



## Cohabitation with a sick partner increases allergic lung inflammatory response in mice



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### ABSTRACT

The bidirectional relationship between the nervous system and the immune system is relevant for homeostatic organism maintenance. Studies from our laboratory showed that 14 days of cohabitation with a sick partner (injected with Ehrlich tumor cells-TAE) produced behavioral, neurochemical, endocrinological and immunological changes. This study analyzes the effects of cohabitation with an Ehrlich tumor-bearing animal on ovalbumin (OVA)-induced lung inflammatory response in mice. Pairs of male mice were divided into three groups: naïve, control and experimental. Animals of the naïve group were kept undisturbed being used for the assessment of basal parameters. One animal of each experimental and control pair of mice was immunized with OVA. On ED<sub>(0)</sub>, these OVA-immunized animals received an OVA booster. At this day (D<sub>(0)</sub>) the experimental mice that were kept undisturbed were inoculated with  $5 \times 10^6$  Ehrlich tumor cells; their immunized cage-mates were then referred as to CSP (“companion of sick partner”). The undisturbed mice of each control pair were i.p. treated on D<sub>(0)</sub> with 0.9% NaCl; their sensitized cage-mates were subsequently referred as CHP (“companion of health partner”). The OVA challenge was performed on CSP and CHP mice on ED<sub>(12)</sub> and ED<sub>(13)</sub>; blood and tissue collection were performed on ED<sub>(14)</sub>. Fourteen days after cohabitation, in comparison to the CHP mice, the CSP mice displayed the following: (1) an increased number of eosinophils and neutrophils in the BAL, (2) a decreased bone marrow cell count, (3) increased levels of IL-4 and IL-5 and decreased levels of IL-10 and IFN- $\gamma$  in the BAL supernatant, (4) increased levels of IgG1-OVA, decreased levels of IgG2a-OVA and no changes in OVA-specific IgE in the peripheral blood, (5) increased expression of L-selectin in the BAL granulocytes, (6) decreased tracheal reactivity to methacholine measured *in vitro*, (7) no changes in plasma corticosterone levels and (8) increased levels of plas-matic noradrenaline. These results suggest that allergic lung inflammatory response exacerbation in CSP mice is a consequence of the psychological stress induced by forced cohabitation with the sick partner. Strong involvement of the sympathetic nervous system (SNS) through adrenaline and noradrenaline release and a shift of the Th1/Th2 cytokine profile toward a Th2 response were considered to be the mechanisms underlying the cell recruitment to the animal’s airways.

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### 1. Introduction

The long-held view that homeostatic mechanisms are integrated by the nervous and endocrine systems has been expanded by information that these systems interact with the immune system (Ader et al., 1990; Dunn and Wang, 1995). Changes in cell-mediated

immune function were reported in individuals undergoing distressing life experiences (Gold, 1988; Nagata et al., 1999). Stressors were reported to decrease immune/inflammatory responses (Elenkov et al., 1999; Costa-Pinto and Palermo-Neto, 2010) and to modulate cytokine and peptide production and release (Besedovsky et al., 1986; Theoharides et al., 1995; Elenkov and Chrousos, 1999a) among others, through Hypothalamic–Pituitary–Adrenal (HPA) axis or Sympathetic Nervous System (SNS) activations.

Ehrlich tumor cells were reported to elicit a strong host inflammatory/immune response (Segura et al., 1997; Palermo-Neto et al., 2003, 2008), a fact that, together with other properties, makes this

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tumor an interesting experimental model for the analysis of tumor growth. The data from our laboratory have shown that 14 days of cohabitation of mice with an Ehrlich tumor-bearing conspecific individual increased their locomotor activity within an open-field apparatus (Morgulis et al., 2004), decreased neutrophil and macrophage oxidative burst and phagocytosis in response to induction by myristate acetate or *Staphylococcus aureus* (Alves et al., 2006, 2007), decreased the animal's host resistance to tumor growth (Morgulis et al., 2004), decreased the levels and increased the turnover rate of noradrenaline within the hypothalamus (Alves et al., 2006), increased plasma levels of noradrenaline and adrenaline (Alves and Palermo-Neto, 2014) and induced no changes in corticosterone serum levels (Alves et al., 2006, 2012). Immune impairment has also been observed in companions of B16F10 melanoma-bearing mice (Tomiyoshi et al., 2009). These effects were discussed as being a consequence of the stress imposed by the housing condition. Indeed, it was shown that odor cues released by Ehrlich tumor-bearing mice are aversive to their partners, increasing their plasma levels of noradrenaline and adrenaline, consistent with a SNS activation hypothesis (Alves and Palermo-Neto, 2014).

The existence of interactions between emotional or psychopathological disorders and allergic and/or chronic diseases such as asthma is well established (Nagata et al., 1999; Vamos and Kolbe, 1999; Rietveld et al., 2000). Asthma is a multifunctional disease characterized by pulmonary cellular infiltration, plasma exudation and airway hyper-responsiveness, the latter of which is globally related to the toxic effects of mediators released into the lungs by alveolar macrophages, neutrophils, eosinophils and mast cells (Bochner and Busse, 2005). Studies concerning emotional impacts on immune responses usually employ animal models of aversive stimulation to evaluate an antigen-induced inflammatory response (Persoons et al., 1995; Costa-Pinto and Palermo-Neto, 2010). Airway inflammation induced by ovalbumin (OVA) aerosol challenge in OVA-sensitized animals mimicked some pathological characteristics of asthma, e.g., peribronchial edema and increased number of lymphocytes and eosinophils in the bronchoalveolar lavage fluid (Portela et al., 2001, 2002). These events were augmented by early-life psychological stress (Chida et al., 2007), unpredictable stressor procedures (Datti et al., 2002), foot shock stress (Portela et al., 2001, 2002) and chemically-induced stressors (Ligeiro de Oliveira et al., 2012; Stankevicius et al., 2012; Hamasato et al., 2013).

