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Cohabitation with a B16F10 melanoma-bearer cage mate influences behavior and dendritic cell phenotype in mice

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ABSTRACT

This study evaluated the effects of cohabitation with a B16F10 melanoma-bearer cage mate on behavior and immune functions in mice. Five different experiments were conducted. In each of them, the female mice were divided into two groups: control and experimental. One mouse of each control pair was kept undisturbed and called "companion of health partner" (CHP). One mouse of each experimental pair was inoculated with B16F10 cells and the other, the subject of this study, was called "companion sick partner" (CSP). On Day 20 of cohabitation, behavior and immune parameters from CHP and CSP mice were analyzed. In comparison to the CHP, the CSP mice: (1) presented an increased general locomotion in the open field and a decreased exploration time and number of entries in the plus-maze open arms; (2) had an enhanced expression of the CD80 costimulatory molecule on Iab^+CD11c^+ spleen cells, but no differences were found on lymph nodes cells; (3) presented an altered differentiation of bone marrow cells in the presence of GM-CSF, IL-4, and LPS in vitro, resulting in a lower percentage of Iab^+CD80^+ cells; (4) had a deficit in the establishment of a Delayed Type of Hypersensitivity to ovalbumin, which was associated to an in vitro proliferation of an IL-10-producing lymphocyte subpopulation after ovalbumin stimulation. Corticosterone levels detected on Day 20 of cohabitation were similar in CHP and CSP mice. It is shown here that DCs phenotype in mice is affected by conditions associated with behavioral alterations indicative of an anxiety-like state induced by the cohabitation with a tumor-bearer conspecific. This phenomenon occurred probably through a nondependent corticosterone mechanism.

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1. Introduction

The Central Nervous System and the Immune System are intimately connected and the bidirectional communication between these systems is the subject of study of the psychoneuroimmunology field (Blalock and Smith, 2007; Dantzer, 2001; Elenkov and Chrousos, 2002; Palermo-Neto et al., 2008). Stress, through the activation of both the Hypothalamus–Pituitary–Adrenal (HPA) axis and the Sympathetic Nervous System, as well as other neuroendocrine pathways, is responsible for many immune alterations (Besedovsky et al., 1986; McEwen et al., 1997; Zorrilla et al., 2001). In both human and animal experimental studies, different kinds of stressors have been found to alter the immune response. The immune alterations observed have been reported to depend on the type of stressor, its duration and frequency, and the temporal relationship between the stress application and the immune system evaluation (Avitsur et al., 2003; Bartolomucci et al., 2005; Fonseca et al., 2005; Palermo-Neto et al., 2003; Queiroz Jde et al., 2008).

It is noteworthy that immunosuppression is not the only outcome observed after stressful situations. Clinical observations indicate that patients with autoimmune diseases, such as lupus erythematosus, present a striking positive correlation between the severity of the disease and psychological stress (Ward et al., 2002), a phenomenon that could depend on stressful products' actions on immunostimulatory molecules (Besedovsky et al., 1986; Chrousos and Gold, 1992; Daynes et al., 1990). Therefore, it seems that individual responses to environmental disturbances also vary according to factors as personal life story (Avitsur et al., 2003) to different neuroendocrine regulations, and may end in either activation or suppression of the observed immune response (Keeney et al., 2006). Therefore, stressful psychological events and environmental disturbances have been considered as important factors to be evaluated, and their neuroimmune consequences have been the subject of several studies (Azpiroz et al., 2008; Palermo-Neto et al., 2008; Vegas et al., 2004).

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Both animals and people need a healthy social relationship and may experience similar responses to chronic psychological stress. Although most consider true empathy to be an exclusive ability of higher primates, empathy may be a phenomenon that reaches all mammals (Langford et al., 2006; Preston and de Waal, 2002). Morgulis et al. (2004) reported that cohabitation for 11 days with a sick cage mate increased the locomotor activity of mice within the open-field apparatus, while the corticosterone serum levels remained unchanged. Further studies showed that these animals companions of sick partners (CSP) presented an increased turnover of hypothalamic noradrenaline (NE), as well as a decreased neutrophil phagocytosis of *S. aureus* (Alves et al., 2006). In the same way, peritoneal macrophages (Onco-BGG- and Ehrlich tumor-activated) from CSP animals presented a decreased phagocytosis of *S. aureus* and CSP mice also presented increased Ehrlich tumor growth (Alves et al., 2007). When familiar mice were given noxious stimuli, their pain behavior was influenced by their neighbor's status bidirectionally; however, the modulation of pain sensitivity was produced solely by exposure to their cage mates, but not to strangers (Langford et al., 2006).

