THE EXPRESSION OF AN INTRASPECIFIC AGGRESSIVE REACTION IN THE FACE OF A STRESSOR AGENT ALTERS THE IMMUNE RESPONSE IN RATS

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ABSTRACT

The repercussion on the immune response of the expression of intraspecific aggressiveness in the face of a stressor agent was investigated in rats. Ninety-day-old animals were divided into three groups: the control group (only immunological measurements were performed), the foot-shock (FS) (animals individually receiving FS), and the intraspecific aggressive response (IAR) group (animals receiving FS and presenting IAR). For immunological measurements, blood samples were collected promptly at 7 and 15 days after FS or IAR. The FS reduced the total leukocyte amount presented. However, aggressiveness triggered not only reduction of the leukocytes, but also lymphocyte decrease and neutrophil increase. Moreover, an elevation in total leukocytes associated with an increase in the humoral immune response was also observed one week after IAR. In this study, the expression of intraspecific aggressiveness in the face of a stressor seemed to activate the immune system and to potentiate the antigen specific humoral response.

Key words: intraspecific aggressiveness, leukocytes, humoral immune response.

RESUMO

A expressão de uma reação agressiva intra-específica diante de um estressor altera a resposta imune em ratos

A repercussão sobre a resposta imune da expressão da agressividade intra-específica diante de um estressor foi investigada em ratos. Aos 90 dias de vida, os animais foram divididos em três grupos: grupo-controle (foram realizadas apenas mensurações imunológicas), choque nas patas (FS) (os animais receberam FS individualmente) e grupo resposta agressiva intra-específica (IAR) (os animais receberam FS e apresentaram IAR). Para as medições imunológicas, amostras de sangue foram coletadas imediatamente, 7 e 15 dias após FS ou IAR. O FS reduziu a quantidade total de leucócitos. Contudo, a agressividade foi acompanhada, além da redução do número de leucócitos, por diminuição de linfócitos e aumento de neutrófilos. Além disso, também foi observada elevação no número de leucócitos associada a aumento na resposta imune humoral uma semana após as IAR. Neste estudo, a expressão da agressividade intra-específica diante de um estressor parece ativar o sistema imune e potencializar a resposta humoral antígeno específica.

Palavras-chave: agressividade intra-específica, leucócitos, resposta imune humoral.

INTRODUCTION

In several species, aggressiveness is crucial to survival, because aggressive behavior guarantees access to food, reproduction, protection of the young, and capacity to confront predators and act in territorial defense (Volvaka, 1995). On the other hand, aggressiveness can result in trauma, injury, and contact with new diseases (Granger *et al*., 2000).

The hypothalamus-pituitary-adrenal axis (HPA) constitutes one of the fundamental elements of the mammalian adaptive response in encounters with the majority of threatening situations (Castelnau & Lôo, 1993). The HPA involvement, along with that of other elements participating in the response to stress, e.g., the sympathetic nervous system (Sgoifo *et al*., 1996; Lawrence & Kim, 2000), is an intimate one. In animals, intraspecific aggressiveness can be induced through painful electric foot-shocks (Eichelman, 1990; Manhães de Castro *et al*., 2001). This kind of electric stimulus is a typical neurogenic stressor (Sawchenko & Ericsson, 2000), which activates the HPA axis as well as the sympathetic-adrenal system.

In the nervous system (NS), the receptors of some chemical messengers have their functional parameters altered by stress situations (Manhães de Castro *et al*., 1996). Moreover, these chemical messengers are implied in the genesis of stress, of anxiety (Dantzer, 1991; Toth, 2003), and of aggressiveness (Manhães de Castro *et al*., 2001; Barreto-Medeiros *et al*., 2001). Stressors can provoke alterations in the immune response, characterizing a possible neuroimmune modulation, and also indicating the direct relationship connecting the immune, nervous, and endocrine systems (Dardenne & Savino, 1996; Lawrence & Kim, 2000). The relationship among these physiological systems is underlined by evidence showing that immune cells can interact with hormones, neuropeptides, and neurotransmitters, and they can also produce them (Blalock *et al.*, 1985; Ottaviani & Franceschi, 1996). However, according to the specific situation, the behavioral and immunological alterations can vary before a stressor (Stefanski & Engler, 1999; Devoino *et al.*, 2003), thereby denoting differences in the responses of these physiological systems.

