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Pro- and anti-inflammatory mediators change leukotriene B_4 and leukotriene C_4 synthesis and secretion in an inflamed porcine endometrium

J. Czarzasta, A. Andronowska, B. Jana*

Division of Reproductive Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, 10-748 Olsztyn, Poland

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ABSTRACT

We studied the effect of lipopolysaccharide (LPS), proinflammatory cytokines (tumor necrosis factor α [TNF-α] and interleukin [IL]-1β), and anti-inflammatory cytokines (IL-4 and IL-10) on leukotriene (LT) A_4 hydrolase and LTC_4 synthase (LTCS) protein expression in, and LTB_4 and LTC_4 secretion from, an inflamed porcine endometrium. On day 3 of the estrous cycle (day 0 of the study), 50 mL of either saline or *Escherichia coli* suspension (10^9 CFU/mL) was injected into each uterine horn of gilts (n = 12 per group). Endometrial explants, obtained 8 and 16 days later, were incubated for 24 h with LPS (10 or 100 ng/mL of medium), TNF-α, IL-1β, IL-4, and IL-10 (each cytokine: 1 or 10 ng/mL of medium). Although acute endometritis developed in all bacteria-inoculated gilts, a severe form of acute endometritis was diagnosed more often on day 8 of the study than on day 16. The amount of the LTA_4 hydrolase (LTAH) protein in the inflamed endometrium on day 8 was greater after applying the lower dose of TNF-α ($P < 0.001$) and both doses of IL-1β ($P < 0.001$) and IL-4 (1 ng, $P < 0.01$ and 10 ng, $P < 0.001$) than in the saline-treated uteri. A similar situation was observed in the case of the inflamed tissue on day 16 in response to LPS (100 ng, $P < 0.01$), TNF-α (10 ng, $P < 0.05$), and IL-4 (1 ng, $P < 0.001$). The content of LTC_4 synthase in the inflamed endometrium on day 8 was reduced by LPS (100 ng, $P < 0.05$), IL-1β (10 ng, $P < 0.05$), IL-4 (1 and 10 ng, $P < 0.05$), and IL-10 (1 ng, $P < 0.01$) but increased after the application of LPS (100 ng, $P < 0.05$) and TNF-α (1 and 10 ng, $P < 0.001$), IL-1β, and IL-4 (1 ng, $P < 0.05$ and 10 ng, $P < 0.001$) on day 16. On day 8, endometrial secretion of LTB_4 from the saline-injected and *E coli*-injected organs was similar in response to all of the used mediators. On the other hand, the contents of LTB_4 in the medium decreased after incubating the inflamed tissues from day 16 with TNF-α (1 ng, $P < 0.05$ and 10 ng, $P < 0.01$), IL-1β (1 ng, $P < 0.01$), and IL-10 (10 ng, $P < 0.05$) compared with the saline-treated ones. Secretion of LTC_4 from the inflamed uteri on day 8 was elevated by the lower doses of TNF-α ($P < 0.01$) and IL-10 ($P < 0.05$), whereas on day 16, such an effect occurred in response to the higher doses of IL-4 ($P < 0.01$) and IL-10 ($P < 0.05$). The obtained results show that pro- and anti-inflammatory mediators participate in the synthesis/secretion of LTs from an inflamed porcine endometrium. Our data suggest that inflammatory mediators may indirectly affect the processes regulated by LTs by influencing LT production.

1. Introduction

Uterine inflammation (endometritis/metritis) is the most frequent reproductive disorder in livestock. The

* Corresponding author. Tel.: +48 89 53574 37; fax: +48 89 5357421.
E-mail address: b.jana@pan.olsztyn.pl (B. Jana).

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consequences of uterine infections are manifold and can range from a complete lack of influence on reproductive performance to perpetual infertility. The most common causes of removing pigs affected by endometritis from herds are abnormal vaginal discharge, no pregnancy, and anestrus [1]. The uteri of sows suffering from endometritis and those free of the condition are a source of bacteria such as *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., and *Corynebacterium* spp. [1–3]. Uterine inflammation causes considerable alterations in the synthesis and secretion of prostaglandin$F_2\alpha$, prostaglandinE_2 [4,5], prostacyclin [6], thromboxane A_2 [7], and nitric oxide [8].

Leukotrienes (LTs) are a family of potent lipid messengers that play a role in the innate immune and inflammatory responses. Their production takes place mainly in immunologic cells. The synthesis of LTs is initiated by the release of arachidonic acid from the cell membrane influenced by phospholipase A_2. Subsequently, arachidonic acid is converted in a two-step process into an unstable intermediate, LTA_4, by a catalytic complex of 5-lipoxygenase (5-LO) and the 5-LO–activating protein. This substance is further transformed by cytosolic LTA_4 hydrolase (LTAH) to LTB_4 or generated to tripeptide glutathione by LTC_4 synthase (LTCS) to give LTC_4. LTC_4 and its metabolites, LTD_4, and LTE_4 are collectively known as cysteinyl-LTs [9–11].