Considering that dendritic cells (DCs) are the key mediators of the initial immune response and that immune response course varies according to DCs phenotype (Banchereau and Steinman, 1998), we now hypothesized whether a subtle environmental disturbance such as cohabitation with a melanoma-bearer cage mate is able to affect mice immunity by altering the DC phenotype. Our results are complementary to the studies earlier done with Ehrlich tumor-bearer cage mate, showing that the alterations found earlier can be observed now using another experimental tumor and mice strain. Our results also indicate that cohabitation with a melanoma-bearer cage mate is able to induce behavioral alterations in mice, and to interfere with immune parameters by both upregulating CD80 expression on DCs taken from spleens, and altering the in vitro DCs differentiation.

2. Material and methods

2.1. Animals

Four- to six-week-old C57Bl/6 female mice were used. Females were chosen since they are less aggressive than males when kept in pairs (Palanza, 2001). Animals were housed in a controlled environment (21 ± 1 °C temperature and 45–65% humidity) and in artificially lighted rooms on a well-defined 12 h light/dark cycle (lights on at 7:00 a.m.) with food and water provided ad libitum. The experiments were performed in accordance with the guidelines of the Bioethical Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil, which are similar to those of the National Research Council, USA.

2.2. Group formation and experimental settings

Group formations was done based on a protocol described elsewhere (Morgulis et al., 2004), but by employing a different tumor in the sick partner. Briefly, the mice were paired (according to their weight) and left undisturbed for five days. After this habituation period, pairs were equally and randomly divided into two groups: control and experimental. One mouse of each control pair was inoculated subcutaneously at the left hind footpad with 50 μL of R-10 (RPMI-1640, supplemented with 10% Fetal Bovine Serum, both from Gibco – Brl™ – Invitrogen™, Carlsbad, CA, USA); the other mouse was kept undisturbed, being designated as "companion of healthy partner" (CHP). In the experimental group, one mouse of each pair was inoculated at the left hind footpad (s.c.) with 10^6 murine B16–F10 melanoma cells suspended in 50 μL of R-10; the other, the subject of this study, was left undisturbed, being designated as CSP. The inoculation day was called experimental day one (ED_1). Cohabitation was carried out for 20 days (ED_1–ED_{20}). CHP and CSP mice were analyzed for estrous cycle periodicity by vaginal cytology from ED_{15}–ED_{20}. The tumor-injected mice were observed for signs of spontaneous pain before and on days 10, 15 and 20 after inoculation of tumor cells. For that, animals were observed during 10 min for the presence or absence of lifting and licking of the injected limb. Tumor growth was visually detected at ED_{10-12} and if no tumor growth was detected, the pair was excluded from analysis.

Five experiments were performed to look for possible differences between CHP and CSP mice. All experiments were carried out in accordance with good laboratory practice (GLP), standardized protocols, and quality control assurance methods. Behavioral analysis was performed on the first experiment, while corticosterone serum levels and DC phenotyping studies were done on the second and third experiments, respectively. The fourth and last experiment was designed to analyze a delayed type of hypersensitivity, lymphocyte proliferation, and IL-10 production.

2.3. Behavioral analysis

Open field and elevated plus-maze behavioral studies were performed at ED_{20} and carried out at the same time of the day (9–11 a.m.), with CHP and CSP mouse intercalated for observations. Sixteen mice (8 CHP and 8 CSP) were used. To minimize the interference of possible odor clues left by a previously observed mouse, the apparatuses were washed with a 5% alcohol–water solution between the observations. Data were collected by a camera mounted vertically above the arena being analyzed by an Ethovision® System software (Noldus Information Technology®, Leesburg, VA, USA) installed on an IBM®-compatible computer, placed in an adjacent room. These protocols were described in detail elsewhere (Faggin and Palermo-Neto, 1985; Fonseca et al., 2002; Morgulis et al., 2004).

2.3.1. Open field studies

The open field used consisted of a round wooden arena virtually divided into four zones: central, intermediary, external, and thigmotactic. The arena is a large circular space with a nonporous white paint floor specially chosen to provide a high-contrast background, enabling video tracking without the need to dye or mark the observed animals (Drai et al., 2001). Each animal was individually placed at the center of the arena and observed for 5 min. Parameters measured included total distance traveled (cm), mean velocity (cm/s), number of zone entries, and exploration time (%).

2.3.2. Elevated plus-maze studies

Analyses in the plus-maze were performed immediately after the end of the open-field trials. Earlier results from this and other laboratories have shown that testing animals in a open field before the plus-maze test significantly elevates the animals' activity within this apparatus, that is, the total number of open- and closed-arm entries, thus resulting in an easier analysis of plus-maze data (Lister, 1987; Pellow et al., 1985). The plus-maze used consisted of two opposing open and closed arms elevated from the ground. Each animal was individually introduced and was allowed a 5 min free-exploration period. The parameters measured were: traveled distance (cm), number of entries, and time spent in open and closed arms. Data were collected and analyzed by the same software described earlier for the open field.

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