Evidence demonstrates that stress and emotional reactions affect the immune system (Stefanski & Engler, 1998; De Castro *et al*., 2000; Gasparotto *et al*., 2002). However, studies about interrelations between aggressive behavior and the immune function in mammals are few, and fewer still on controlling stress-factor interference. The present work investigates the repercussion, or lack thereof, in the immune response of the intraspecific aggressiveness expression in rats facing a stressor.

MATERIAL AND METHODS

Animals and experimental groups

All manipulations were performed in male Wistar rats. The animals were kept under controlled conditions (*ad libitum* access to water and food, 12 h light-dark cycle, and 23 ± 1ºC room temperature) throughout the study period. At ninety days after birth, the animals were distributed in three groups: a control group (n = 16, in which only the immunological measurements were done), a foot-shock (FS) group (n = 14, composed of animals that individually received foot shocks), and an FS group + intraspecific aggressive response (IAR) (n = 16; composed by animals boxed in pairs that received foot-shocks and presented aggressive response).

Stress induced by foot-shocks

Rats were submitted to stress induced by foot shocks administered in an isolated room, using a box, 20 x 20 x 20 cm, whose bottom consisted of parallel metallic bars (interbar distance: 1.3 cm) connected to a scrambled current source. The test consisted of placing one rat in the box, where it was subjected to an electric stimuli session. Each stimulus (an electrical foot-shock) consisted of a 1.6 mA – 2 sec current pulse. Each session lasted 20 min and was composed of 5 stimuli interspersed by a 4 min interval, during the first 3 min of which the behavioral response was analyzed. Observations were noted and equipment readings were done in the last minute of each interval.

Intraspecific aggressive response study

The aggressiveness test consisted in placing a pair of rats matched by weight of the aggressive response group in the box, where they were subjected to a session of electric stimulus under the same conditions referred to above. The aggressive response was defined as the presentation of at least one of the following two behaviors: a) the animals stood on their hind paws, facing one another, in a threatening posture but without direct contact, or b) they maintained evident physical contact (besides scratching, exhibition of teeth, and emission of characteristic vocalization).

Blood sampling

Blood samples were collected promptly 7 and 15 days after submitting the rats to the stress induced by foot-shocks or after the aggressiveness test. Blood was collected by the tail clip method as previously described by Dhabhar *et al*. (1996). The samples were used for leukocyte (about 20 µl, 3% EDTA) and antibody titer analysis.

White blood cells (WBC) and leukocyte subsets

Total WBC number was determined by a hemocytometer. The percentage of lymphocytes and neutrophils was determined with a microscope (May-Grün-Wald/Giemsa staining).

Immunization

The animals were immunized immediately after the stress induced by foot shocks or following aggressive behavior. Sheep red-blood cells (SRBC) were prepared by washing citrated sheep blood three times in sterile saline. Animals were immunized intraperitoneally with 10^8 cells/ml in a volume of approximately 0.5 ml.

Determination of antibody titer

Blood samples were collected before (negative control of antibody titer anti-SRBC) and 7 e 15 days after immunization. Samples were subsequently centrifuged at 3,000 rpm for 5 min and the supernatant collected. Serum complement was then inactivated at 56ºC for 30 min and stored at –20ºC. Twofold serial dilutions of inactivated serum, saline, and a 1% SRBC solution were then made using microwell glass. The highest dilution at which aggregation of SRBCs was still evident was considered to be the antibody titer.

Statistical analysis

For statistical comparisons, data were previously tested for normality (Kolmogorov-Smirnov test) and variance homogeneity (Levene median test). Previous verification showed that most of the data did not correspond to at least one of these criteria. So the results were compared by Kruskal-Wallis one-way analysis of variance followed by Dunn's test for multiple comparisons; statistical significance was defined as $p \leq 0.05$.

