

Figure 1: Primary bovine mammary epithelial cells

INTRODUCTION

Epithelial cells play an important role in the initial immune response to invading pathogens into the mammary gland during mastitis. Here we describe a cryopreserved bovine mammary epithelial cell model to study the infection response to different host-pathogen interactions

The model provides biological reproduction where all cells are simultaneously exposed to a defined infection pressure.

MATERIAL AND METHODS

- Primary mammary epithelial cell cultures isolated from milk of eight healthy lactating Brown Swiss were passaged twice, and frozen in liquid nitrogen
- To confirm epithelial origin, cryopreserved cells were grown on collagen coated cover slips and immunologically stained for different cytokeratins (clone MNF 116, DaKo Cytomation)
- Cryopreserved cells were grown in 12-well plates for 24h, then treated with 0.5 or 10 bacteria per cell (MOI; multiplicity of infection) of heat inactivated *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus uberis*. Sterile plates were included as controls. Total RNA was harvested 1, 6 and 24h or 90 min after start of treatment
- Immune modulators like cytokines, chemokines and acute phase proteins mRNA expression was detected by quantitative real time PCR using SYBR Green on RotorGene 3000 (Corbett Research)

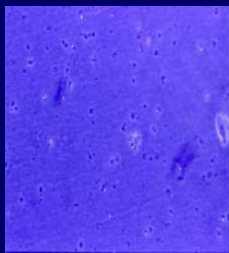


Figure 2: Day 1 of cultivation

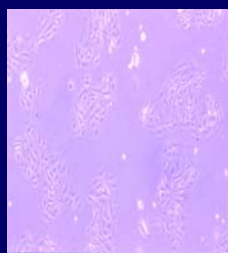


Figure 3: Day 16 of cultivation

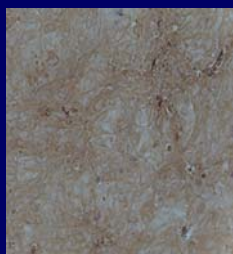


Figure 4: Immunocytochemical staining of mammary epithelial cells (clone MNF 116, DaKo Cytomation)

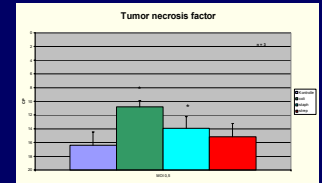
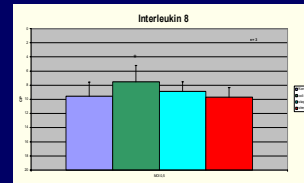


Figure 5+6: qRT – PCR for tumor necrosis factor and Interleukin 8 m-RNA expression after treatment with 0.5 MOI of heat inactivated *E. coli*, *S. aureus* and *Str. uberis* for 90 min

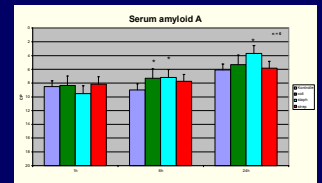
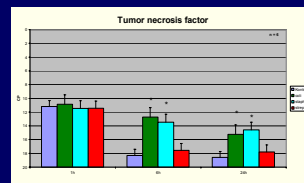
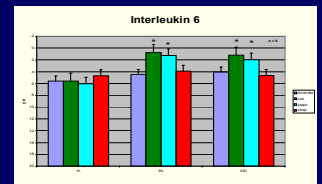
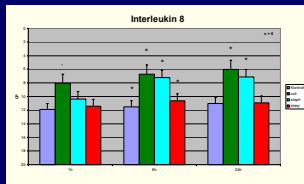


Figure 7 – 10: qRT – PCR for Interleukin 6, 8 TNF and SAA m – RNA expression at 1, 6 and 24h after the start of treatment with 10 MOI of *E. coli*, *S. aureus* and *Str. uberis*

RESULTS

- An exposure with 0.5 MOI *E. coli* and *S. aureus* for 90 min led to a significant increase of interleukin-8- (IL8) and tumor necrosis factor alpha (TNF)-mRNA expression (Figure 5+6).
- For *Str. uberis*, treatments of 10 MOI were needed for a significant increase of cytokine mRNA.
- Treatments over different time periods showed a faster significant increase of interleukin-6- (IL-6), IL-8-, and TNF-mRNA expression in *E. coli* treatment (Figure 7 – 9).
- mRNA expression of measured Cytokines were not significantly higher compared to *S. aureus* treatment.
- Serum amyloid A (SAA) mRNA concentration after a 24h exposure was only with this bacterium significantly increased (Figure 10).
- The immune response to the same amount of *Str. uberis* was always lower compared to the other bacteria.

DISCUSSION

The treatment of cultured cells from different cows with different mastitis pathogens is a useful method to investigate the role of epithelial cells in the early immune response of the mammary gland. With qRT-PCR differences in the immune response could be detected. *E. coli*, a bacterium, which causes predominantly an acute course, *S. aureus*, predominantly responsible for chronic and subclinical mastitis, and *Str. uberis*, which can cause both kinds of mammary gland infections, were confronted in this model. All strains induced an increased mRNA expression of the measured cytokines. Interestingly, *Str. uberis* always induced weaker reactions, also compared to the subclinical infection inducing *S. aureus*. However, *E. coli* seems to induce a faster immune response and this is likely related to the acute cause of infections with this bacterium.

The biological replication is an advantage over experiments with an immortalized cell line. Cells from one cow can be treated with different pathogens at the same time.