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Relations between different coping strategies for social stress, tumor development and neuroendocrine and immune activity in male mice

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Abstract

This study analyzes the effects of acute social stress and different coping strategies employed in response to it on the development of B16F10 melanoma pulmonary metastases, the activation of the HPA axis and the NKG2D receptor expression. To this end, male OF1 mice were subjected to 24 h of social stress using the sensorial contact model. This model includes three 5-min sessions of direct social interaction with resident cagemates selected for consistent levels of aggression. Subjects' behavior was videotaped and assessed. Six days after the first social interaction (1st social stress), the animals were inoculated with tumor cells or vehicle, and six days later, both tumor-bearing and non tumor-bearing mice were subjected to a second 24 h sensorial contact social stress session (2nd social stress). One hour after the 2nd social interaction, corticosterone levels and NKG2D receptor expression were determined. Lung metastatic foci numbers were determined 21 days after inoculation (15 days post-stress). Social stress increased the number of pulmonary metastases and the serum corticosterone level. A combination of cluster and discriminant analyses established the existence of two types of coping strategies: (1) a passive–reactive strategy characterized by subjects dedicating a greater percentage of time to submission, flee and avoidance behaviors; and (2) an active–proactive strategy, characterized by subjects dedicating a greater percentage of time to attack and non social exploration behaviors. Subjects belonging to the passive–reactive group were found to have a higher number of tumor foci, a higher level of corticosterone and a lower NKG2D receptor expression than subjects in the active–proactive group. These data indicate the relationship between different coping strategies for social stress and tumor development.

Keywords: Coping strategies; Social stress; Tumor development; Corticosterone; NK cells; NKG2D

1. Introduction

Various studies in humans indicate that psychological factors may affect the development and progression of cancer (Kiecolt-Glaser and Glaser, 1999; Spiegel et al., 1998; Thomas et al., 2002). Similarly, different stable behavioral traits (e.g. temperament, personalities) may affect the course of this illness in different ways (Eysenck, 1990; Hansen et al., 2005; Nakaya et al., 2006; Segerstrom, 2003; Sturmer et al., 2006; Temoshok, 1987). Nevertheless, the contribution of psychological factors such as stress or depression, as well as certain stable behavioral characteristics such as neuroticism, assertiveness and aggressiveness, etc., are not well established. The use of different animal models is proving highly effective in clarifying some of these phenomena.

It has been demonstrated that social isolation and social stress both increase the development of tumor metastases in animal models (Giraldi et al., 1994; Rowse et al., 1992; Stefanski and Ben-Eliyahu, 1996; Strange et al., 2000; Wu et al., 2000). This effect of social stress on tumor development has been attributed to the immunosuppressive effects of stress on the activity of different immune parameters, mainly the cytotoxic activity of NK cells (Bartolomucci, 2007; Ben-Eliyahu et al., 1991; Palermo-Neto et al., 2003; Stefanski and Ben-Eliyahu, 1996).

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Furthermore, it has been observed that social stress may also have immune consequences that depend on the subject's behavioral characteristics. In previous studies (Cacho et al., 2003; Fano et al., 2001; Sa-Rocha et al., 2006), it has been observed that the neuroendocrine and immune changes produced by social stress in defeated subjects depend on the behavioral characteristics shown during social interactions. There is also evidence to indicate the existence of different neuroendocrine responses produced by stress that, in association with different coping strategies, have different immune consequences (Bartolomucci et al., 2001; Bohus et al., 1993; Gasparotto et al., 2002; Marsland et al., 2002; Strauman et al., 2004). Furthermore, associations have been found between individual differences in behavior and angiogenesis, as well as metastatic tumor development (Sajti et al., 2004). With regard to social stress, Stefanski and Ben-Eliyahu (1996) did find a positive correlation between expressed defensive behaviors in defeated intruder rats and the retention of tumor cells in their lungs. Others have linked the expression of aggressive behaviors to a lower level of tumor metastasis (Amkraut and Solomon, 1972; Lemonde, 1959; Sklar and Anisman, 1979). It is therefore possible to hypothesize that tumor development may also depend on the behavioral strategies that the subject uses to cope with a situation of social stress.

Previous studies in our laboratory show that social stress increases the pulmonary metastatic development of B16 melanoma, and point to a greater degree of tumor development in subjects which employ a more passive coping strategy in response to stress (Vegas et al., 2006a). We should remember, however, that in this study, social behavior was recorded various days after inoculation, and since the presence of the tumor itself may alter behavior (Vegas et al., 2004), it was not possible to attribute the differences in tumor development to the different behavioral strategies employed in response to the social stress.

