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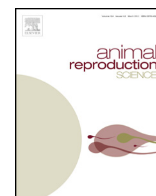

The upregulation of pro-inflammatory cytokines in the rabbit uterus under the lipopolysaccaride-induced reversible immunoresponse state

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The reproductive organs are more likely to develop gram-negative bacterial infection than other internal organs because of direct access to the body surface. The objective of this study was (1) to provide a suitable intravenous injection dose of lipopolysaccharides (LPS) instead of gram-negative bacterial infection in order to induce a reversible immunoresponse state and (2) to examine the expression levels of pro-inflammatory cytokines in the uterus of rabbits while in an immunoresponse state. Two series of experiments were performed to accomplish these objectives. In the first series, 20 healthy New Zealand White female rabbits were divided into 5 homogeneous groups (n = 4), and intravenously injected with 0, 0.5, 1, 2, or 4 mg/kg body weight (BW) of LPS derived from *Escherichia coli* dissolved in 2 ml of sterile saline (LPS carrier). The control group received only saline. The concentrations of IL-1β, IL-6, and TNF-α in serum and the white blood cell count changed with time after LPS stimulation, and certain doses of LPS led to the death of some rabbits. The results suggested that a dose of 0.5 mg/kg of LPS induced a reversible immunoresponse state. In the second series, 4 rabbits were not injected (0 h), 16 rabbits were injected with 0.5 mg/kg LPS, and 16 rabbits in the control group were injected with 2 ml of sterile saline. Tissues of the uterine horn, uterine body, and cervix from the 36 rabbits were collected at 0, 1.5, 3, 6, and 12 h (n = 4) postinjection for examination of the expression levels of IL-1β, IL-6, and TNF-α by quantitative real-time PCR (qRT-PCR). The results suggested that 0.5 mg/kg of LPS upregulated the expression levels of IL-1β, IL-6 and TNF-α in the uterine body and uterine horn, and IL-6 in the cervix. In conclusion, the expression levels of IL-1β, IL-6 and TNF-α were upregulated in the uterus of rabbits under the reversible immunoresponse state induced by 0.5 mg/kg of LPS-injection.

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1. Introduction

The genital tracts of female animals are more likely to encounter invasion by microorganisms than other internal organs. The infections caused by gram-negative bacteria have been associated with dysfunction of the uterus in these animals such as intrauterine fetal death, fetal reabsorption, premature birth, abortion, and intrauterine growth retardation (Aisemberg et al., 2007; Ogando et al., 2003; Xu et al., 2007). In addition, most systemic infections of the genital tract caused by gram-negative bacteria

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have no representative symptoms and undergo a reversible immuneresponse (Brecchia et al., 2010; Priscilla, 2010; Angela, 1997).

It is well known that lipopolysaccharide (LPS), a component of gram-negative bacteria, is recognized by toll-like receptor 4 (TLR-4) on the cell surface, which then induces the production of the pro-inflammatory cytokines, interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), via the mediations of MyD88 and NF-κB cascade signal pathways (Imler et al., 2000; Matsuguchi et al., 2000). Therefore, IL-1β, IL-6, and TNF-α act as innate immune mediators in the uterus (Matsuguchi et al., 2000). In the immunoresponse process, immune cells differentiated from white blood cells (WBCs) are recruited and induced by pro-inflammatory cytokines and migrate towards inflammatory sites to clear pathogenic microorganisms. Meanwhile, immune cells secrete pro-inflammatory cytokines. The different types of white blood cells, such as mononuclear macrophage and natural killer (NK) cells, secrete IL-1β, IL-6, and TNF-α, in order to regulate lymphocyte proliferation and differentiation, and finally to participate in the initiation and development of inflammation (Netea et al., 2015; O'Neill et al., 2013; Abdelsalam et al., 2011). Therefore, the concentrations of IL-1β, IL-6, and TNF-α in peripheral serum and the number and type of WBCs in the blood are important indicators of the immunoresponse after LPS induction. TNF-α is first produced after LPS stimulation, and it induces various cells to secrete IL-1β, IL-6, and IL-8 (Sozen and Arici, 2002). Simultaneously, IL-1β enhances the production of IL-6 and TNF-α, and IL-6 downregulates the production of IL-1β and TNF-α (Orsi and Tribe, 2008). The mutual regulation of IL-1β, IL-6, IL-8, and TNF-α establishes an interactive network to maintain a moderate immunoresponse and avoid excessive immunodefense, and so a reversible process of immunoresponse is suitable for controlling immune stimulation, such as by using LPS.

