Responses of Bovine Endometrial Epithelial Cells to Pathogens

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Abstract

In dairy cows, clinical uterine infection (metritis) and subsequent persistent inflammation of the endometrium (endometritis) are major causes of infertility. *Escherichia coli* is a prevalent bacteria in metritis and endometritis and promotes infection with bovine herpes virus type 4 (BoHV-4) through mechanisms involving liposaccharide endotoxins (LPS). In this thesis, interactions between *E. coli* LPS, BoHV-4 and the endometrial epithelium were studied using *in vitro* models following characterisation of tissue samples used for culture. Examination of cell proliferation, survival and apoptosis after challenges with various doses of LPS revealed that cow and tissue characteristics did not influence proliferation of bovine endometrial epithelial cells (bEEC) in response to LPS. However, *E. coli* LPS stimulated proliferation of bEEC (maximum observed at 8 µg/mL LPS). The strong increase in cell numbers by 72 h was not associated with an increase in apoptosis, but this occurred with higher LPS doses. Analysis of protein profiles revealed de-regulation of 38 proteins belonging to many pathways, some related to the process of implantation. Morphological studies and ELISA were used to characterise the survival of cells and the cytokine response of bEEC to BoHV-4. In infected samples, the number of living cells started to decrease by Day 4 post-challenge and by Day 7 the number was lower than in controls. This change was associated with viral replication between Day 0 and Day 5, as demonstrated by immunofluorescence, titration and qPCR results and changes in IL-8 and TNF-α profiles. Moreover, the results showing strong pathogenic effects of BoHV-4 on endometrial epithelial cells pave the way for future studies on sexual transmission of BoHV-4 at time of insemination.

The results obtained led to development of reliable models to study interactions between uterine epithelial cells and pathogens, which could be of translational use. In a time- and dose-dependent manner, *E. coli* LPS increased and BoHV-4 decreased the survival of bovine bEEC *in vitro*, while LPS induced strong alterations of protein profiles, especially those related to pathways activated at time of implantation. Such deregulations may be part of the mechanism by which persistent inflammation following infection impairs fertility. This information can be exploited to identify new diagnostic markers of persistence of inflammation in the endometrium.

Keywords: cow, LPS, BoHV-4, endometrium, cell culture, proteomic, cell proliferation, endometritis, oestrous cycle

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