RESULTS

Effect of stress induced by foot shocks and of the intraspecific aggressive response in WBC

Compared to the control group, the animals that received foot shocks presented an immediate reduction in the total leukocyte count. However, there was no difference 7 and 15 days after the foot shocks. In the same way, the aggressive response group presented an immediate reduction in the total leukocyte count. However, 7 days after the aggressive response, an increase was observed in the total number of those cells (Fig. 1).

Effect of stress induced by foot shocks and of the intraspecific aggressive response in leukocyte subsets

There was no difference in the lymphocyte and neutrophil percentages between control and foot-shock groups. However, aggressiveness reduced the lymphocyte percentage and increased the neutrophil percentage immediately after the expression of aggressive behavior. Moreover, 7 and 15 days after the induction of the aggressive response, no difference was observed in lymphocyte and neutrophil percentages (Figs. 2 and 3).

Effect of stress induced by foot shocks and of the intraspecific aggressive response on humoral immunity

Compared to the control, there was no alteration in the humoral immune response of the animals submitted to the foot shocks. However, intraspecific aggressiveness increased the titers of anti-SRBC antibodies 7 days after immunization. Besides, 15 days after immunization, no difference was found in the humoral immune response between the aggressive response and control groups (Fig. 4).

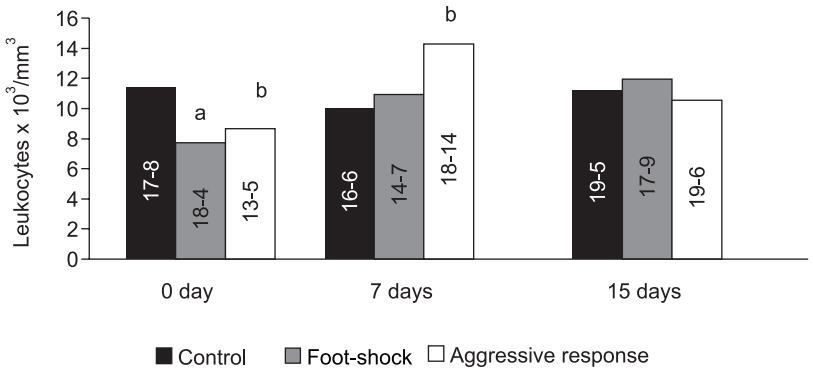

Fig. 1 — Leukocytes were counted immediately, and 7 and 15 days after submitting the rats to stress induced by foot shocks or after aggressive behavior, as described in Methods. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between (a) Control x Foot shock; (b) Control x Aggressive Response were made by Dunn's test ($p < 0.05$).

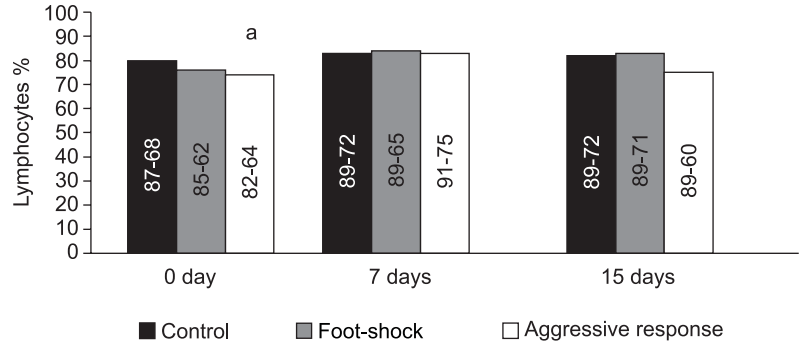

Fig. 2 — Lymphocytes were analyzed immediately, and 7 and 15 days after submitting the rats to stress induced by foot shocks or after aggressive behavior, as described in Methods. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between (a) Control x Aggressive Response were made by Dunn's test ($p < 0.05$).

