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Endocrine and immune system interactions during pregnancy

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ABSTRACT

Pregnancy is known to induce a transient depression of maternal cell-mediated immunity, to prevent rejection of the fetus, while at the same time it keeps adequate maternal host defense mechanisms to fight infection. Presently, the aim of this paper was to investigate a possible endocrine and immunologic alteration observed during a successful pregnancy. This study consistently showed that plasma corticosterone levels were significantly higher ($P < 0.0001$) in pregnant Wistar rats than in virgin female. An increased number of peritoneal macrophages was also detected in pregnant females when compared to non-pregnant ones. Macrophages play an important role in the production of bioactive proteins and lipids such as nitric oxide. Then, in support of the latter, the present study showed increased levels of endogenous NO in pregnant rats when compared to non-pregnant ones, thereby mediating the vasodilatation process of normal gestation. Furthermore, our FACS analysis clearly indicated the correlation between reduced CD161 expression on NK cells ($P < 0.0001$) in pregnant rats when compared to virgin females. It was found that pregnancy appears to be associated with depressed cell immunity, as evidenced by a significant inhibition of lymphocyte proliferation. Understanding the immunological paradox of maternal tolerance, as well as the hormonal modulation of the immune environment during pregnancy is essential for future studies to investigate the potential for these processes to be modulated by diet or effective therapeutics during pregnancy.

Introduction

Pregnancy is known to induce a transient depression of maternal cell-mediated immunity, to prevent rejection of the fetus (Raghupathy, 1997). This immunological environment may result in increased susceptibility to infection. The state of pregnancy is still considered an immunological enigma during which the body must prevent rejection of the antigenically foreign fetus while at the same time it keeps adequate maternal host defense mechanisms to fight infection.

During pregnancy, levels of distinct hormones and other serum factors that may modulate lymphocyte or macrophage synthesis, activation, and/or function shift considerably. The placenta synthesizes estrogens from fetal and maternal precursors and secretes them into fetal and maternal circulations. At term, estradiol, the most active estrogen, is present at ~0.1 μM in human maternal serum and at ~0.25 μM in the intervillous blood (Varner et al., 1983). The latter value is ~250 times greater than the amount present in sera of non-pregnant young women at midcycle (Hoff et al., 1983). Progesterone firstly produced by corpus luteum, then by trophoblasts is secreted by cells into the maternal circulation. In pregnant mice, it is present at concentrations of 39.0 ng/ml in serum (Mccormack and Greenwald, 1974).

The plasma corticosterone concentrations, the major circulating glucocorticoids in rats, are also increased as pregnancy progressed. Glucocorticoids appear to play an important role in trafficking and in the function of T and B cells (Dhabhar et al., 1996). Endogenous glucocorticoids as well as progesterone also have been demonstrated to play an important role in suppressing immune responses (Franchimont et al., 2000).

In contrast to depletion of T and B cells several studies have suggested that heightened innate immunity plays an essential role in the maintenance of host defense throughout gestation. Some other papers describe that female steroids may affect macrophage

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functionality, probably by regulating surface receptors that are involved in phagocytic activity (Vernon-Roberts, 1969; Baranao et al., 1992).

Macrophages are cells with an impressive range of functions. It has been reported that macrophages, constituting approximately 90% of cells in the peritoneal cavity, act as a source of mitogenic factors and paracrine regulators (Rappolee et al., 1988), stimulating the proliferative activity of granulosa cells in growing follicles (Fukumatsu et al., 1992). Another important aspect is that these versatile cells act influencing the functions of neighboring cells such as invasive cytotrophoblast cells, glandular epithelial cells, and arterial endothelial cells (Nehemiah et al., 1981; Reister et al., 2001).

During pregnancy some changes in peripheral NK cell numbers, phenotype and activity take place, suggesting that NK cells are hormonally regulated (Dosiou and Giudice, 2005). In addition to their primary function of killing virally infected or transformed cells, they also assist in the initiation and development of adaptive immune responses by producing cytokines or by direct cell-to-cell interactions, with a series of surface receptors that recognize different cellular ligands on potential target cells.

