THE EFFECT OF *STAPHYLOCOCCUS AUREUS* BACTERIA TO PROPORTION OF GAMMA DELTA T-LYMPHOCYTES FROM BOVINE MAMMARY GLAND

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ABSTRACT

The aim of this thesis was study of lymphocytes, especially subpopulation γδ T-lymphocytes. The experiment was conducted on five clinically healthy heifers. Heifers were crossbred Holstein and Czech Pied. Their mammary glands were experimentally infected by bacteria *Staphylococcus aureus*. Bacterial suspension was applied in amout of 5 ml and the concentration was 800 CFU/ml. Application of inoculum was designed after control lavage with phosphate buffered saline (PBS). Samples of cell populations were obtain by lavage of the mammary gland in 4 intervals (24, 48, 72 and 168 hrs) after initiation of experirimental infection. Percentage of lymphocytes and subpopulation γδ T-lymphocytes were detected by flow cytometry. Stimulation of mammary gland by *S. aureus* led to percetnage increase of lymhocytes from 9.62% detected in 24 hrs to 27.36% in 168 hrs after aplication of inoculum. Percentage increase of γδ T-lymphocytes was detected too. 24 hrs after stimulation by *S. aureus* there was 10.5% γδ T-lymphocytes detected out of the total number of lymphocytes. Proportion of γδ T-lymphocytes continually increased up to 16.46% in 168 hrs. The results show that γδ T-lymphocytes play an important part in inflammatory response to Gram positive bacteria in heifers´s mammary gland.

Key words: *γδ* T-lymphocytes, *Staphylococcus aureus*, mammary gland, mastitis, heifer

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INTRODUCTION

Mammary gland is protected by many nonspecific and specific defensive substances and mechanisms and by its anatomical structure. Lymphocytes and macrophages are cells which protect mammary gland against pathogens. They are prevalent population of resident cells. During an infection, recruitment of leukocytes is induced, especially neutrophils from blood. (Miller et al., 1991).

γδ T-lymphocytes in adult cows represent 10 – 25 % of circulating T-lymphocytes . (Toman et al., 2009). Wilson et al. (1996) report that this number can be up to 40 % at calves. Davis et al. (1996) report that this number can increase up to 60 % at calves. T-cells expressing γδ TCR are called unconventional, innate or transient T- cells. That is because these cells have both features – innate as well as specific immune response (Guznam et al., 2012). γδ T-lymphocytes have been shown to recognize non-peptide antigens, pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). These cells appear to be „pre-activated" as shown by the expression of memory and activation markers and this may enable rapid induction of effector functions (Boneville et al., 2010). It has been recently shown that the interaction of MIC (proteins which are expressed in response to cellular stress) and NKG2D immune receptor (Natural Killer group 2D) expressed in bovine $W1^{+}$ γδ T-cells induces their activation in the absence of any other stimulus. This supports the role of γδ T-cells as members of innate imune (Guzman et al., 2010). Response of γδ T-cells to stimulation with cytokines is characterized by the release of interferon gamma (IFN-γ) (Price et al., 2007). γδ T-cells may also have a role as a „killer" cells, throught expression of granulolysin (Endsley et al., 2004) and perforin (Alvarez et al., 2009).

The aim of this diploma thesis was study of lymphocytes and choice of an appropriate method of study of lymphocytes. The study concentrated to subpopulation of γδ T-lymphocytes of bovine mammary gland after stimulation by bacteria *Staphylococcus aureus*. *S. aureus* is one of the most common factors causing mastitis in cows (Hogan and Smith, 2003; Bannerman et al., 2004).

The experiment was conducted on clinically healthy heifers which are endangered by mastitis in the same way as dairy cows. Heifers with healthy mammary gland are a basis of succesful bred of dairy cows.

MATERIAL AND METHODS

The experiment was carried out on five clinically healthy heifers, crossbred Holstein and Czech Pied. Heifers were 16 – 18 months old. All of them were free of intramammary infections, as demonstrated through a bacteriological examination of mammary lavages.

Before experimental infection, the mammary glands were treated with phosphate buffered saline (PBS) and control samples were taken. They were taken in the same time intervals as following samples of populations of lymphocytes after stimulation with inoculum. The time intervals were: 0 hrs – 24 hrs (left-front quarter of mammary gland) – 48 hrs (left-rear) – 72 hrs (right-front) and 168 hrs (right-rear).

There was used bacterial strain *S. aureus* Newbould 305 (CCM 6275, Czech Collection of Microorganisms, Masaryk University, Brno). The inoculum of *S. aureus* was prepared by growing the organism on ram blood agar (BA) medium. It was cultivated under continuous rotation (30 rpm/min) for 18 hrs at 37 °C. The stock culture was stored at 4 °C. After dilution of the bacterial suspension to 800 CFU/ml, the inoculum was adjusted in the syringes. It was used 5 ml of bacterial suspension. The proportion of lymphocytes in the cell suspension obtained from the lavages was asessed through flow cytometry (FCM) (FACS Calibur apparatus, Becton Dickinson, CA, USA). Arithmetic means and standard deviations were used to describe the proportions of lymphocytes

and γδ T-lymphocytes. Statistically significiant differences in the proportions were determined using the paired *t*-test. The data were processed using STATISTICA 7.1. software (StatSoft CR Ltd, Prague, Czech Republic).

