary metastases at 21 days after inoculation; therefore, the animals were divided into 2 subgroups according to their tumor development: large ($n = 12$; tumor area > 85.73 mm^2) and small ($n = 22$; tumor area < 85.73 mm^2) relative to the median.

3.1. Effects of tumor development on depressive behaviors

3.1.1. Novel Palatable food (NPF) test

In an analysis of the consumption data obtained 20 days after tumor inoculation, the ANOVA only showed a trend effect for the group factor ($F[1, 40] = 3.573$, $p = 0.066$). The tumor-bearing mice consumed a smaller amount of peanut than did the control mice. No differences were found between the large and small tumor-bearing groups and the control group.

3.1.2. Tail Suspension test (TST)

The analysis of immobile bout time during the TST at 14 days after tumor inoculation showed a significant effect for the group factor ($F[1, 41] = 4.471$, $p = 0.041$). The tumor-bearing mice exhibited more immobility than did the control mice (Fig. 1A). As shown in Fig. 1B, significant differences were also observed with respect to tumor development ($F[2, 40] = 4.702$, $p = 0.015$). Specifically, the mice with increased tumor development exhibited greater immobility than did the control mice ($p = 0.014$). No differences were observed between the small tumor-bearing group and the other groups.

3.2. Effects of tumor development on central and peripheral immune activity

No significant effects of tumor development were observed on cytokine mRNA expression in the hypothalamus. However, the hippocampal data analysis revealed a significant effect of the group factor for IL-6 ($F [1, 38] = 4.820$, $p = 0.034$) but not for TNF-α or IL-1β. Therefore, tumor-bearing mice exhibited higher levels of hippocampal IL-6 mRNA expression than did control mice (Fig. 2A). Furthermore, the cytokine expression analysis according to tumor development (large vs. small) revealed significant differences in the expression levels of TNF-α ($F[2, 39] = 4.809$, $p = 0.014$) and IL-6 ($F [2, 37] = 7.946$, $p < 0.001$) but not IL-1β mRNA in hippocampus. When TNF-α mRNA expression was compared among the 3 groups, the large tumor-bearing mice expressed higher levels of this cytokine

than did the small tumor-bearing ($p = 0.018$) and control mice ($p = 0.05$; Fig. 3B). Similarly, the large tumor-bearing mice expressed higher levels of IL-6 mRNA relative to those in small tumor-bearing ($p = 0.010$) and control mice ($p = 0.002$; Fig. 2B). In this sense, although no group factor effect was identified regarding the plasma IL-6 and TNF-α levels, a functional effect was observed for IL-6 with regard to tumor development. The *post hoc* analysis (Fig. 4B) revealed that the large tumor-bearing mice exhibited higher plasma IL-6 levels than did small tumor-bearing ($p = 0.003$) and control mice ($p = 0.041$).

3.3. Effects of tumor development on central monoaminergic activity

The central monoamine analysis revealed significant group factor effects with regard to the levels of DOPAC ($F[1, 41] = 5.305$, $p = 0.026$; Fig. 5A1), DA ($F[1, 41] = 4.825$, $p = 0.034$; Fig. 5A2) and 5-HT ($F[1, 41] = 8.746$, $p = 0.005$; Fig. 6A) in the striatum and the level of 5-HIAA ($F[1, 41] = 5.268$, $p = 0.027$; Fig. 7A1) and the ratio of 5-HIAA/5-HT ($F[1, 41] = 12.224$, $p < 0.001$; Fig. 7A2) in the prefrontal cortex. In all cases, the tumor-bearing mice presented with lower levels of these monoamines relative to those in control mice.

The analysis of the group differences according to tumor development revealed significant differences in the level of 5-HT in the striatum ($F[2, 40] = 5.079$, $p = 0.011$) and the level of 5-HIAA ($F[2, 40] = 4.562$, $p = 0.016$) and the 5-HIAA/5-HT ratio ($F[2, 40] = 6.679$, $p = 0.003$) in the prefrontal cortex. The *post hoc* analyses revealed that the large tumor-bearing group exhibited lower levels of 5-HT in the striatum relative to the control group ($p = 0.010$; Fig. 6B) whereas in the prefrontal cortex, the control group exhibited higher levels of 5-HIAA relative to the large tumor-bearing group ($p = 0.014$; Fig. 7B1) and a higher 5-HIAA/5-HT ratio relative to both the small ($p = 0.014$) and large ($p = 0.004$) tumor-bearing groups (Fig. 7B2).

