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Differential somatic cell count in milk before, during, and after lipopolysaccharide- and lipoteichoic-acid-induced mastitis in dairy cows

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ABSTRACT

Intramammary infections induce the initiation of the inflammatory response, resulting in an increase in somatic cell count (SCC) in milk. The SCC includes several different types of cells but does not differentiate between them. On the contrary, the new differential somatic cell count (DSCC) parameter allows for the differentiation between 2 groups of cells: polymorphonuclear neutrophils (PMN) and lymphocytes versus macrophages. Therefore, the aim of this paper was to describe the changes of both DSCC and SCC during mastitis induced by cell wall components from typical mastitis-causing pathogens [lipopolysaccharide (LPS), *Escherichia coli*; lipoteichoic acid (LTA), *Staphylococcus aureus*] known to trigger different severities of mastitis. In addition, the effect the glucocorticoid prednisolone (PRED), which is known to attenuate the immune response in the mammary gland, was investigated. Twenty dairy cows were equally divided into 5 groups and treated with LPS, LTA, LPS+PRED, LTA+PRED, or a saline control. Milk samples were taken at the following time points: baseline (d −3, −2, and −1), right before treatment (d 0), 5 h after treatment (d 0.2), early cure phase (d 1 and 2), and late cure phase (d 3, 4, 5, 6, 7, and 14) and analyzed for DSCC and SCC. Mean DSCC values increased significantly from <60% at baseline and right before treatment to >81% 5 h after treatment and the early cure phase in all groups, except for the groups control and LTA+PRED. This increase clearly reflects a shift in cell populations to predominantly PMN. The SCC increased significantly following the stimulation, too, as expected. Interestingly, we observed cases where SCC increased moderately only whereas DSCC showed an evident increase, meaning that the shift in cell populations occurred even at low SCC levels. The PRED clearly lowered the cell migration in group LTA+PRED. This is the first ever study investigating DSCC during induced mastitis under controlled conditions. The combination of DSCC and SCC could be employed for the earlier detection of mastitis by revealing the shift in cell population independent from the SCC level. Furthermore, combining DSCC and SCC information could help to determine the stage of mastitis because we observed high DSCC and SCC results in the early stage of mastitis but evidently lower DSCC and high SCC in the cure phase. Hence, our results offer the first fundamental insights on how mastitis monitoring could be improved in the frame of dairy herd improvement programs.
Key words: mastitis, udder health, differential somatic cell count, *Escherichia coli*, *Staphylococcus aureus*

INTRODUCTION

Mastitis is mostly caused by bacterial pathogens invading the mammary gland. Typical pathogens, namely *Escherichia coli*, a gram-negative bacterium usually associated with acute, clinical mastitis, and *Staphylococcus aureus*, a gram-positive bacterium often associated with chronic mastitis, can cause differential activation of the immune system (Bruckmaier and Wellnitz, 2017).

Bacteria have specific cell wall components embedded in their cell wall, namely LPS on *E. coli* and lipoteichoic acid (**LTA**) on *S. aureus.* Intramammary injection of these bacterial cell wall components can be used to induce inflammation equal to that occurring during mastitis (Wellnitz et al., 2013). Furthermore, glucocorticoids have been known to affect the bovine immune system (Roth and Kaeberle, 1981, 1982), and specifically, the glucocorticoid prednisolone (**PRED**) can influence the recruitment of neutrophils given that it has been known to affect the recruitment of immune cells to the site of infection (Schwiebert et al., 1996; Sipka et al., 2013).

Somatic cell counts in milk provide an indication of the inflammatory response in the mammary gland and hence a proxy for measuring IMI. The optimal cut-off

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point to distinguish between infected and uninfected quarters at the cow level has been established at 200,000 cells/mL (IDF, 2013). Besides the well-established SCC, cell differentiation measurements provide more detailed insights on the actual inflammatory status of mammary glands and thus udder health status (e.g., Pilla et al., 2013). Whereas SCC represents the total number of cells, cell differentiation refers to the proportions of individual cell populations such as lymphocytes, macrophages, and PMN that play an important role in inflammatory responses within the mammary gland (Paape et al., 1979; Sordillo and Nickerson, 1988; Sordillo et al., 1997).

