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Brief and long maternal separations decrease corticosterone secretion in a lupus-prone strain: Dissociation from disease-related parameters

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Abstract

Neonatal manipulations are known to alter the activity of the immune system and the hypothalamus–pituitary–adrenal (HPA) axis. This study was performed in order to examine whether brief and long maternal separations (BMS and LMS, respectively) interfere with the onset and development of murine lupus in NZB/NZWF1 females, and to determine whether the pattern of corticosterone (CORT) secretion throughout life is associated to the expression of the disease. Maternal separation was performed daily during postnatal days 1–14, lasting 15 min in the BMS group and 3 h in the LMS group. Blood was sampled from the retro-orbital plexus on the 9th week, and every other week, from 10th to 34th weeks of life, for detection of anti-nuclear antibodies (ANA) and anti-double-strand DNA (anti-dsDNA) antibodies, and for determination of CORT serum levels. Urine samples were collected on the 21st, 27th, 33rd and 37th weeks of life. There were no group differences in regard to disease-related parameters, but LMS females presented a tendency for late onset of anti-dsDNA antibodies. BMS and LMS mice exhibited reduced CORT levels compared to non-manipulated (NM) animals. There was a strong negative correlation between total mean CORT concentration and onset of ANA, and a strong positive correlation between total mean CORT concentration and life span only in the NM group. Neonatal manipulations appeared to eliminate these correlations; hence, both BMS and LMS modified basal CORT secretion and the association between glucocorticoids and immune activity in the NZB/NZWF1 mouse strain.

Keywords: Autoimmunity; Maternal Separation; NZB/WF1 mice; HPA axis; Systemic lupus erythematosus (SLE); Early life stress; Anti-nuclear antibodies (ANA); Anti-double-strand DNA (anti-dsDNA) antibodies; Proteinuria

1. Introduction

Dam–pup interaction is essential for the development of the infants and maternal care is a main regulator of a number of physiological processes in the pup (Levine et al., 1991; Rosenfeld et al., 1993; Suchecki et al., 1993). Disruptions of this interaction, such as brief and long maternal separations (BMS and LMS, respectively) during the first weeks of life are known to affect systems as the hypothalamus–pituitary–adrenal (HPA) axis. On one hand, BMS has been shown to decrease ACTH and corticosterone (CORT) responses to a wide variety of stressors, and rats submitted to this manipulation also present a more efficient HPA axis negative feedback (Meaney et al., 1985, 1996). On the other hand, LMS promotes an increase in HPA axis stress responsiveness and a decrease in the CORT negative feedback (Ladd et al., 2004; Meaney et al., 1996).

Glucocorticoids (GCs) play a major regulatory role on the immune system (IS), since they act as immunomodulators, interfering with transcription genes involved in the inflammatory response. At physiological levels, these hormones induce a shift from the Th1 cytokine production pattern (proinflammatory) to the Th2 pattern (anti-inflammatory). Proinflammatory and anti-inflammatory cytokine balance is essential, and loss of this balance leads to

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numerous neoplasic, infectious, allergic, inflammatory and autoimmune diseases (AD) (reviewed in Elenkov and Chrousos, 2002). A classical example of the influence of HPA axis on AD is evidenced in the Lewis and Fischer rat strains. Lewis rats are susceptible to some inflammatory diseases, in part due to their defective HPA axis response. Conversely, the Fischer strain displays a hyperesponsive HPA axis and is relatively resistant to the same autoimmune inflammatory diseases (Sternberg et al., 1989a, b).

Neonatal manipulations, which affect HPA axis responsiveness, also alter the susceptibility to experimental allergic encephalomyelitis (EAE), an AD with Th1 cytokine pattern. Studies have shown that BMS increases while LMS decreases susceptibility to EAE (Laban et al., 1995a, b). However, there seems to be some controversy, since Stephan and colleagues (2002) reported an early onset and worsening of EAE in female Lewis rats submitted to a daily 2 h-maternal separation with a concomitant decrease in CORT levels, compared to control rats. In addition, 2 h-neonatal handling with periodic touching also aggravates EAE in Lewis female rats (Manni et al., 1998) and rats submitted to 24 h-maternal deprivation on day 9 of life exhibit, as adults, a much higher EAE clinical score and reduced LPS-induced TNF-α and increased nitrite production (as a measurement of nitric oxide production) (Teunis et al., 2002).

