

Leucocytes (WBC) are recruited from peripheral blood into milk as part of the inflammatory response, mediated through cytokines or interleukins synthesized by mammary tissue and the milk somatic cells (SC). The inflammatory response is related to the concentration of SC and the cytokines produced. To investigate and to compare the kinetics of cytokine production in SC and WBC during inflammation, cell culture models were established, where SC and WBC were cultured in parallel (n = 3). In addition, macrophages or monocytes were isolated from milk and blood with antibody-coated magnetic beads and cultivated separately. Isolated cells were pure, unaltered and viable. Cultures were activated with 10 µg/ml LPS. After 0, 1, 2, 3, 4 and 8 h cells were harvested for RNA isolation. Cytokine (TNFα, IL-1β, IL-6) mRNA expression responses and transcriptional activity of CD14 and lactoferrin (LF) were quantified via a one-step real-time RT-PCR.

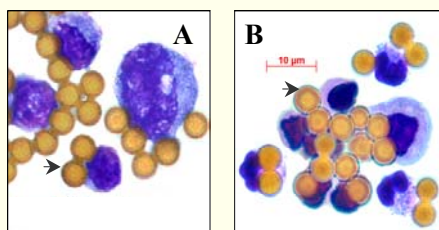


Fig. 1. Pictures of milk macrophages (A) and blood monocytes (B) bound to Dynabeads stained according to Papanheim. Dynabeads are indicated by the arrowheads.

MATERIAL AND METHODS

- Three Brown Swiss dairy cows were selected for this study based on somatic cell counts (SCC). SCC ranged from 4×10^5 to 1×10^6 cells/ml milk. Fresh milk was collected from one quarter of each cow and blood was taken from a jugular vein of the identical animals.
- Immuno-magnetic cell separation of monocytes and macrophages was performed with Dynabeads M-450 (Dyna) coated with a monoclonal antibody BAQ151A (VMRD).
- To determine the purity of isolated monocytes and macrophages a Papanheim staining was performed. Viability of the separated cells, was determined by trypan blue staining
- WBC, SC and immuno-magnetic separated monocytes and macrophages were incubated as duplicates in medium and stimulated by addition of 10 µg/ml LPS following a 24 h culture period. After 0, 1, 2, 3, 4 or 8h total RNA was extracted from milk- and blood-derived and antibody treated cells.

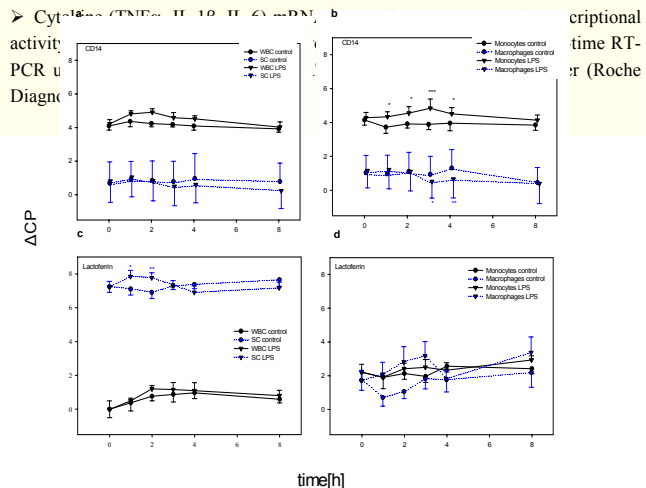


Fig. 2. Kinetics of CD14 and Lactoferrin mRNA expression in LPS (10µg/ml) induced WBC and SC (a, c) or monocytes and macrophages (b, d). The CD14 (a, b), Lactoferrin (c, d) mRNA expression was assessed by real time one-step RT-PCR using GAPDH as house keeping gene. The mean ± SEM from three separate experiments are indicated (n=6). *p<0.05, **p<0.01, ***p<0.001.

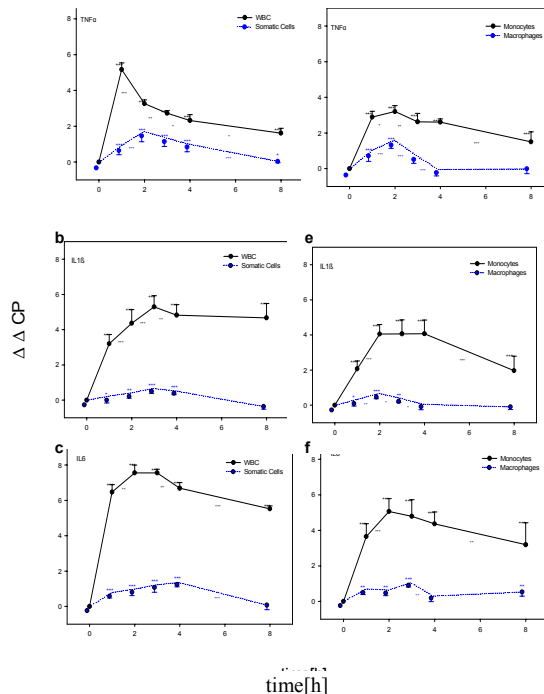


Fig. 3. Kinetics of cytokine mRNA expression in LPS (10µg/ml) induced WBC and SC (a, b, c) or monocytes and macrophages (d, e, f). The TNFα (a, d), IL-1β (b, e) and IL-6 (c, f) mRNA expression was assessed by real time one-step RT-PCR using GAPDH as house keeping gene. The mean ± SEM from three separate experiments are indicated (n=6).

RESULTS AND DISCUSSION

The transcriptional activity of CD14, Lactoferrin (LF) and various cytokines was compared in lipopolysaccharide (LPS) activated bovine leucocytes (WBC), monocytes, SC and milk macrophages. Significant cytokine mRNA increases were found in all four cell culture types and genes, with peaks after 1 and 2 h (TNFα > IL-6 > IL-1β). In WBC or monocytes higher LPS responses and longer persistence (IL-1β > IL-6 > TNFα) were observed than in corresponding milk cells. This may be ascribed to the role of CD14 and LF on the cytokine production of the investigated cells. The constitutive transcription of CD14 mRNA in WBC and monocytes was found to be 6- to 15-fold higher than in adequate milk cells, whereas mRNA expression levels for LF were strongly up-regulated (14-fold up to 153-fold) in SC during the whole cell culture period compared to monocytes, macrophages and WBC. The membrane bound CD14 (mCD14), expressed on the cell surface of monocytes and macrophages and, to a lesser degree, on neutrophils, binds the LPS-LBP complexes and thus mediates the activation of those phagocytes. Since CD14 mRNA levels of the monocytes and macrophages were similar to corresponding WBC and SC, the mononuclear cells appear to be the major source of CD14 mRNA expression. LF is a cationic metal binding glycoprotein produced by epithelial cells and leucocytes that is present in high concentration in milk, especially in colostrum and during involution or inflammation. It plays a role in host defense mechanisms via its bacteriostatic and bactericidal effects and in iron binding. The ability of LF to bind free LPS and to interfere with the LBP/CD14 pathway have been suggested to contribute to the anti-inflammatory activities of LF by restraining the cytokine production (TNFα, IL-1β, IL-6) *in vivo* and *in vitro*.

CONCLUSIONS

The present study demonstrates a time-dependent effect of the cytokine mRNA expression in immuno-separated and LPS-treated milk and blood cells. We also show a lower response of SC and macrophages to bacterial endotoxins than corresponding blood cells. The strength of the immune response in the blood system is much more prominent than in the mammary gland. This may be ascribed to the role of CD14 and LF on the cytokine production of the investigated cells, or may be caused by the blood-to-milk diapidesis.