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# *Short communication:* Differential immunoglobulin transfer during mastitis challenge by pathogen-specific components

**O. Wellnitz,[1] E. T. Arnold, M. Lehmann, and R. M. Bruckmaier**
Veterinary Physiology, Vetsuisse Faculty, University of Bern, CH-1725 Posieux, Switzerland

## ABSTRACT

Mastitis induced by *Escherichia coli* is often characterized by severe clinical signs, indicating a more powerful combat of the immune system against the pathogen compared with *Staphylococcus aureus* infections, which are often represented by chronic and subclinical diseases. The aim of this study was to test the major pathogenic component lipopolysaccharide (LPS) from *E. coli* and lipoteichoic acid (LTA) from *Staph. aureus* for their effects on blood-milk barrier integrity and the related transfer of immunoglobulins and lactate from blood into milk. A similar somatic cell count (SCC) increase was achieved by intramammary challenge of 1 quarter of 5 cows with 20 μg of LTA, and 8 cows with 0.2 μg of LPS (maximum log SCC/mL: 7). Milk $IgG_1$ concentrations increased in LPS- but not in LTA-challenged quarters. Milk $IgG_2$ concentrations were increased in treated quarters at 3 h after LPS, and 6 h after LTA challenge. Higher maximum levels of $IgG_2$ were reached in milk of LPS-treated quarters (173 ± 58 μg/mL) than of LTA-challenged quarters (62 ± 13 μg/mL). Immunoglobulin $G_1$ and $IgG_2$ levels did not change in control quarters. L-Lactate concentrations in milk increased 4 h after LPS and 5 h after LTA challenge and reached higher maximum levels in LPS- (221 ± 48 mg/L) than in LTA-treated quarters (77 ± 18 mg/L). In conclusion, a mammary inflammation on a quantitatively similar level based on SCC increase achieves a more efficient transfer of blood components such as $IgG_2$ via the blood-milk barrier if induced by LPS from *E. coli* than by LTA from *Staph. aureus*. This pathogen-specific difference may play an important role in the cure rate of the respective intramammary infection, which is usually lower in *Staph. aureus*- than in *E. coli*-induced mastitis.

**Key words:** mastitis, blood-milk barrier, lipoteichoic acid, lipopolysaccharide

## Short Communication

Intramammary infection with *Escherichia coli* usually causes acute clinical mastitis (Hogan and Smith, 2003), indicating a powerful combat of the immune system against the pathogen. In contrast, intramammary *Staphylococcus aureus* infections are often characterized by chronic and subclinical diseases (Sutra and Poutrel, 1994), and the pathogen seems to be able to prevent significant activity of the immune system. Lipoteichoic acid (**LTA**) and LPS are cell wall components of *Staph. aureus* and *E. coli*, respectively, which are generally accepted as major bacterial components that induce the mammary immune defense. These cell wall components are experimentally used to investigate the mammary immune response (Schmitz et al., 2004; Werner-Misof et al., 2007; Rainard et al., 2008). Choosing dosages to standardize the immune response quantitatively based on a similar SCC increase allowed the study of qualitative differences between these pathogenic components (Wellnitz et al., 2011). Differences in the induction of the mammary immune response by intramammary challenge with LPS and LTA were shown by a different induction of expression of different immune factors (Wellnitz et al., 2011), which most likely plays a role in the development of different mastitis severities.

During inflammation of the mammary gland, a massive leakage of blood constituents into milk occurs due to blood-milk barrier alteration (Burton and Erskine, 2003). Besides SCC, the concentrations of several other parameters increase in milk in response to inflammation of the mammary gland. Not all of these parameters may contribute to the immune response. Immunoglobulin G is the major immunoglobulin in ruminant milk (Butler, 1983). The subclass $IgG_1$ is the predominant antibody type in milk from healthy quarters because of an active, selective $IgG_1$ transport across the blood-milk barrier via the neonatal Fc receptor (FcRn) system (Baker et al., 2009). In mastitic milk, $IgG_2$ becomes the predominant antibody (Caffin and Poutrel, 1988). It is considered to be the main opsonin supporting neutrophil phagocytosis in the bovine mammary gland and, therefore, plays an important role in the combat against mastitis pathogens (Burton and Erskine, 2003).

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[1]Corresponding author: olga.wellnitz@vetsuisse.unibe.ch

L-Lactate (hereafter referred to as lactate) is another blood component that increases in milk during mastitis and is proposed to be used as an early indicator to detect mastitis (Davis et al., 2004). Leukocytes as a source of lactate in milk during an immune response have been considered (Davis et al., 2004). Recently, we described the blood as a major source of milk lactate that leaks into milk as a result of the impairment of the blood-milk barrier during the immune response (Lehmann et al., 2013). The aim of the current study was to investigate the transfer of immunoglobulins and lactate from blood into milk due to a change in the blood-milk barrier integrity after an intramammary challenge with LPS from *E. coli* and LTA from *Staph. aureus* with a comparable SCC increase in milk.

