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Prenatal stress has pro-inflammatory consequences on the immune system in adult rats

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Summary
The *in utero* environment is critical for initiating the ontogeny of several physiological systems, including the immune surveillance. Yet, little is known about adverse early experiences on the offspring's immunity and vulnerability to disease. The present work aimed at investigating the impact of restraint prenatal stress (PS) on the development and responsiveness of *in vitro* and *in vivo* cellular and humoral immunity of male progeny aged 7 weeks and 6 months. In adult 6-month-old rats, we detected increased circulating $CD8^+$-expressing and NK cells in PS rats as compared to controls, associated with higher mRNA expression of IFN-γ. In addition, *in vitro* stimulation with phytohemagglutinin-A induced an increase in both the proliferation of T lymphocytes and the secretion of IFN-γ in PS rats. Interestingly, these alterations were undetectable in younger PS rats (7-week old), except for a slight increase in the mRNA expression of several pro-inflammatory cytokines in peripheral blood mononuclear cells. Moreover, *in vivo* neutralization of IFN-γ in young rats had no effects in PS group. In conclusion, we report for the first time long-lasting pro-inflammatory consequences of PS in rats.

1. Introduction

Early life environment is crucial for the development of an individual. In particular, gestational stress increases maternal circulating glucocorticoids (GC), stress hormones capable of crossing the placental barrier and potentially

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reaching the fetus (Barbazanges et al., 1996a). If chronic, these hormonal changes can result in permanent adverse short- and long-term neuroanatomical, biological and behavioral modifications in the offspring (Maccari et al., 2003).

In rats, restraint prenatal stress (PS) induces perturbations of the hypothalamic–pituitary–adrenal (HPA) axis (Maccari et al., 1995, 2003; Weinstock, 2005), likely altering the immune reactivity of rats. Indeed, immune cells express receptors for a variety of hormones (Roszman and Brooks, 1997) and GC are known to affect the development and function of the immune system (McEwen, 1998). Moreover, increased GC levels have been reported during the course of an immune response (Reul et al., 1994).

It is generally acknowledged that acute stress at adulthood enhances, whereas chronic stress suppresses the immune function (Dhabhar and McEwen, 1997). However, few studies have investigated consequences of maternal stress over the immune system of the offspring. Yet, results obtained so far depend on the animal species, the nature of the stressor, the duration of stress, the intensity and persistence of the stressor, as well as the immune compartments investigated and the age of animals. Indeed, several studies reported immunosuppressive effects of various PS procedures in neonate or juvenile rats (Bakker et al., 1995; Sobrian et al., 1997), monkeys (Coe et al., 2002) and suckling piglets (Tuchscherer et al., 2002). Conversely, immunoenhancing effects of PS have been documented in pre-term lambs (Kallapur et al., 2003), neonate Rhesus monkeys (Coe et al., 1999) and young adult rats (Klein and Rager, 1995). Finally, some authors observed no effects of PS on the humoral immune response of neonate and juvenile rats (Bakker et al., 1998) or both immunosuppression and increased production of pro-inflammatory cytokines in juvenile rats (Laviola et al., 2004). Therefore, even if PS convincingly alters cellular and humoral immunity in rats, whether these effects are anti- or pro-inflammatory is currently a matter of debate.

In view of these divergent data and considering that, to our knowledge, none of these reports investigated long-term consequences of PS on the immune function, the present work aimed at deciphering the impact of PS on the immunocompetence of the adult male offspring.

2. Materials and methods

2.1. Animals and PS procedure

Experimental protocols used in the present study were designed to minimize animal suffering and were carried out in accordance with the guidelines of laboratory animal care published by the French Ethical Committee and the rules of the European Communities Council Directive (10/24/1986; 86/609/EEC). Animals used in our experiments were derived from three different reproductions performed at separated seasonal periods throughout the year. Adult (6-week old) virgin female Sprague–Dawley rats (Harlan, France) were housed by groups of 10 for 2 weeks to coordinate their estrous cycles, before being mated with 8-week-old males (Harlan) at the ratio of one male per female. From day 1 of fecundation, determined by the presence of vaginal plugs, gestating females were transferred to individual cages with *ad libitum* access to food and water and housed in a temperature- ($24 \pm 1\,^{\circ}C$) and humidity (60%)-controlled room on a 12 h light/dark schedule (lights on: 0800 h). Control dams ($n = 12$) were left undisturbed except for routine body weight checks once a week, whereas stressed dams ($n = 12$) were subjected to a repeated restraint stress procedure as previously described (Maccari et al., 1995). Figure 1 shows the time-course of our experimental paradigm. Briefly, pregnant females of the stressed group were daily restrained in a transparent plastic cylinder exposed to bright light for 45 min three times per day, during the last 11 days of gestation. After birth, pups were left undisturbed with their biological mothers until weaning at postnatal day 21. Only litters of 8–13 pups with similar *sex ratio* were kept (Chapman and Stern, 1979). From weaning on up to the age of 2 months, animals were housed by gender then transferred by pairs to smaller housing cages and experiments were exclusively performed on the male offspring at the ages of 6–7 weeks and 6 months on different sets of animals. A maximum of two males per litter were used for each treatment to avoid any litter effect.

2.2. PBMC isolation

Blood was collected from the tail vein of each individual rat of the offspring ($n = 6$–8 for each group), either at the ages of 7 weeks or 6 months for experiments assessing immune parameters in young or adult animals respectively, diluted in PBS (v/v) and layered on a Ficoll density gradient. After centrifugation (15 min at 100*g*, at 4 °C), peripheral blood mononuclear cells (PBMC) were collected from the interface and washed twice in RPMI-1640 medium (Gibco Invitrogen, France). After enumeration, cells were further adjusted to 2×10^6/ml in culture medium (RPMI-1640+10% FCS, 1 mM sodium pyruvate (Gibco), 2 mM L-glutamine (Merck,

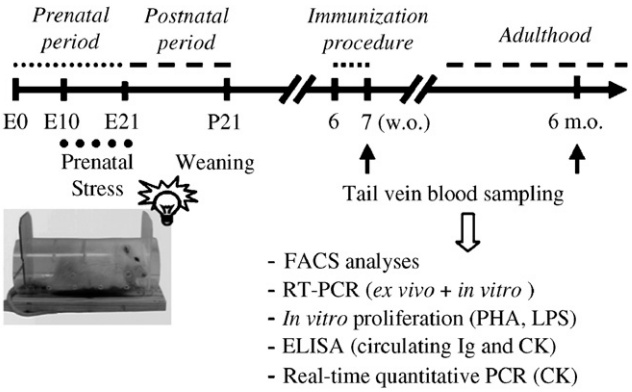

Figure 1 Experimental design. Prenatal stress procedure was performed during the last 11 days of gestation as previously described (Maccari et al., 1995). Analysis were performed on the male offspring either aged of 6–7 weeks for assessing immune parameters in young animals or at 6 months of age for studying the same parameters at adulthood. Note that experiments in young and adults animals were performed on different sets of rats. E: embryonic day; P: postnatal day; Ig: immunoglobulin; CK: cytokine; PHA: Phytohemagglutinin; LPS: Lipopolysaccharides.