A new parameter for cell differentiation in milk, the differential somatic cell count (**DSCC**) parameter, has recently been described (Damm et al., 2017). Briefly, DSCC indicates the percentage of PMN combined with lymphocytes. Proportions of macrophages can be calculated by 100 − DSCC. Percentages of DSCC were described to vary in a broad range in the low SCC range but increase as SCC increases (Damm et al., 2017).

To our knowledge, this is the first study to examine DSCC during the occurrence of mastitis under controlled conditions. Therefore, the primary objective of this study was to describe the changes of the new parameter, DSCC, as well as SCC, before, during, and after mastitis induced by LPS from *E. coli*, or LTA from *S. aureus*, with or without administration of the glucocorticoid PRED. Furthermore, the interrelation of DSCC and SCC in different types of milk samples (i.e., quarter foremilk, quarter composite, and cow composite) was investigated. The aim of this study was to contribute to the basic understanding of the practical application of the new DSCC parameter (e.g., determination of the stage of mastitis).

MATERIALS AND METHODS

Animals

All animal trials were approved and permitted by the Committee of Animal Experiments, Canton of Fribourg, Switzerland, and all experimental procedures followed the Swiss law of animal protection. Twenty dairy cows (Holstein Friesian) in mid-lactation [DIM (on challenge day) = 195.8 ± 18.1; mean ± SD] were selected. Parities of experimental cows ranged from 1 to 6 and cows were producing >15 kg of milk/d (mean milk yield = 20.5 ± 0.8 kg). All cows had a SCC <150,000 cells/mL in all 4 quarters during the 3 d before the experiment and showed no signs of clinical mastitis. Cows were examined by a veterinarian, and overall health status was determined by a blood glutaraldehyde coagulation test (Sandholm, 1976; Tøllbøll and Jørgensen, 2003) before the experiment. The glutaraldehyde test was considered negative if no clotting occurred within 10 min. Cows were housed at the Agroscope research station (Posieux, Canton of Fribourg, Switzerland) in straw and sawdust bedded tiestalls throughout the experiment. Cows were fed roughage ad libitum and 1 kg of energy concentrate daily. Water was also available ad libitum. Cows were machine milked regularly, twice daily at 0530 and 1600 h.

Experimental Procedures and Treatments

On the day of the experiment, cows were randomly allocated to 5 treatment groups (group A, control, n = 4; group B, LPS, n = 4; group C, LPS+PRED, n = 4; group D, LTA, n = 4; and group E, LTA+PRED, n = 4), and mammary glands were challenged according to Figure 1 and as described elsewhere (Wall et al., 2016). Briefly, immediately following morning milking, 2 quarters from each cow were injected via the teat canal and each quarter received a co-injection of 2 treatments from separate sterile syringes. The injections were performed by sterilizing each teat with gauze soaked in 70% ethanol and inserting a sterilized teat cannula. A 15-s massage in the cisternal direction was performed immediately after injection.

Treatments were prepared as follows: 0.2 μg of LPS (from *E. coli* serotype O26:B6, Sigma-Aldrich, St. Louis, MO) diluted in 10 mL 0.9% sterile saline; 20 μg of LTA (from *S. aureus*, Sigma-Aldrich) diluted in 10 mL of 0.9% sterile saline; 30 mg of PRED (prednisolone sodium phosphate, Santa Cruz Biotechnology, Dallas, TX) diluted in 10 mL of double distilled water; the control treatment was 10 mL of 0.9% sterile saline. Time of injection was designated as time/d 0. Each cow had 1 treatment and 1 control quarter (Figure 1).

Sampling Procedures

Quarter Foremilk Samples. Before milking, teats and teat ends were cleaned using a 1-way cloth and the first 2 squirts of milk were discarded. Volumes of 10 mL of milk were collected per udder quarter. All samples were preserved with Broad Spectrum Micro tabs (Advanced Instruments Inc., Norwood, MA) to a final concentration of bronopol and natamycin of 0.27 and 0.01 mg/mL of milk, respectively. Only treatment and control quarters were sampled from each cow.

Sampling Schedule. Quarter foremilk samples were taken from all 4 quarters on d −3, −2, and −1 at the morning milking to establish a baseline. On the day of the challenge, samples were collected at 0 and 5 h postchallenge (i.e., d 0 and 0.2). Whereas samples were collected both at morning and evening milkings on d 1