Stressors are known to shift the Th1/Th2 cytokine balance toward a Th2 cytokine profile, thus suppressing Th1 and upregulating Th2 responses (Elenkov and Chrousos, 1999b), and deregulation of the Th1/Th2 cytokine balance is a key to the pathogenesis of asthma and atopic diseases (Marshall et al., 1998; Ngoc et al., 2005). The over-production of Th2 cytokines results in the recruitment and activation of inflammatory mediators, including mast cells, basophils, and eosinophils, further resulting in airway obstruction (Finkelman et al., 2010). The present study was thus specially designed to analyze the effects of 14 days of cohabitation with an Ehrlich tumor-bearing conspecific on OVA-sensitized and challenged mice, considering the following: cell trafficking into the bronchoalveolar lavage fluid, Th1 and Th2 cytokine production, immunoglobulin plasma levels, adhesion molecule expression, *in vitro* tracheal reactivity, corticosterone serum levels and plasma catecholamine levels.

## 2. Materials and methods

### 2.1. Animals

Male Balb/c mice (25–35 g, 8 weeks) from our departmental facilities were used. The animals were maintained under a

controlled temperature (22–24 °C) and a 12-h light/dark cycle with free access to food and water. The animals were housed in plastic cages and were handled and used in accordance with the guidelines of the Bioethical Committee for the Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine of the University of São Paulo, Brazil (protocol No.2595/2012); these guidelines are similar to those of the National Institutes of Health (NIH), USA.

### 2.2. Group formation and induction of allergy

Fig. 1 depicts the protocol used in this study. Experiments were performed according to Good Laboratory Practice (GLP) standards and quality assurance methods. After a habituation period of 8 days, twenty pairs of mice were at random divided into three groups: naïve (4 pairs) control (8 pairs) and experimental (8 pairs). Care was taken to avoid possible stress induced by social hierarchies' establishment. For that, the paired mice were taken from the same cage colony. Fights and/or confrontations were not seen throughout the 8 days of previous cohousing. Animals of the naïve group (N) were kept undisturbed from ED<sub>(-8)</sub> (Experimental day-8) to ED<sub>(13)</sub>. On ED<sub>(-7)</sub>, one animal of each experimental and control pair of mice was subcutaneously (s.c.) immunized with a suspension of 10 µg OVA (Egg Albumin Grade V, Sigma Chemical Company®, USA) plus 10 mg of aluminum hydroxide. On ED<sub>(0)</sub>, these OVA-immunized animals received a s.c. OVA booster injection (10 µg OVA plus 10 mg Al(OH)<sub>3</sub>). At this day (D<sub>(0)</sub>) the experimental mice that were kept undisturbed were inoculated with  $5 \times 10^6$  Ehrlich tumor cells intraperitoneally (i.p.); their immunized cage-mates were then referred as to CSP ("companion of sick partner"). The undisturbed mice of each control pair were i.p. treated on D<sub>(0)</sub> with 0.9% NaCl; their sensitized cage-mates were subsequently referred as CHP ("companion of health partner"). The pairs of mice were left to cohabitate in the same cage for 14 days; confrontations or fights were not seen during this time period. On ED<sub>(12)</sub> and ED<sub>(13)</sub>, the CHP and CSP mice, the subjects of this study, were anesthetized with isofluorene and subsequently intranasally challenged with two drops of a 1% OVA solution, as suggested by (Zosky and Sly, 2007). On ED<sub>(14)</sub>, the Ehrlich tumor-injected mice were scored for Ehrlich tumor clinical signs and symptoms as suggested elsewhere (Tomiyoshi et al., 2009; Alves et al., 2010, 2012). Tissues were collected from the N, CSP and CHP mice on ED<sub>(14)</sub>.

### 2.3. Leukocyte recruitment in the bronchoalveolar lavage fluid (BAL)

On ED<sub>(14)</sub>, the lungs of the N, CSP and CHP mice were lavaged with 1.5 mL phosphate-buffered saline (PBS) solution through a cannula inserted into the trachea. The recovered BAL (approximately 1 mL) was centrifuged (250g for 5 min at 20 °C), and the resulting cell pellet was suspended again in 1 mL of PBS. Cell suspensions (90 µl) were stained with 10 µl of 0.2% crystal violet, and the total cell number was determined in Neubauer chambers using a light microscope. Differential cell counts were performed with cytocentrifuge preparations (Cytospin, Fanem, São Paulo, Brazil) that were stained with Rosenfeld's dye using standard morphological criteria.

### 2.4. Quantification of blood leukocytes and bone marrow cells

Blood aliquots taken from the abdominal aorta of N, CSP and CHP mice on ED<sub>(14)</sub> were diluted 1:20 in Turk's fluid (3% acetic acid) for total white blood cell counting with an automatic cell counter (ABC Vet®, São Paulo, Brazil). The total number of bone marrow cells taken from the mice was quantified from the femoral marrow lavage fluid. The recovered bone marrow lavage (5 mL) was centrifuged (250g for 5 min at 20 °C). The supernatant was

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