TWO PHENOTYPES OF BOVINE NEUTROPHILS DURING LACTATION: FICTION OR FACT?

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Periparturient (E) *E.coli* mastitis in cows is accompanied by a large variation in systemic symptoms varying from mild and moderate, to severe. Severely affected cows suffer from unbalanced inflammation. During mid lactation (M), intramammary infections with *E. coli* cause less variable and less severe symptoms because of a well-regulated inflammatory reaction. A few years ago we demonstrated that pre-infection capacity of blood neutrophils (PMN) to produce reactive oxygen species (ROS) is negatively correlated with the severity of systemic symptoms during experimental *E.coli* mastitis in E-cows, indicating that pre-infection PMN may play a pivotal role in variation of severity of periparturient *E.coli* mastitis. This correlation lasts for 24 h and subsides after a few days. According to the cows' stage of lactation and parity, we observed two PMN chemiluminescence (CL) phenotypes, after stimulation with Zymosan (Zym) and phorbol-myristate-acetate (PMA). In a first step we wanted to know if typical kinetics of PMN CL activation could be explained through the differential expression of isoforms of genes that are known to regulate ROS production in PMN. If this would be the case the high sensitivity of some cows to develop severe *E.coli* mastitis could be related to one or more isotypes. Therefore we selected a small group of genes : *CAT* (catalase), *SOD2* (superoxide dismutase 2), *CYBA* and *CYBB* (cytochrome b245, alpha and beta polypeptide), *NCF1* and *NCF4* (neutrophil cytosolic factor 1 and 4) and *RAC1* and *RAC2* (ras related C3 botulinum toxin substrate 1 and 2). In contrast to the afore-mentioned studies, each cow was sampled two times over time: first sample within 24 h after parturition (E), and second sample 135 days later (M). Thirteen primi- and twelve multiparous lactating dairy Holstein cows were used. Four groups of observations can thus be distinguished: primiparous cows sampled around parturition (PrE) and established lactation (PrM), and multiparous cows (2-5 calvings) at parturition (MuE) and established lactation (MuM). PMN were isolated and ROS-production was measured after stimulation with Zym and PMA. We herewith present some preliminary results. In the current study similar differences between ROS-kinetics (phenotypes) were detected in E- and M-cows as previously described with the different populations of cows. Isoforms of the afore-mentioned genes were not detected. A particular PMN phenotype and probably also the high sensitivity of some cows to develop severe systemic symptoms during *E.coli* mastitis can therefore not be explained by the afore-mentioned gene isoforms. In a second step of this study, we are studying the gene expression and its epigenetic regulation during lactation.

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INTRODUCTION

Periparturient (E) *E.coli* mastitis in cows is accompanied by a large variation in systemic symptoms varying from mild and moderate, to severe. During mid lactation (M), intramammary infections with *E. coli* cause less variable and less severe symptoms because of a well-regulated inflammatory reaction.

In this study we evaluated reactive oxygen species (ROS) production by blood neutrophils (PMN) in both E- and M-cows with different parities. Also, we evaluated if the typical kinetics of ROS production could be explained through the differential expression of isoforms of genes that are known to regulate ROS-production in PMN. We herewith present some preliminary results.

MATERIALS AND METHODS

Twenty-five lactating dairy Holstein-Friesian cows were used as blood donors. The first samples were taken < 24 h post partum and the second samples during mid lactation at 135 DIM (range 126 to 141 days).

We selected a small group of genes involved in ROS production. Exon-overlapping DNA primers were designed using Primer3Plus and taking the predicted isoforms into acount.

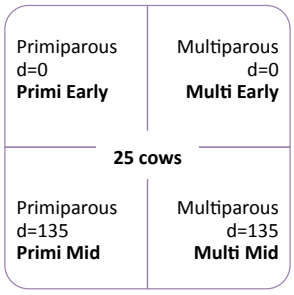

Fig 1. Different subpopulations used in this study

PMNs were isolated and ROS-production was measured after stimulation with Zymosan (Zym) and phorbol-myristate-acetate (PMA). Four cows were selected on the basis of their divergent ROS phenotypic results.

CAT	*catalase*
SOD2	*superoxide dismutase 2*
CYBA	*cytochrome b245 alpha polypeptide alpha*
CYBB	*cytochrome b245 beta polypeptide*
NCF1	*neutrophil cytosolic factor 1*
NCF4	*neutrophil cytosolic factor 4*
RAC1	*ras related C3 botulinum toxin substrate 1*
RAC2	*ras related C3 botulinum toxin substrate 2*

Table 1. Genes of interest involved in ROS production

RESULTS

We observed divergent PMN chemiluminescence (CL) phenotypes, after stimulation with Zym and PMA, according to the cows' stage of lactation and parity. This effect was seen in particular after stimulation with Zym. Cows in mid lactation showed a significantly higher and faster ROS production by neutrophils.

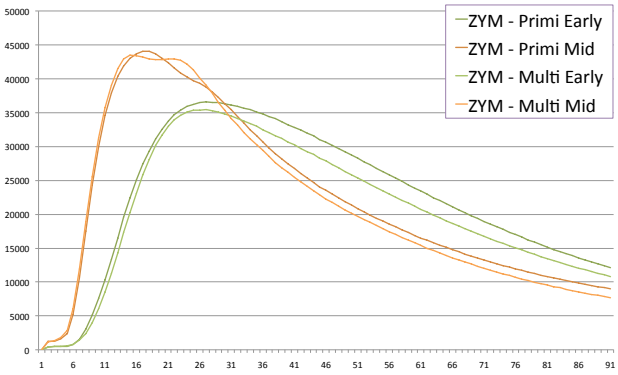

Fig 2. ROS production of bovine neutrophils after stimulation with Zym

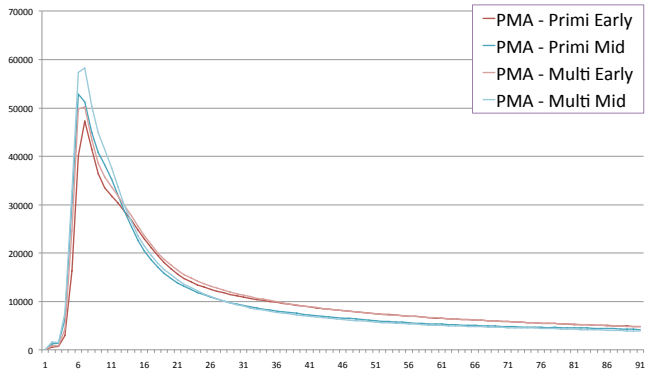

Fig 3. ROS production of bovine neutrophils after stimulation with PMA

Isoforms of the afore-mentioned genes were not detected and thus the high sensitivity of cows to develop severe *E. coli* mastitis is not related to one or more isotypes.

DISCUSSION

We previously demonstrated that pre-infection capacity of PMN to produce ROS is negatively correlated with the severity of systemic symptoms during experimental *E.coli* mastitis in E-cows, indicating that pre-infection PMN may play a pivotal role in variation of severity of periparturient *E.coli* mastitis. The particular PMN phenotype around parturition as shown by stimulation of neutrophils with Zym, and the high sensitivity of some cows to develop severe systemic symptoms during *E.coli* mastitis can not be explained by the afore-mentioned gene isoforms. In a second step, we are studying the gene expression and its epigenetic regulation during lactation.