RESULT AND DISCUSSION

The presence of lymphocytes was detected in control samples as well as in samples after bacterial stimulation. 24 hrs after stimulation by *S. aureus* the lowest percentage of lymphocytes out of the total number of leukocytes was noticed. The percentage of lymphocytes was 9,62 %. 48 hrs after bacterial challange it was increased to 20,78 %. Subsequently it incerased up to 26,2 % - 72 hrs after stimulation. At the end of the experiment, 168 hrs after stimulation the percentage of lymphocytes was 27,36 %. We can say the lymphocytes were stimulated by bacteria *S. aureus* to migration into beginning inflammatory area.

At the population of γδ T-lypmhocytes there was gradual increase of percentage detected as well. 24 hrs after bacterial challenge 10,5 % of γδ T-lypmhocytes out of total number of lymphocytes was detected. This is a statisticaly significant result comparing to the control sample ($P<0,05$). After 48 hrs it was 11,38 % of γδ T-lypmhocytes and at 72 hrs the percentage was increased up to 16,92 % ($P<0,01$). In the latest sample at 168 hrs the detected percentage was 16,46 % ($P<0,05$). T-cells expressing γδ TCR are called unconventional, innate or transient T-cells. That is because these cells have both features – innate as well as specific immune response (Guznam et al., 2012) They can recruit from blood or they are present in tissue for immediate protect from pathogens. We can assume that increasing percentage of this population is caused by influx of these cells from blood and from surrounding tissue. Moreover, Collins et al. (1998) report that γδ T-lypmhocytes of bovine are able to present antigen. This claim would support consideration that their timely presence can provide fast presence of antigens to αβ T-lymphocytes. This would lead to providing help with specific immune response. Some authors report (Shen et al., 2002; Dieli et al., 2003) that γδ T-lypmhocytes can even have a memory function. These considerations are excluded in this case because clinmically healthy heifers were used.

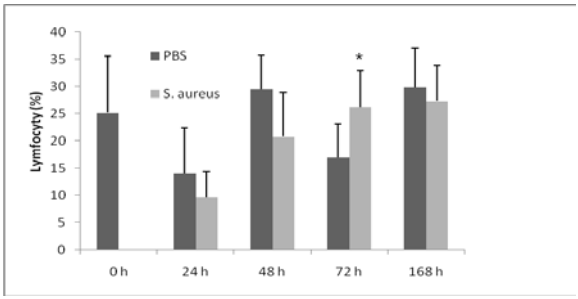

Fig. 1. Proportion of lymphocytes during experinmental infection.

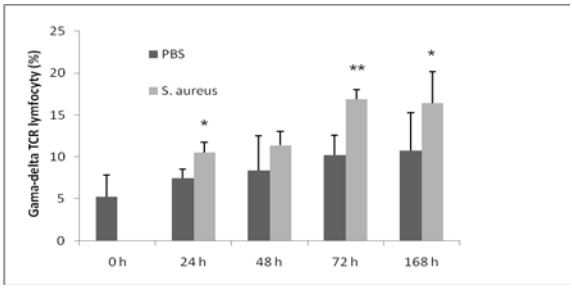

Fig. 2. Proportion of gamma-delta T-lymphocytes during experinmental infection.

CONCLUSIONS

The aim of this thesis "Influence of bacteria *Staphylococcus aureus* to population of bovine mammary gland´s lymphocytes" was study of lymphocytes, especially γδ T-lymphocytes.

The experiment was conducted on five clinically healthy heifers (20 mammary glands), at the age of 16-18 months. Application of inoculum was designed after control lavage with phosphate buffered saline (PBS). Samples of cell populations were obtained by lavage of the mammary gland in 4 intervals (24, 48, 72 and 168 hrs) after initiation of experirimental infection. Percentage of lymphocytes and subpopulation γδ T-lymphocytes were detected by flow cytometry. Stimulation of mammary gland by *S. aureus* led to percetnage increase of lymhocytes from 9,62 % detected in 24 hrs to 27,36 % in 168 hrs after aplication of inoculum. Percentage increase of γδ T-lymphocytes was detected too. 24 hrs after stimulation by *S. aureus* there was 10,5 % γδ T-lymphocytes detected out of the total number of lymphocytes. The proportion of γδ T-lymphocytes continually increased up to 16,46 % in 168 hrs.

Not all functions of γδ T-lymphocytes are known yet, however, the results of this experiment show that γδ T-lymphocytes play an important part in inflammatory respons to Gram positive bacteria in heifers´s mammary gland.

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