Among several events that happen during pregnancy, nitric oxide (NO) also plays an important role. NO, a tiny lipophilic molecule enzymatically originated from cleavage of terminal guanidino nitrogen from L-arginine by a family of NOS, plays essential role in distinct biologic events (Moncada and Higgs, 1993). Cell-mediated immune responses are considered as classical NO-mediated actions (Nathan, 1997). The activation of the NO system triggers macrophage cytotoxicity being considered the first line of defense against invading pathogens eliciting cellular apoptosis in different systems (MacMicking et al., 1997). Interestingly, the susceptibility of several bacterial and viral infections is greatly increased in knockout mice lacking iNOS or treated with inhibitors of NO synthase as compared to wild-type mice (MacMicking et al., 1997). Inside the wide range of its biological functions, NO is considered as a potent vasodilator and immune modulator being its concentrations enhanced during pregnancy (Conrad and Vernier, 1989). These data raise the possibility that NO may favor to maternal vasodilatation and uterine immune suppression of pregnant woman and other mammals such as the rat (Gilson et al., 1992; Conrad 1992).

Other authors describe a cardiac involvement in which cardiovascular changes ultimately assure the adequate delivery of oxygen and nutrients to the fetus in a way that, for a successful pregnancy outcome, an immunosuppressive environment of the uterus must be kept, preventing rejection of the fetoplacental semiallograft (Feinberg and Gonik, 1991).

To understand the immunological mechanisms during the pregnancy an immunological follow-up of pregnant rats may provide valuable information on the actual role and contribution of immune cells, such as macrophages and NK, as well as functional assays of activation or cytotoxicity would be useful to confirm the events that govern the control and development of the pregnancy.

Based on the premises above described we focused the aims of this paper on the role of NK cells through the evaluation of CD161 expression by flow cytometry, corticosterone and NO concentrations, macrophage counts and splenocyte proliferation in pregnant females comparing with their roles in non-pregnant rats.

Materials and methods

Pregnancy

Female Wistar rats, weighing 180–200 g, were housed two to a cage. One male Wistar rat was introduced into each cage and was allowed to mate with the females. The presence of a vaginal plug indicated that mating had occurred. Females presenting vaginal plugs were separated from the males and considered as being at day 1 of gestation. Rat pad was changed 3 times/week to avoid concentration of ammonia from urine. The protocol of this study was approved by the local Ethics Committee protocol number 01.193.53.4.

Euthanasia

On day 18 of gestation, pregnant ($n = 5$) and virgin females rats ($n = 5$) were decapitated with prior anesthesia using 2.5% tribromoethanol.

Corticosterone concentrations

Plasma corticosterone concentrations were measured by using commercially available ELISA kits (Immuno-Biological Laboratories, IBL-America, Minneapolis, MN, USA) according to the manufacturer's specifications with reference standard curves. All samples were processed individually and assayed in duplicate, with plates being read at 450 nm.

Counting peritoneal macrophages

Macrophages were assessed by the injection of 5 mL of cold RPMI-1640 medium (LGC) into the peritoneal cavity. The cells were centrifuged for 15 min and pellet was resuspended in ice-cold RPMI-1640 medium. Ten microliter of each harvested peritoneal exudate cell suspension were added to 990 μL of Turkey Solution, and the macrophages cell counting was proceeded in a Neubauer chamber.

Measurement of NO production

NO production was measured according to Brazão et al. (2010), as accumulated supernatant nitrite (a stable breakdown product of NO) in pregnant and virgin animals by the Griess reaction. Cells were collected from the peritoneal cavity, ressuspended in RPMI-1640 medium (LGC). The cells were centrifuged for 15 min and pellet was resuspended in ice-cold RPMI-1640 medium. The cells were adjusted to 2×10^6 cells/mL. Volumes (100 μL) of cell suspensions were plated onto each well of 96-well flat-bottom culture plates (Corning) with or without LPS (10 μg/mL). Plates were incubated at 37 °C for 48 h in 5% CO_2 atmosphere. Subsequently, 100 μL of the supernatant was collected and transferred to a new 96-well flat-botton culture plates. The supernatants were incubated with 100 μL of Griess reagent (50 μL of 1% sulfanilamide (Sigma) plus 50 μL of 0.1% N-1-naphthylethylenediamine (Sigma) in 5% phosphoric acid solution) at room temperature for 5 min. The absorbance was determined in triplicate at 540 nm and expressed as micromoles.

Flow cytometry

Spleens of five animals per group were collected for cell proliferation and phenotyping. Spleens were aseptically removed and single-cell suspensions were prepared in RPMI-1640 with 10% fetal calf serum-FCS (Gibco, USA). 2×10^6 cells from the suspension of each organ from each experimental group were placed in 96-well round-bottom plates for cytofluorometric analysis. Following Fc receptor blocking, cells were incubated with color combinations of the monoclonal antibodies anti-CD161 - phycoerythrin as well as immunoglobulin isotype-matched controls. Stained cells were stored for analysis in PBS containing 0.01 m sodium azide and 1% paraformaldehyde, in sealed tubes held in the dark. All steps were performed at 4 °C. Analysis of these cells was performed using a

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