The systematic immunoresponse process of LPS in the rabbit is very similar to that following a gram-negative bacterial infection (Tilders et al., 1994). Therefore, LPS can act as a model for a pathogen-associated molecular pattern of immunoresponse instead of gram-negative bacteria. However, few studies have investigated the expression of pro-inflammatory cytokines in the uterus of the rabbit under a reversible immunoresponse state, and one in which the appropriate dose of LPS induced this reversible immunoresponse state. Therefore, the objective of this study was to determine a suitable dose of LPS for inducing a reversible immunoresponse in the rabbit in order to elucidate the effect of LPS on the expressions of IL-1β, IL-6, and TNF-α in the cervix, uterine body, and uterine horn of rabbits under a reversible immunoresponse state.

2. Materials and methods

2.1. Farming condition of animals and the approval of the experimental procedures

Fifty-six healthy female New Zealand White rabbits (3.25 ± 0.25 kg and aged 5–6 months) were kept in individual cages under a 14-h light and 10-h dark regimen and at temperatures ranging from 16° to 25°. All animals were fed ad libitum on a standard diet (De Blas and Wiseman, 1998).

All the experimental procedures described below were approved by the Animal Ethics Monitoring Committee of Sichuan Agricultural University, and carried out in accordance with the Guidelines of Animal Welfare in China.

2.2. Experiment 1

To provide a suitable LPS-injection dose for the model of the reversible inflammatory state, an immunoresponse experiment was performed by injecting a gradient dose of LPS. Twenty rabbits were divided randomly into five groups, and each group (n = 4) was intravenously injected with 0 (2 ml sterile saline, LPS vehicle), 0.5, 1, 2, or 4 mg/kg body weight (BW) of LPS (*Escherichia coli* 055:B5 extracted by phenol; Sigma, USA), respectively. An indwelling needle was inserted in the marginal ear vein of each rabbit, and 0.5 ml venous blood was collected at 0 h (before injection) and at 1, 3, 6, 12, and 24 h post injection. In addition, the symptoms of illness, such as fever, lethargy, and shivering, were observed, and the number of dead rabbits was counted during the process of the first experiment.

2.3. Leukocyte and cytokine measurement

The WBC count was measured using an automatic hemocytometer (Medonic CA-620, Sweden). A 20-μl blood sample was immediately diluted 20-fold with anticoagulant buffer solution, and then the number of WBCs (Nw) and total blood cells (Nb) were measured. The WBC count was calculated according to the formula (Nw/Nb) × 100%. The serum was isolated by a centrifugation of 2000*g* for 15 min. The concentrations of IL-1β, IL-6, and TNF-α in serum were measured using an ELISA kit (BBI, UK) following the manufacturer's protocol.

2.4. Experiment 2

Based on the results of the first experiment, 36 rabbits were divided into three groups: 1) in the untreated group, four animals were left uninjected for samples taken at 0 h, 2) in the control group, 16 animals were intravenously injected with 2 ml sterile saline, and 3) in the LPS group, 16 animals were intravenously injected with 0.5 mg/kg LPS. The endometrial tissues of the cervix, uterine body, and uterine horn were collected at 0, 1.5, 3, 6, and 12 h post injection.

2.5. RNA isolation and cDNA synthesis

The tissues were homogenized immediately in 1-ml RNAiso Plus (Takara, Japan) after collection and processed to extract total RNA according to the manufacturer's protocol. The integrity of the total RNA was detected on 1.5% agarose gel by electrophoresis and then the concentration of total RNA was measured with a SmartSpec™ Plus spectrophotometer (GE, Sweden). The purified RNA samples were reverse-transcribed using PrimeScript® RT reagent

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