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Determination of selected parameters for non-specific and specific immunity in cows with subclinical endometritis

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ABSTRACT

Endometritis in dairy cow herds is a serious economic problem all over the world due to the large economic losses. The aim of the study was a comparative evaluation of selected indicators of non-specific and specific immunity in cows with subclinical endometritis and in cows without inflammation of the uterus. The study was performed on 40 cows on day 65 after delivery. Based on the results of cytological tests, the cows were divided into two groups: experimental (subclinical endometritis) and control (20 cows in each group). A flow cytometric analysis was performed for the leukocyte surface molecules CD4, CD8, CD14, CD21, CD25. Moreover the phagocytic activity of granulocytes and monocytes/macrophages in peripheral blood and uterine washings was determined. It has been demonstrated that the percentage of phagocytic granulocytes and monocytes/macrophages in both the peripheral blood and uterine washings was significantly lower for cows with subclinical endometritis when compared to cows undergoing a normal puerperal period ($p < 0.001$). A significant ($p \leq 0.001$) decrease in the percentage of CD4+, CD14+, CD25+ and CD4 + CD25+ leukocytes was also observed in peripheral blood of the cows from the experimental group. In uterine washings a significant decrease ($p < 0.001$) in CD21+ and increase in CD8+ lymphocytes was detected. The results indicate that dysfunction of cell immunity coexisting with subclinical endometritis may be the main factor causing advanced inflammation of the uterus. Knowledge of immunological mechanisms observed in cows with subclinical endometritis could aid in choosing the right adjuvant therapy using immunomodulating agents.

1. Introduction

Compared to other types of inflammation of the uterus, subclinical endometritis is diagnosed late, usually when insemination becomes ineffective, due to the lack of noticeable clinical symptoms related to the reproductive system. Despite numerous studies, implementation of new diagnostic methods and the use of different therapeutic methods, endometritis in dairy cows remains a serious economic problem all over the world. This is mainly due to the large economic losses caused by the low rate of artificial insemination and the necessity to cull animals in the herd (Galvão, 2012; LeBlanc, 2008; Lee and Kim, 2007). Inflammation of the uterus in cows causes changes in the endometrium, which disturbs fertilization, hampers implantation, and may even lead to early miscarriages (Gilbert, 2011; Lee and Kim, 2007; López-Gatius et al., 1996). It also causes ovarian cycle disorders, including the formation of ovarian cysts, prolongation of the luteal phase and delay of first postpartum estrus (Herath et al., 2009;

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Mateus et al., 2002; Sheldon and Dobson, 2004; Sheldon et al., 2009b).

The essential role in the development and persistence of subclinical endometritis is played by cellular and humoral mechanisms of non-specific and local specific anti-infective immunity of the uterus. The uterine endometrium can selectively produce IgG and IgA, whose level in the uterine secretion reflects the intensity of inflammation and is a measure of the health status of this organ (Beutler et al., 2003; Gautam et al., 2010; Hansen, 2011; Sheldon et al., 2009a). In the process of phagocytosis and intracellular killing, the role of "professional" phagocytes is played by neutrophils and macrophages. These phagocytes under the influence of cytokines released by endometrial cells and lymphocytes cross the blood–endometrium barrier and participate in the removal of necrotic and apoptotic uterine cells and microbes (LeBlanc et al., 2011; Singh et al., 2008; Turner et al., 2012).

The aim of the study was a comparative evaluation of selected parameters of non-specific and specific immunity in the peripheral blood and uterine washings from cows with subclinical endometritis and from cows without inflammation of the uterus, in terms of their usefulness in therapy as diagnostic and prognostic factors.