Earlier studies have shown that in immunologic cells, interleukin (IL) 4 and IL-13 stimulate the expression of 5-LO, LTAH, and LTCS [12,13], whereas lipopolysaccharide (LPS) augments the production of LTB_4 [14]. In turn, the tumor necrosis factor α (TNF-α) is able to enhance 5-LO messenger RNA level in the intestinal epithelium [15], whereas IL-1β and IL-6 promote the release of an LO product, 5-hydroxyeicosatetraenoic acid, from human gestational tissues [16]. Conversely, an inhibitory effect of IL-4 and IL-13 on LTB_4 release from human peripheral monocytes has been noted [17]. Large quantities of TNF-α, IL-1β, IL-1, IL-6, IL-8, and IL-10 are known to be synthesized within and secreted from the uterus during inflammation [18–22]. Moreover, our previous studies revealed that the inflammation of the uterus in gilts was connected with an increase in the contents of LTB_4 and LTC_4 in peripheral blood and uterine tissues and washings, which was in turn associated with the enhanced expression of 5-LO, LTAH, and LTCS [23]. Additionally, under physiological conditions, functional receptors for TNF-α and IL-1β were found in the bovine [24] and porcine [25] endometrium, respectively, and ones for IL-4 in human uteroplacental tissues [26]. The expression of IL-10 receptors was revealed in human adenomyotic tissues [27]. Based on the previously mentioned findings, we hypothesize that pro- and anti-inflammatory factors may indirectly affect processes regulated by LTs by modulating LT synthesis/secretion in an inflamed uterus. Because the mechanisms involved in controlling the production and release of LTs in an inflamed uterus are not completely recognized, the present study was conducted to determine the influence of LPS and proinflammatory (TNF-α and IL-1β) and anti-inflammatory (IL-4 and IL-10) inflammatory cytokines on the whole manuscript (1) the amounts of LTCS and LTAH proteins in an inflamed porcine endometrium and (2) the release of LTB_4 and LTC_4 from this tissue.

2. Materials and methods

2.1. Animals and experimental procedures

All animals were kept and treated in accordance with procedures which had the consent of the local ethics committee (conforming to the principles of animal care, NIH publication No 86–23, revised in 1985, Agreement No 31/2010). The study was performed on 30 gilts (Large White × Landrace) aged 7 to 8 mo and weighing between 107 and 122 kg. Behavioral estrus was determined with the aid of a tester boar. The animals originated from a herd which was characterized by no disturbances in reproductive processes.

The experimental procedure concerning the induction of endometritis had been described earlier in greater detail [6]. To provide a brief summary of the method, 24 pigs were randomly chosen and designated into either a saline-treated group (SAL, n = 12) or *E coli*-treated group (*E coli*, n = 12) on day 3 of the estrous cycle (day 0 of the experiment). Next, the animals were subjected to mid-ventral laparotomy under general anesthesia, and either 50 mL of saline solution or 50 mL of *E coli* suspension (strain O25:K23/α/:H1; Department of Microbiology, National Veterinary Research Institute, Puławy, Poland), containing 10^9 CFU/mL, was administered into each uterine horn. The first subset of the saline-treated animals (n = 6) and *E coli*-treated animals (n = 6) was slaughtered on day 8 and the second on day 16 after treatment (expected days 11 and 19 of the estrous cycle), at which time the uteri were collected. In addition to this, the uteri of pigs from the control group (CON, n = 6, not subjected to any investigative procedures) were collected on day 0 (day 3 of the estrous cycle) to determine the basal values of the tested variables. The results of macroscopic and histopathologic examination of uteri used in the present study had been reported in an earlier work [6].

2.2. Treatment of endometrial fragments

To conduct in vitro studies, the uteri were transported on ice to the laboratory within 30 min and then washed twice with sterile phosphate-buffered saline (137 mM NaCl, 27 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4; pH 7.4). Next, a fragment of the uterine wall was taken from the middle part of each horn, and the endometrium was separated from the myometrium by careful scraping, using a scalpel blade. Subsequently, the fragments of the endometrium were cut and sliced (100–110 mg) and washed with Medium 199 (Sigma, No. A2058). Single explants of the endometrium were placed into glass vials containing 2 mL of Medium 199 supplemented with 0.1% bovine serum albumin (Sigma, No. A2058) and antibiotics (100 IU/mL of penicillin and 10 mg/mL of streptomycin, both from Sigma). The tissue fragments were preincubated and incubated in a shaking water bath at 37°C in a humidified atmosphere of 95% air and 5% CO_2. After a 90-min preincubation period, the endometrial explants were treated for 24 h with fresh (control) medium or with the addition of 10 and 100 ng/mL of LPS (Sigma, No. L2880) and 1 and 10 ng/mL of TNF-α (Sigma, No. T6674), IL-1β (Sigma, No. SRP3083), IL-4