Behavioural Brain Research 272 (2014) 83–92

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Melanoma tumors alter proinflammatory cytokine production and monoamine brain function, and induce depressive-like behavior in male mice

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HIGHLIGHTS

- Tumor development affects behavior during the tail suspension test and IL-6 expression in the hippocampus.
- Tumor-bearing mice exhibited a decrease in dopaminergic activity in the striatum.
- Serotonergic activity in the prefrontal cortex was reduced in melanoma-bearing mice.
- The expression levels of IL-6 and TNF-α in the brain increased in melanoma-bearing mice.
- The IL-6 levels in the plasma increased in melanoma-bearing mice.

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 23 June 2014
Accepted 24 June 2014
Available online 1 July 2014

Keywords:
Anhedonia
Cytokines
Inflammation
Melanoma tumor
Monoamines
Tail suspension test

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 (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 resulted in increasing the immobility in TST and the expression level of IL-6 in the hippocampus. 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. Our findings suggest that these alterations in inflammatory cytokines and monoaminergic system function might be responsible for the manifestation of depressive-like behaviors in tumor-bearing mice.

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 similarity with *sickness behavior*. Sick individuals experience depressive-like behaviors such as anhedonia, fatigue, activity reduction, loss of interest in social activities,

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http://dx.doi.org/10.1016/j.bbr.2014.06.045
0166-4328/

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 interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α) act on the brain and exert effects on neurotransmission, neuroendocrine function, and behavior [7]. Moreover, administration of IL-1, IL-6, and TNF-α [8,9] or agents (*e.g.*, lipopolysaccharides (LPS) and endotoxins) that promote the production of these cytokines, have been found to induce 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. 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].

Our previous studies 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 × 24.5 × 15 cm. Food and water were available *ad libitum*. The holding room was maintained at a constant temperature of 20 °C 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 two groups. After recording the body weights of all mice, one 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 °C prior to measuring IL-1β, 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 °C 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 30½ 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 ($n = 3$).