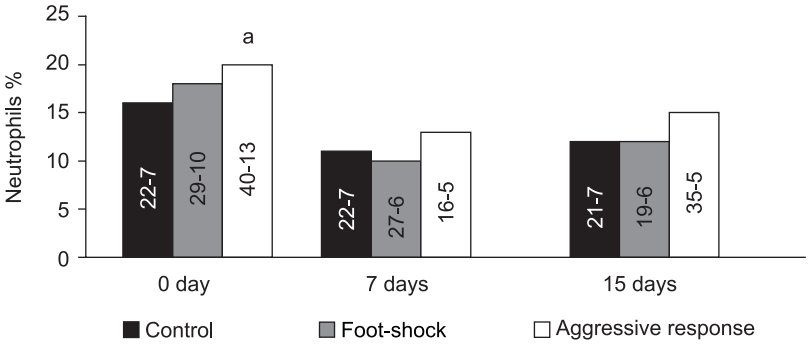

Fig. 3 — Neutrophils were analyzed immediately, 7, and 15 days after submitting the rats to stress induced by foot shocks or after aggressive behavior, as described in Methods. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between (a) Control x Aggressive Response were made by Dunn's test ($p < 0.05$).

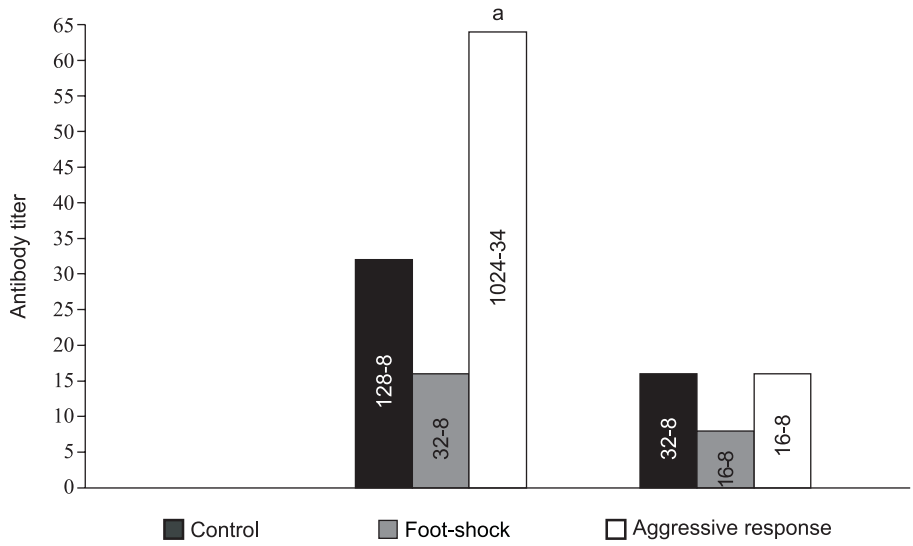

Fig. 4 — The antibody titers were analyzed 7 e 15 days after immunization, as described in Methods. The animals were immunized immediately after stress induced by foot shocks or after aggressive behavior. Columns represent the medians; maximum and minimum values are inside columns. Comparisons between (a) Control x Aggressive Response were made by Dunn's test ($p < 0.05$).

DISCUSSION

Studies about interrelations between aggressive response and the immune function in mammals are few; even rarer are those focused on controlling the interference of the stress factor. Thus, in this study, the possibility that the expression of intraspecific aggressiveness before a stressor agent alters the immune response in adult rats was evaluated. The results demonstrated that the stress induced by the foot-shocks markedly reduced leukocyte amounts in the blood stream. However, these alterations were reverted a week after stress ceased. Our data corroborate experimental evidence indicating a reduction of the total leukocyte number in animals submitted to acute stress (Dhabhar *et al.*, 1994; Dhabhar *et al.*, 1995). It seems that stressing experiences suppress the ability of the immune system to respond to foreign agents (Dhabhar & Mcewen, 1996).