With the basic aim of responding to this question, this study reanalyzes the possible relations between different coping strategies for social stress and tumor development. Some of the possible neuroendocrine and immune mediators involved in this relationship were also measured. To this end, we used the sensorial contact social stress model (Kudryavtseva et al., 1991), and a melanoma as an experimental tumor model which is particularly immunogenic and commonly used in studies focusing on tumor immunology (Houghton et al., 2001; Jensen et al., 1999). Given that in studies using animals it has been demonstrated that NK cell activity is especially important for the modulation of metastasis formation (Barlozzari et al., 1985; Stefanski and Ben-Eliyahu, 1996; Wiltrout et al., 1985), and that the importance of NK cells in the specific resistance to B16F10 metastasis has been reported (Seaman et al., 1987), even in cases in which these cells are allogeneic with respect to the host B6F3B1 mice (Wu and Pruett, 1999), we analyzed NKG2D receptor expression as an indicator of the level of activation of cytotoxic lymphocytes (Upshaw and Leibson, 2006).

With the aim of determining whether or not a relationship exists between different neuroendocrine activation profiles and coping strategies, we analyzed serum corticosterone levels as an indication of the activation of the hypothalamic-pituitary-adrenal axis.

2. Materials and methods

2.1. Subjects and husbandry

Six-week-old male OF-1 mice (CRIFFA, Barcelona, Spain) arrived at our laboratory and were individually housed for 10 days in transparent plastic cages measuring $24.5 \times 45.5 \times 15$ cm^3. Food and water were available ad libitum. The holding room was maintained at a constant temperature of 20 °C with a 12 h light/dark cycle (white lights on from 20:00 to 08:00 h). The light cycle was reversed to facilitate behavioral assessment during the animals' active (dark) phase. All experimental procedures were conducted under dim red light conditions in a room adjacent to the holding facility. All procedures involving mice were carried out according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18 March 1986) as well as to related secondary and supplementary legislation.

2.2. Experimental design

On the 10th day of individual housing, animals ($n = 257$) were randomly allocated to two groups, stressed ($n = 198$) and non stressed ($n = 59$) (stress factor).

Animals in the stressed group were exposed (1st social stress) to the sensory contact social stress model (Kudryavtseva et al., 1991) for 24 h. Social interaction involved contact with highly aggressive trained and selected subjects (Vegas et al., 2004). During this 24 h period, subjects were only exposed to direct physical interaction for three 5 min intervals, separated by two approximately 12 h periods. The rest of the time, intruders were separated from residents by perforated methacrylate barriers, which bisected the cage and allowed sensory (non-physical) contact outside the direct confrontation periods. The separator prevented injuries that may have triggered the immune system and distorted the results. Although during the direct interaction period, subordinate subjects received some bites, no wounds were evident. The non stressed control group remained in isolation during the entire 24 h period, but a methacrylate separator was introduced into the cages during these two intervals in order to monitor the effect of the separator itself and the resulting reduction in space.

Six days after the application of the social stress model, both groups (stressed and non stressed) were separated into two new subgroups. One of these subgroups was inoculated with B16F10 melanoma cells ($n = 146$) and the other with vehicle ($n = 111$) (tumor inoculation factor). Thus, the following experimental groups were obtained: stressed-inoculated ($n = 99$), stressed-non inoculated ($n = 99$), non stressed-inoculated ($n = 47$) and non stressed-non inoculated ($n = 12$).

Six days after inoculation, stressed subjects (inoculated and non inoculated with tumor cells) were subjected to a second social stress session (2nd social stress), following the same procedure as in the first social interaction. One hour after the second social stress session (day 7), 12 animals from each of the experimental groups were put down in order to enable an analysis of their physiological measurements. The other animals remained housed individually until 21 days after inoculation with the tumor, at which point they were put down in order to enable an analysis of tumor development.

This experiment was carried out in three phases, and in each one the described experimental design was applied.

Fig. 1 shows a schematic representation of the experimental design.

2.3. Experimental tumor induction

Tumors were induced by B16F10 melanoma murine cells, allogeneic to the OF1 mice strain. The B16F10 cells were maintained in vitro by subculturing the tumor cells at 37 °C in a humidified atmosphere of 5% CO_2 at a