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Single early prenatal lipopolysaccharide exposure prevents subsequent airway inflammation response in an experimental model of asthma

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ABSTRACT

Aims: There has been emerging interest in the prenatal determinants of respiratory disease. In utero factors have been reported to play a role in airway development, inflammation, and remodeling. Specifically, prenatal exposure to endotoxins might regulate tolerance to allergens later in life. The present study investigated whether prenatal lipopolysaccharide (LPS) administration alters subsequent offspring allergen-induced inflammatory response in adult rats.
Main methods: Pregnant Wistar rats were treated with LPS (100 μg/kg, i.p.) on gestation day 9.5 and their ovariectomized female offspring were sensitized and challenged with OVA later in adulthood. The bronchoalveolar lavage (BAL) fluid, peripheral blood, bone marrow leukocytes and passive cutaneous anaphylaxis were evaluated in these 75-day-old pups.
Key findings: OVA sensitized pups of NaCl treated rats showed an increase of leucocytes in BAL after OVA challenge. This increase was attenuated, when mothers were exposed to a single LPS injection early in pregnancy. Thus, LPS prenatal treatment resulted in (1) lower increased total and differential (macrophages, neutrophils, eosinophils and lymphocytes) BAL cellularity count; (2) increased number of total, mononuclear and polymorphonuclear cells in the peripheral blood; and (3) no differences in bone marrow cellularity or passive cutaneous anaphylaxis.
Significance: In conclusion, female pups treated prenatally with LPS presented an attenuated response to experimentally-induced asthma. We observed reduced immune cell migration from peripheral blood to the lungs, with no effect on the production of bone marrow cells or antibodies. It was suggested that inflammatory events such as exposure to LPS in early fetal life can attenuate allergic inflammation in the lung, which is a common symptom in asthma.

Introduction

Asthma is an inflammatory lung disease characterized by cellular recruitment, plasma exudation and airway hyper-responsiveness. These hallmarks of asthma are induced by inflammatory mediators, which are released from sensitized mast cells after antigen challenge (Bryce et al., 2006; Busse and Lemanske, 2001).

The so-called 'hygiene hypothesis' or microbial deprivation hypothesis suggests that a lack of exposure to microbial stimulation early in childhood is a major factor involved in the increasing prevalence of allergy and asthma (Conrad et al., 2009; Kalliomaki et al., 2010). Although traditional postnatal 'hygiene hypothesis' risk factors play a clear role in the development of asthma and atopic disease, there has been emerging interest in the prenatal determinants of this respiratory disease (Kumar, 2008). There may be in utero and early-life factors that play a role in airway development, inflammation, and remodeling (Kumar, 2008). Specifically, it has been found that a number of the epidemiological protective factors of the so-called 'hygiene hypothesis' are related to the effects of endotoxin and other microbial exposure (Braun-Fahrlander et al., 2002; Gehring et al., 2002) via innate immune system activation (Gern et al., 2004). For example, in utero exposure to farming environments elicited protective effects on asthma phenotypes (Douwes et al., 2008). Furthermore, it has been reported that prenatal exposure to immunological factors may be even more important than early-life exposures (Douwes et al., 2008; Kumar, 2008).

In fact, contact with lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, in early infancy is associated with decreased allergen sensitization (Gerhold et al., 2002; Wang and McCusker, 2006).

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As far as we know, all studies performed on prenatal LPS exposure and asthma have been performed in late pregnancy, or throughout pregnancy (Blumer et al., 2005; Cao et al., 2010; Datti et al., 2008). Thus, we believed that it would be relevant to study the effects of a single early prenatal LPS exposure on gestation day (GD) 9.5 on allergic lung inflammatory response in adult female rats. To this end, ovariectomized female pups were then sensitized and challenged with ovalbumin (OVA) in adulthood. The bronchoalveolar lavage (BAL) fluid, peripheral blood, bone marrow leukocytes and passive cutaneous anaphylaxis were evaluated in these pups. GD 9.5 was selected for LPS exposure since this date coincides with the beginning of the pivotal pseudoglandular stage of prenatal lung development in rats. This stage is most likely involved with the susceptibility to developing asthma in later life (Walters et al., 1987; Warburton et al., 2000).