3.4. Effects of tumor development on body weight

No differences in body weight were observed between the groups at the end of the experiment.

4. DISCUSSION

As stated in our hypothesis, tumor development induces behavioral changes indicative of a depressive state. This hypothesis was supported by the immobility observed during the TST, a well-established standardized paradigm for analyzing the extent of "despair" in animals that tolerate an immobile and passive posture without attempting to escape from an unpleasant situation [48]. Tumor-bearing subjects exhibited greater immobility than did the control subjects. However, although the tumor-bearing animals ate less during the NPF test relative to the control subjects, this difference was not found to be significant. This finding could be interpreted as the lack of an effect of tumor development on these behaviors or as the insufficient recording of information regarding consumption. Another notable factor about this result concerns the reinforcement characteristics of the selected test. In this regard, Pyter et al. (2009), using a sucrose consumption test to measure anhedonia in an animal model of ovarian cancer, observed sucrose solution concentration-dependent differences in the amounts consumed by the tumor-bearing animals versus the control animals [25].

The manifestation of this depressive behavior might be consequent to the increase in IL6 and TNF-α. Animals with greater tumor development exhibited significant increases in immobility along with increased hippocampal IL-6 and TNF-α mRNA expression and plasma IL-6 levels. Various studies performed in animals and humans have shown the effects of IL-6 and TNF-α on the pathogenesis of depression [49-52]. Additional evidence has been provided from studies on the administration of antidepressants. These studies have shown a direct relationship between manifestation of depressive symptoms and IL-6 y TNF- α plasmatic levels [53, 54]. In agreement with the immobility behavior examined in our study, Krügel et al. (2013) used an animal model of chronic mild stress to observe that treatment with the antidepressant etanercept, a TNF-α blocker, significantly reduced depressive behaviors as determined by the reduced immobility time during the forced swimming test (FST) and increased climbing behavior [55].

Growing evidence indicates that inflammatory stimuli produce changes in activity of multiple brain regions including basal ganglia, prefrontal cortex and hippocampus, that are involved in behavioral changes associated with depressive-like behaviors [56-59]. Moreover, a cytokine-induced changes in monoaminergic activity, dopaminergic and serotonergic activity have been established as a possible mediatory mechanism [10, 34-37]

We found that mice presenting with tumor development exhibited reduced dopaminergic activity in the striatum coincided with an increase in immobility observed in these subjects during the TST. Previous studies have provided evidence that alterations in basal ganglion function and DA activity can contribute to the development of the psychomotor and anhedonic symptoms observed in depressive patients [34, 60-63].

Interestingly, tumor development significantly reduced 5-HT turnover in the prefrontal cortex in tumor-bearing mice. This decrease in serotonergic function might arise from a decrease in the availability of tryptophan (TRP). Immune activation is known to associate with the induction of indoleamine-2-3-dioxygenase, an enzyme that directs the metabolism of TRP toward kynurenine (KYN) and quinolinic acid synthesis, thus reducing 5-HT availability [64]; this pathway might favor the development of depression [65, 66]. This hypothesis has been supported by data concerning the effects of IFN-α treatment on hepatitis C patients. IFN-α treatment led to increases in the IL-6 level and the ratio of KYN to kynurenic acid, which have been associated with depressive disorders [66, 67], as well as a negative correlation between the concentrations of IL-6 and 5-HIAA, which have also been correlated with depression severity [59]. Furthermore, cancer patients undergoing cytokine therapy were also found to exhibit significant decreases in blood TRP levels that correlated with the severity of depressive symptoms during the course of therapy [37].