The electrical foot-shock is a classic stress model (Sawchenko, 2000), well known for provoking increases in biosynthetic and secretory activity of chemical messengers in the HPA axis (Kant *et al.*, 1984) and in the sympathetic-adrenal system (McCarty & Kopin, 1978). Thus, it is very probable that substances such as adrenaline and glucocorticoids mediated the stress effects on the leukocytary pattern observed in the present work. Corroborating this hypothesis, some studies have demonstrated that organs and cells of the immune system express receptors for these chemical messengers and are, therefore, regulated by them (Blalock *et al.*, 1985; Dantzer, 1991). Glucocorticoids are physiologic agents that have the potential to inactivate macrophages as well as other cells of the immune system (Celada & Nathan, 1994). A recent article reports that under stress situations, a decrease was observed in superoxid production by alveolar macrophages activated with phorbol myristate acetate (PMA), associated with an increase of the serum corticosterone levels (De Castro *et al.*, 2000).

Regarding the humoral immune response, this study showed no alteration in the titers of anti-SRBC antibodies as a result of the foot shocks. However, a previous study had demonstrated that rats

submitted immediately, and 24 hours after, immunization to a session of electrical foot-shocks (16 shocks, with 1.6 mA, 5s long, and 4 min interval), presented an increase in the humoral immune response (Wood *et al*., 1993). It must be pointed out that, in the present study, the stress session consisted of 5 shocks in the paws, with 1.6 mA, 2 s long, and 4 min interval. Moreover, the animals were immunized immediately after the stress session and the humoral response was evaluated 7 and 15 days following immunization. Thus, it is possible that methodological differences such as the number and duration of electric stimuli, as well as the period of stressor application of the in relation to administration of the antigen, are responsible for the discrepancy among existing studies.

In this study, the foot shock followed by the expression of the aggressive response was also accompanied by lymphocyte decrease and neutrophils increase, besides the characteristic reduction due to stress of the number of leukocytes. Moreover, an elevation in the leukocyte number associated to an increase in the humoral immune response was observed a week after the aggressive interactions. The HPA axis and also other elements involved in the response to stress are directly related to the expression of aggressive behavior (Sgoifo *et al*., 1996; Lawrence & Kim, 2000). Thus, we can suppose that, in the present work, immunological alterations induced after the expression of the aggressive behavior were mediated by neuroendocrine components specifically associated to this behavioral response. Thus, a specific neuroendocrine profile (synthesis and liberation of several chemical messengers) associated to the activation of the aggressiveness neural circuits, which are distinct from those of stress, would affect in a specific way the different immunological components. This hypothesis is plausible and also corroborates existing evidence that immune cells can interact with hormones, neuropeptides, and neurotransmitters (Blalock *et al.*, 1985; Ottaviani & Franceschi, 1996).

The number and the proportion of leukocytes in the blood represent in a significant way the state of leukocyte distribution in the organism as well as that of the immune system state of activation (Dhabhar *et al*., 1995). Thus, in the present work, the alterations observed in the leukocytary pattern, specifically related to the presence of aggressiveness, as opposed to those of the stress, can be the result of differential activation of the immune system in the presence of a new behavioral component, which in turn may be an adaptive response of the organism resulting in a more effective immune defense.

In this study, it is of interest that, 7 days after the aggressive behavior, there was an increase in titers of anti-SRBC antibodies. The largest production of antibodies, which was found in the aggressive group, reinforces the hypothesis that intraspecific aggressiveness can activate the immune system, increasing its capacity to react against strange antigens. Corroborating this hypothesis, Cohen *et al*. (1997) observed that primates with higher levels of aggressive behavior presented lower susceptibility to respiratory infection. In the same way, Devoino *et al*. (2003) observed that the occurrence of aggressive behavior in previously submissive rats produced immune stimulation.

Another important aspect to consider is that aggressiveness is also modulated by neurotransmitters systems, among which, the dopaminergic system stands out (Eichelman, 1990). Moreover, there are several indications of dopamine involvement in immune system modulation (Devoino *et al*., 1994; 1997). Thus, it is possible that the immunological alterations observed in the rats that expressed aggressiveness have also been mediated by dopaminergic mechanisms. However, this subject requires further study.

In conclusion, the expression of intraspecific aggressiveness before a stressor seems to activate the immune system and to potentiate the antigen-specific humoral response. This suggests that aggressive behavior can increase an organism's capacity to respond to noxious agents, and thus, favors the survival of the species.

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