Materials and methods

Animals

Pregnant Wistar rats from our own colony, weighing 216–263 g each, were used (GD 0 = spermatozoa in the vaginal smear). Dams were individually housed in polypropylene cages (38×32×6 cm) at controlled room temperature (22±2 °C), humidity (65–70%), and artificial lighting (12-hour light/12-hour dark cycle, lights on at 6:00 a.m.) with free access to Nuvilab® rodent chow (Nuvital Co., São Paulo, SP, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as animal bedding. The dams were randomly distributed into two control groups and one experimental group (n=10/each group). These rats were allowed to give birth and nurture their offspring normally. No cross-fostering procedure was used. The day of birth was considered as postnatal day (PND) 1. No handling was performed on PND 1, but on PND 2, 8 offspring (4 males and 4 females) were randomly selected. Litters smaller than 8 pups were culled. These eight pups were kept with each dam until weaning (PND 21). On PND 21, the littermates were separated, housed together by sex and grouped in the same laboratory conditions as their parents. One female pup of each litter was used for the tests in adulthood; the male offspring were separated, to be used in other experiments (Kirsten et al., 2010b). All experiments were performed between 7:00 and 12:00 am. The animals used in this study were kept in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil (protocol No. 925/2006, FMVZ–USP). These guidelines are similar to those of the National Institutes of Health, Bethesda, MD. Experiments were carried out in accordance with the GLP protocols and with quality assurance methods.

Treatment

LPS (from *Escherichia coli*, Sigma®, serotype 0127: B8) was dissolved in sterile saline (50 μg/ml of LPS in 0.9% NaCl solution) and was administered intraperitoneally (i.p.) to pregnant rats at a dose of 100 μg/kg on GD 9.5. This dose was chosen because it has been reported to (1) elicit sickness behavior in the dams, (2) induce endocrine alterations, (3) increase cytokines at the placental level, and (4) impair offspring viability and reduce the social behavior of male offspring during infancy and adulthood (Kirsten et al., 2010b; Spencer et al., 2007; Wang et al., 2006). The control group consisted of pregnant rats submitted to the same treatment schedule, but with sterile saline (0.9% NaCl). Each dam was treated with 0.1 ml/100 g saline solution. An additional control group of ovariectomized female rats were used as a negative control group, here called basal group. These animals were not submitted to any treatment, i.e. prenatal and OVA-sensitized and challenged in adulthood.

Ovariectomy and validation of ovariectomy

Twenty female rat pups (1 from each litter, n=10/each group) were ovariectomized on PND 53 to minimize the variables of the experiment, since female sex hormones modify the course of allergic pulmonary inflammation (Riffo-Vasquez et al., 2007). The ovariectomy and validation of ovariectomy were performed as described elsewhere Ligeiro de Oliveira et al. (2004)). Briefly, animals were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg) by i.p. injection. Upon laparotomy, the ovaries were surgically removed. The effectiveness of ovariectomy was determined by analysis of the vaginal smear and by quantification of uterine weight. Only successfully ovariectomized females in the diestrus phase were used in the experiments.

Rat model of allergic lung inflammation

The rat model of allergic lung inflammation was performed as described elsewhere (Ligeiro de Oliveira et al., 2008). Briefly, after 7 days of ovariectomy (PND 60), the female pups were sensitized with OVA (Egg Albumin Grade II, Sigma Chemical Company® USA). Seven days later (PND 67), the animals were boosted with OVA, and seven days later (PND 74), challenged with OVA aerosol.

Bronchoalveolar lavage (BAL) fluid analysis

On PND 75, BAL was performed in female pups as previously described by (Ligeiro de Oliveira et al., 2008). Briefly, the animals were anesthetized with ketamine plus xylazine, sacrificed, and their lungs were flushed for the total leukocyte and differential cell counts.

Peripheral blood analysis

Immediately before BAL collection, blood samples were taken from the animal's abdominal aorta. The total number of cells and the differential leukocyte counts were performed as described elsewhere (Ligeiro de Oliveira et al., 2008).

Bone marrow analysis

The total number of bone marrow cells was quantified in the femoral marrow lavage (FML) fluid obtained as described elsewhere (Ligeiro de Oliveira et al., 2008). Briefly, after BAL and peripheral blood collections, rat femurs were removed, the cells were collected and analyzed for total leukocyte counts.

Passive cutaneous anaphylaxis

Passive cutaneous anaphylaxis (PCA) reactions were performed as described elsewhere (Ligeiro de Oliveira et al., 2004). Briefly, sera from sensitized female pups were serially diluted and injected in the shaved dorsal skin of unsensitized naive rats. After 24 h, these animals received OVA and Evans blue in saline. Thirty minutes later, the rats were killed, the skin was removed and the diameter of the dye stain on the inner surface of the skin was measured. The PCA (IgE) titers represent the highest dilution of the serum that gave a dye stain>5 mm in diameter.

Statistical analysis

Results were expressed as mean±SEM. One female rat pup from each dam was considered in the offspring studies to avoid litter effects. Homoscedasticity was verified through the F test. Normality was verified through the Kolmogorov–Smirnov test. Thus, parametric data were analyzed by Student's *t*-test or by ANOVA, followed by the