2. Materials and methods

2.1. Experimental animals

The study was approved by the Local Ethics Committee at University of Life Science in Lublin. The examinations were performed in a herd of 252 dairy Holstein-Friesian cows which were in different stages of lactation. Feeding was based on the TMR (total mixed ration) system and included maize silage, grass silage, hay, straw, grain meal, soy meal, mineral supplements and protein supplements. The nutrition of cows was adjusted to actual milk productivity and gestation period. The reproductive system control by rectal examination combined with ultrasonography was conducted regularly at monthly intervals. In cows which had no complications during parturition and no signs of inflammation, the synchronization protocol of estrus and ovulation (presynch–ovsynch protocol) and artificial insemination (AI) with frozen semen were applied. However, cows with detected uterine inflammation were properly treated and subsequently subjected to synchronization protocol of estrus and ovulation and AI. The cows with ovarian cycle disturbances were treated individually according to the recognized cause. The investigations were conducted from February to December 2012. Preliminary 86 cows with clinical signs of endometritis were included. These animals between 21 and 47 DPP were treated with intrauterine infusion of cefaphirin (Metricure®, Intervet International B.V, Boxmeer, Netherlands). Subsequently, on day 65 after parturition, from 86 cows 12 had clinical signs of endometritis and cytological examination revealed subclinical endometritis in 20 cows which were subjected to further study. From the group of 54 healthy cows without any signs of clinical or subclinical endometritis, 20 were chosen as the control group. Their body condition was good or very good (body condition score 3.5–4.0), and during the previous lactation period they produced over 35 l of milk per day. The study included the assessment of the clinical health status of the animals and a detailed evaluation of the reproductive system by means of ultrasound using a Honda 1500 Ultrasound (Honda Electronics CO., LTD., Toyohashi, Japan) with a dual frequency 5.0/7.5 MHz intrarectal transducer. In addition, cytological tests were performed and bacteriological cultures of smears from the uterus were made. The tests were conducted on day 65 after delivery. Based on the uterine cytology, 20 cows with subclinical endometritis were selected (neutrophils count >5%), they represented the experimental group. The control group consisted of 20 cows without inflammation of the uterus (neutrophils count <5%). The cow selection process was based on literature (Kasimanickam et al., 2004, 2005; Gilbert et al., 2005; Sheldon et al., 2006). Cytological, bacteriological and immunological tests were performed in both groups of animals. The material for bacteriological tests was collected with swabs and the material for cytological tests was collected with an intrauterine brush adapted for cows Directa pro (Jiangsu Yada Technology Group Co., Ltd, Jiangsu, China). Within an hour, the biological material collected for laboratory tests was sent on transport medium (Meus s.r.l., Piove di Sacco, Italy) to the laboratory. The material for cytometric analysis included the peripheral blood and uterine washings. Blood samples (9 ml) were collected from the external jugular vein in EDTA or heparinized tubes, whereas uterine washings were collected in Vacutest standard tubes (Vacutest Kima srl, Arzergrande (PD), Italy). The washings were collected using a Foley catheter (Jorgen Kruuse, Marslev, Denmark) with a syringe, and 50 ml of isotonic phosphate-buffered saline (PBS) was injected into the uterus. The uterus was massaged through the rectum for a few minutes, and the saline solution was aspirated by drawing back the syringe plunger to create negative pressure in the catheter. In most cases, approximately 40 ml of fluid was recovered, while some of it remained in the uterus.

2.2. Bacteriological tests

The cultures were made from uterine swabs. Tests were performed on standard substrates: Columbia blood agar, Schaedler blood agar and McConkey agar (BioMérieux SA, Marcy L'Etoile, France), intended for the culture of aerobic and anaerobic bacteria. The cultures were incubated at 37 °C for 24–48 h under aerobic and anaerobic conditions. Results of bacteriological tests were evaluated based on the time of growth and morphology of the colony, and biochemical characteristics of the isolates using API tests (Appareils et Procédés d'Identification: BioMérieux SA, Marcy L'Etoile, France).

2.3. Cytological tests

Imprint cytology specimens were prepared from the cytological brushes, which after drying were fixed and stained by means of the Hemacolor method (Merck, Darmstadt, Germany). The preparations were evaluated under a microscope (CX 41 – Olympus Corporation, Tokyo, Japan)