Systemic lupus erythematosus (SLE) is a multi-systemic AD, characterized by overproduction of Th2 cytokines, and disease remissions and bursts. The better known immune alteration in lupus is the production of numerous antibodies against self-antigens, including antibodies against DNA, histones, nucleosomes and ribonucleoproteins, the so-called anti-nuclear antibodies (ANA). The main markers for SLE are the anti-double-strand DNA (anti-dsDNA) and anti-nucleosome antibodies, which are implicated in the formation and deposition of immune complexes in target organs, including the kidney (Manson and Rahman, 2006). The female-to-male incidence ratio of this disease is approximately 9:1, and it affects mainly women at the reproductive age, essentially due to the influence of sex hormones (Wilder, 1995). Other factors are also associated with the onset and worsening of SLE, such as pregnancy, stress and a variety of drugs (Ostensen, 1999; Peralta-Ramirez et al., 2004; Rubin, 2005).

The New Zealand Black/New Zealand White F_1 (NZB/WF1) mouse strain spontaneously develops an AD that closely resembles immunological and clinical characteristics of human SLE, such as a high production of ANA, including anti-dsDNA antibodies, lymphadenopathy, arthritis, hemolytic anemia, vasculitis, and a variety of histopathological manifestations, of which glomerulonephritis is the most prominent (Theofilopoulos and Dixon, 1985). As in humans, the incidence is higher and severity of the disease is worse in females (Theofilopoulos and Dixon, 1981). Data from our laboratory show that this strain exhibits an earlier onset of the disease when submitted to sleep deprivation (Palma et al., 2006), and that animals exposed to this kind of stress present sustained elevated CORT levels for several weeks afterwards (Palma et al., 2007), suggesting an important role of stress on manifestation of AD and HPA axis activity in NZB/WF1 mice.

Since neonatal manipulations modify HPA axis responsiveness and the course of AD in some animal models, we hypothesized that a spontaneous AD could also be affected by disruptions in the dam–pup interaction. Therefore, this study was performed in order to examine whether BMS and LMS modify the onset and development of murine lupus in NZB/WF1 females, and to associate the pattern of corticosterone (CORT) secretion to the expression of the disease.

2. Materials and methods

2.1. Animals

All procedures were carried out in accordance with the guidelines on animal care of the National Institutes of Health and were approved by the Ethics Committee in Research of the Universidade Federal de São Paulo (CEP 0881/03).

Female NZB and male NZW mice were purchased from Universidade de São Paulo (São Paulo, Brazil), and then matted in the Research Laboratory of the Department of Psychobiology of Universidade Federal de São Paulo to generate NZB/WF1 hybrids. Both parents remained with the litter until weaning. After weaning, mice were housed in plastic cages in groups of three to eight animals and kept at a constant temperature (21 ± 2 °C) and under a 12/12 h light/dark cycle (lights on at 7:00 h). Food and water were available *ad libitum*. Once females are more affected by murine lupus than males, only this gender was used in the present study (Theofilopoulos and Dixon, 1981).

2.2. Maternal separations

The day of delivery was set as post-natal day (PND) 0. Maternal separations were performed daily from PND 1 to 14, from 13:00 h to 16:00 h. Whole litters were removed from the nest and placed in separate cages during 15 min (BMS) or 3 h (LMS) in an adjacent room. Pups were kept warm during the separation period by heating pads set at 33 °C. The non-manipulated (NM) group was not disturbed, except for cage cleaning once a week. Weaning was performed between days 21 and 23, resulting in three groups: NM ($n = 19$), BMS ($n = 15$) and LMS ($n = 17$).

2.3. Blood sampling and antibody determination

After a rapid anesthesia by ether vapors (approximately 15 s), blood was sampled from the retro-orbital plexus on the 9th week, and every two weeks, from 10th to 34th weeks of life. After centrifugation (2500 rpm for 7 min), serum was separated and stored at −20 °C until analysis. Blood sampling was carried out between 9:00–11:00 h.

ANA was determined by a standard indirect immunofluoresce (IIF) technique using HEp-2 cells as the substrate (Hemagen Diagnostics, Inc., Columbia, MD, USA). Briefly, the slide with the substrate was incubated with diluted serum for 30 min at 37 °C, allowing autoantibodies in the sample to bind to antigens in the substrate. Excess serum from the slide was removed by PBS wash. Substrate was then incubated with fluorescein-conjugated (FITC) rabbit anti-mouse IgG, kindly donated by BioLab, for 30 min at 37 °C. New PBS wash removed the excess of FITC and slide was coverslipped and checked for fluorescent patterns with a fluorescent microscope. Positive and negative controls were added in each assay. Starting dilution of serum in PBS was set in 1/50 and was progressively increased, until no antibodies were detected in the sample. Proportional numeric values (1, 2, 10, 20, 40 and 80) were attributed to dilutions