RESEARCH PROGRESS REPORT SUMMARY

Grant 02118-A: Targeting the Mechanism of Bacterial Adherence during Pyometra to Develop an Effective, Non-Invasive Treatment for Disease

Principal Investigator: Dr. Cordula Bartel, PhD

Research Institution: University of Veterinary Medicine of Vienna

Grant Amount: $10,368.00

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Progress Report: End-Year 1 (FINAL)

Report Due: 12/31/2015 **Report Received:**

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Pyometra is the most common uterine disease in intact bitches leading to potentially life-threatening complications via the systemic inflammatory response syndrome (SIRS) and sepsis. Different bacterial strains of Escherichia coli are the most abundant isolated infectious agents causing pyometra in bitches. In a previous study we characterized epithelial foam cells in the canine endometrial surface occurring in metestrus, the cyclic stage with the most common presence of pyometra. Foam cell formation seems to be a physiological process as preparation for the implantation of an embryo. The foamy appearance of the epithelial cells is caused by lipid droplet accumulation and the uptake of lipids from the blood is accomplished via special lipid receptors of these cells. These lipid receptors are named scavenger receptors (SR) and SR-B1 is one of the most prevalent receptors in epithelial cells. Unfortunately, SR-B1 is also a strong binding partner for bacteria. In different animal models and human patients increased levels of SR-B1 were observed in SIRS but a selective blocking of this receptor led to extenuated progress of infection and reduced clinical signs of SIRS. Based on this knowledge we assume SR-B1 to be involved in the development of endometrial epithelial foam cells and canine pyometra. Multidisciplinary approaches will be used to identify the lipid composition in the epithelial foam cells and to determine expression levels of SR-B1 in the healthy and pyometra-affected canine endometrium. The identification of SR-B1 in the canine

endometrium might open doors for a new non-invasive pyometra treatment via blocking this receptor.

Publications:

In preparation.

Report to Grant Sponsor from Investigator:

Pyometra is the most common uterine disease in intact bitches leading to potentially life-threatening complications via the systemic inflammatory response syndrome and sepsis. Different bacterial strains of Escherichia coli are the most abundant isolated infectious agents causing pyometra in bitches. In a previous study we characterized epithelial foam cells in the canine endometrial surface occurring in metestrus, the cyclic stage with the most common presence of pyometra. Foam cell formation seems to be a physiological process as preparation for the implantation and the nourishment of an embryo. The foamy appearance of the epithelial cells is caused by lipid droplet accumulation and the uptake of lipids from the blood is accomplished via special lipid receptors of these cells. These lipid receptors are named scavenger receptors (SR) and SR class B type 1 (SR-B1) is one of the most prevalent lipid receptors in epithelial cells. Unfortunately, SR-B1 is also a strong binding partner for bacteria. In different animal models and human patients increased levels of SR-B1 were observed in SIRS but a selective blocking of this receptor led to extenuated progress of infection and reduced clinical signs of SIRS. Based on this knowledge we assumed SR-B1 to be involved in the development of endometrial epithelial foam cells and canine pyometra.

Therefore, the foamy lipid droplet accumulating epithelial cells were isolated from uterine tissue samples that were obtained during routine ovariohysterectomy. Via laser capture microdissection, a technique to isolate a specific cell type from a whole tissue sample, these cells were collected and SR-B1 was identified to be expressed on genomic level in the endometrial epithelial foam cells. To elucidate a potential function of this receptor in the epithelial cells the SR-B1 protein had to be verified because in the cell there are several steps between the expression of a gene and the effective production of the respective protein. Therefore, immunohistochemistry was applied to identify the SR-B1 protein to be expressed in the epithelial cells. This technique visualizes the respective protein and its localization in the cells. SR-B1 protein was expressed in the basal and in the apical regions of the epithelial cells. In the basal regions SR-B1 is important for lipid uptake from the blood which courses in the capillaries beneath the epithelial cells. The function of apical SR-B1 expression was not explainable and therefore, we compared the localization of the SR-B1 protein in the metestrous epithelial cells to the epithelial cells in the glandular chambers of canine placenta. In the placenta epithelial SR-B1 expression has an important role for lipid transport from the maternal to the fetal site and therefore, apical and basal SR-B1 expression was identified in

these cells. Based on these results we postulate that the expression of SR-B1 in the metestrous epithelial cells is a preparation for a potentially implanting embryo because implantation of the embryo occurs between day 16 and 20 after the surge of the luteinizing hormone (metestrus). But if the bitch is not served, apical epithelial SR-B1 expression might become a problem because of its high affinity to bind bacteria. During the metestrus bacterial infection of the uterus is frequently observed and the highly secreting state of the metestrous uterus facilitates reproduction of the bacteria. Additionally we assumed high levels of SR-B1 in pyometra affected uteri which increase bacterial adherence, reproduction and the progress of infection. Therefore, we compared SR-B1 levels of healthy metestrous and pyometra affected uteri and verified increased levels of SR-B1 in the pyometra-affected uteri. A high number of SR-B1 expressing epithelial cells of the uterine luminal surface were identified by means of immunohistochemistry. Therefore, the increased levels of SR-B1 in pyometra affected uteri indicate a potential role of this scavenger receptor in endometrial bacterial adhesion.

Follow-up studies will be performed in the laboratory with cell culture experiments (in vitro) using uterine tissues obtained from routine ovariohysterectomies. A cell culture model of the canine endometrial surface epithelium will help to investigate the influence of hormones, cytokines and bacteria on SR-B1 expression and allows the experimental blocking of SR-B1 without animal testing. In the follow-up study we hypothesize that blocking of SR-B1 in vitro will lead to decreased bacterial binding and inflammatory response via cytokine release as it was observed in other pathological conditions in human diseases and murine models. If these studies are successful we suggest flushing of the canine uterus with a SR-B1 blocking solution as a preventative procedure in exposed bitches or as additional treatment in conservative pyometra therapy.