In 13 dairy cows, a similar SCC increase (maximum log SCC/mL: 7) was achieved by intramammary challenge of 1 quarter with 20 μg of LTA (n = 5) from a *Staph. aureus* strain that induced a chronic bovine mastitis, or with 0.2 μg of LPS (n = 8) from *E. coli* that induced acute bovine mastitis, as previously described (Figure 1; Wellnitz et al., 2011). In plasma (jugular vein) and milk samples (~10 mL) taken hourly from challenged and control quarters, $IgG_1$ and $IgG_2$ concentrations were analyzed using ELISA (bovine $IgG_1$/$IgG_2$ ELISA Quantitation Set; Bethyl Laboratories Inc., LuBioScience GmbH, Lucerne, Switzerland). The procedure was performed according to the manufacturer's protocol. A blocking reagent consisting of fish gelatin [1 mL of fish skin gelatin (G7765; Sigma-Aldrich, Steinheim, Germany) in 20 mL of bidistilled water] was used to avoid matrix effects. Coefficients of variation, calculated using a control sample on each plate, were 10 and 20% within and between assays, respectively. Lactate concentrations were measured using the test kit Lactate PAP (bioMérieux, Marcy l'Étoile, France) with an automated analyzer (Cobas Mira; Roche Diagnostics International AG, Rotkreuz, Switzerland) according to the manufacturer's instructions.

Data are presented as means ± standard error of the mean. Lactate concentrations are presented and statistically evaluated on a logarithmic scale ($\log_{10}$) to ensure normal distribution. Differences within treatment group to time point 0 and between-LPS and -LTA treatments within each time point (hourly) were tested for significance ($P < 0.05$) by ANOVA using PROC MIXED SAS (1999–2001, release 8.02; SAS Institute Inc., Cary, NC). The model included time, treatment, and their interaction as fixed effects, and quarter within cow as repeated subject. A Tukey-Kramer adjustment was used to compensate for multiple comparisons. The significant ($P < 0.001$) Pearson correlation coefficient (SigmaPlot v11; Systat Software Inc., Chicago, IL) between SCC and $IgG_1$ and $IgG_2$ in LPS-challenged quarters was 0.42 and 0.33, respectively, and 0.45 and 0.68 between SCC and $IgG_1$ and $IgG_2$ in LTA-challenged quarters, respectively.

In blood $IgG_1$ and $IgG_2$, concentrations were 16.5 ± 1.1 mg/mL and 35.4 ± 6.8 mg/mL, respectively, and did not change throughout the experiment. Although IgG concentrations in serum are known to be variable due to different factors such as age and lactational stage (Mallard et al., 1983), these are relatively high values compared with those in other studies where concentrations around 10 mg/mL were found for both immunoglobulins (Butler, 1983; Caffin and Poutrel, 1988). Reasons for that remain unclear. The test kits were validated according the manufacturer recommendations. Milk $IgG_1$ concentrations (Figure 2A) were 68 ± 6, 63 ± 5, and 83 ± 12 μg/mL in control, LPS-, and LTA-challenged quarters before (0 h) challenge, respectively. In LPS-challenged quarters, $IgG_1$ concentrations increased at 4 and 5 h and from 7 h after challenge until the end of the experiment. The maximum of 105 ± 13 μg/mL was reached 5 h after challenge. In control and LTA-challenged quarters, milk $IgG_1$ concentrations did not significantly increase.

Milk $IgG_2$ concentrations (Figure 2 B) were 30 ± 6, 32 ± 8, and 23 ± 8 μg/mL, in control, LPS-, and LTA-challenged quarters before (0 h) challenge, respectively. Milk $IgG_2$ concentrations increased at 3 h in LPS-challenged quarters, reached the maximum of 173 ± 58 μg/mL at 6 h after challenge, and stayed elevated until the end of the experiment. In LTA-challenged quarters, $IgG_2$ was increased at 6 h, reached a maximum of 67 ± 9 μg/mL at 8 h, and stayed elevated until 11 h after

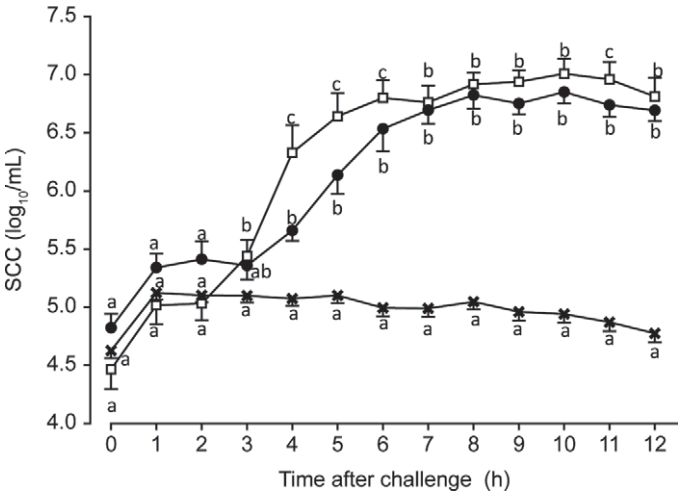


**Figure 1.** Milk SCC in LPS-challenged quarters (□; n = 8), in lipoteichoic acid (LTA)-challenged quarters (●; n = 5), and in control quarters (x; n = 13). Means without common letters (a–c) are significantly different between groups within a time point ($P < 0.05$). Data are presented as means ± SEM. Reproduced with permission from Wellnitz et al. (2011).