In summary, the results obtained from this study indicate that tumor development, especially during advanced stages, produces an increase in cytokine expression as well as alterations in dopaminergic and serotonergic transmission in the brain that might be responsible for depressive behaviors. Although a causal relationship could not be established, our data indicate the following possible mechanism by which proinflammatory cytokines can produce

depression: altered 5-HT turnover in regions such as the prefrontal cortex, which is a key structure in the manifestation of depressive behaviors, as well as decreased dopaminergic transmission in the striatum in correlation with the increased immobility observed in the tumor-bearing subjects.

When analyzing the results of the present study, it is important to emphasize a few of its limitations. First, an allogenic tumor was used, likely responsible of the wide variability of tumor development found. Second, we performed few only a behavioral tests to determine the subjects' states of depression. Despite these limitations, it should be noted that few studies have simultaneously examined tumor development and analyzed a variety of biological parameters (brain, systemic, and behavioral); therefore, this study will certainly contribute an interesting perspective to the study of the possible mechanisms underlying the relationship between cancer and depression.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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FOOTNOTES

Fig. 1. Mean (± SEM) immobile bout time during the tail suspension test. (A) Tumor-bearing mice (n = 34) had a significantly higher immobile bout time than did control mice (n = 9). (B) Large tumor-bearing mice (n = 12) had significantly higher immobile bout times than did control mice (n = 9). * $p < 0.05$.

Fig. 2. Mean values (± SEM) of IL-6 mRNA relative gene expression in the hippocampus. (A) Tumor-bearing mice (n = 32) had significantly higher IL-6 expression levels than did control mice (n = 8). (B) Large tumor-bearing mice (n = 11) had significantly higher IL-6 expression levels than did control (n = 8) and small tumor-bearing mice (n = 21). * $p < 0.05$; ** $p < 0.01$.

Fig. 3. Mean values (± SEM) of TNF-α mRNA relative gene expression in the hippocampus. (A) Tumor-bearing mice (n=33) exhibited no significant differences from control mice (n=9). (B) Large tumor-bearing mice (n = 12) had significantly higher TNF-α expression levels than did control (n = 9) and small tumor-bearing mice (n = 21). * $p < 0.05$.

Fig. 4. Mean plasma levels (± SEM) of IL-6 (pg/ml). (A) Tumor-bearing mice (n=33) exhibited no significant differences from control mice (n=8). (B) Large tumor-bearing mice (n = 11) had significantly higher IL-6 levels than did control (n = 8) and small tumor-bearing mice (n = 21). * $p < 0.05$; ** $p < 0.01$.

Fig. 5. Mean levels (±SEM) of DA and its metabolite DOPAC in the striatum (ng/mg of wet tissue). (A1) Tumor-bearing mice (n=34) exhibited significantly lower levels of DOPAC than control mice (n=9). (A2) Tumor-bearing mice (n=34) exhibited significantly lower levels of DA than control mice (n=9). (B1) In the case of DOPAC levels, no differences were observed between the three groups (control (n=9), small (n=12) and large (n=22). (B2) In the case of DA levels, no differences were observed between the three groups (control (n=9), small (n=12) and large (n=22)). * $p < 0.05$.

Fig. 6. Mean levels (±SEM) of 5HT in the striatum (ng/mg of wet tissue). (A) Tumor-bearing mice (n = 34) had significantly lower levels of 5HT than did control mice (n = 9). (B) Large tumor-bearing mice (n = 12) had significantly lower levels of 5HT than did control mice (n = 9). * $p < 0.05$; ** $p < 0.01$.

Fig. 7. Mean levels (±SEM) of 5HIAA and 5HIAA/5HT rate in the prefrontal cortex (ng/mg of wet tissue). (A1) In the case of 5HIAA, tumor-bearing mice (*n*=34) exhibited no significant differences from control mice (*n*=9). (A2) Tumor-bearing mice (*n*=12) exhibited significantly lower 5HIAA/5HT rate than control mice (*n*=9). (B1) Large tumor-bearing mice (*n*=12) exhibited significantly lower levels of 5HIAA than control mice (*n*=9). (B2) Small (*n*=22) and large (*n*=11) tumor-bearing mice exhibited significantly lower 5HIAA/5HT rate than control mice (*n*=9). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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