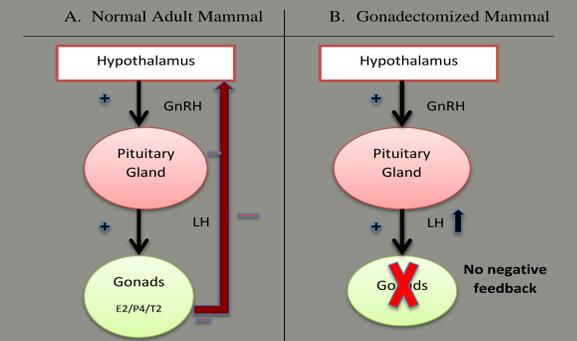


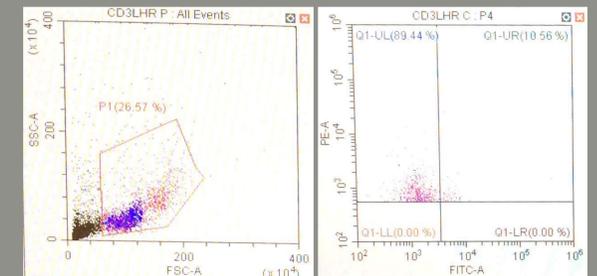
# LUTEINIZING HORMONE RECEPTOR EXPRESSION IN CANINE T-LYMPHOMA CELL LINES

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## FIGURES



**Figure 1.** A: In the normal adult mammal, the hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to release of luteinizing hormone. Luteinizing hormone (LH) stimulates the secretion of gonadal steroid hormones (testosterone (T2) in males and estrogen (E2)/progesterone (P4) in females). These gonadal steroid hormones then negatively feedback to the hypothalamus and anterior pituitary to decrease the secretion of GnRH and LH, respectively. B: In the gonadectomized mammal, there is no negative feedback, which results in supraphysiologic circulating concentrations of LH.



**Figure 2.** Representative flow cytometry results from the EMA T-lymphoma cell line. A: The cell population that expressed LHR was smaller in size (forward scatter; FSC) and more granular (side scatter; SSC). B: The EMA cell line had the fewest neoplastic T-lymphocytes that expressed LHR (an average of 10%).

## INTRODUCTION

- ❖ Canine lymphoma is a common malignant tumor in dogs.
- ❖ Lymphoma is 3-4 times more common in spayed female and neutered male dogs compared to intact females and males.<sup>1,2</sup>
- ❖ Circulating luteinizing hormone (LH) concentrations are up to 20 times higher in spayed dogs than in intact dogs because of a lack of negative feedback to the anterior pituitary from the gonads (**FIGURE 1**).<sup>3</sup>
- ❖ Luteinizing hormone receptors (LHR) are present in reproductive tract and non-reproductive tract tissues (including adrenal cortex, lower urinary tract, skin, thyroid).<sup>4</sup>
- ❖ LHR have also been found in normal and neoplastic canine lymphatic tissue.<sup>5</sup>
- ❖ LHR have also been found in human leukemia cell lines.<sup>6</sup>

## HYPOTHESES

- ❖ We hypothesized that LHR would be present in canine T-cell lymphoma lines.
- ❖ We also hypothesized that each cell line would express a different quantity of LHR.

## OBJECTIVE

- ❖ The objective of this study was use immunocytochemistry and flow cytometry to measure LHR expression in three cell lines of cultured canine T-lymphoma cells.

## MATERIALS & METHODS

- ❖ Three T-lymphoma cell lines established from spontaneously occurring canine lymphomas were generously donated from Yamaguchi University.
- ❖ Cells were cultured in R10 complete medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified 5% CO<sub>2</sub> incubator.
- ❖ Fresh cell culture media was replaced every 2-3 days at a ratio of 1:4 (EMA, CLK) or 1:9 (CLC).
- ❖ After reaching 75% confluence, cells were transferred to a 50 mL conical tube and centrifuged at 400 x g for 15 minutes to remove the culture media.
- ❖ The supernatant was discarded and the cell pellet was transferred to Eppendorf tubes.
- ❖ The cells were washed in ice cold PAB (1% bovine serum albumin and 0.05% sodium azide in phosphate buffered saline) and then resuspended in PAB at final dilution 10,000,000 cells/mL.
- ❖ Nonspecific antibody binding was blocked using a Mouse Seroblock FcR (BUF041A, Bio-Rad) at a 1:10 dilution for 10 minutes on ice.
- ❖ With the exception of the negative controls from each cell line, cells were incubated with goat polyclonal LHR (SC-26341, Santa Cruz Biotechnology) at a 1:50 dilution for 20 minutes at room temperature.
- ❖ Cells were washed in PAB and then incubated with both goat f(ab')<sub>2</sub> IgG negative control:RPE in a 1:10 dilution and mouse anti-dog CD3:FITC (MCA1774F; Bio-Rad) for 20 minutes on ice in the dark.
- ❖ Cells were washed in PAB, pi pipette onto a 96-well plate, and analyzed on a CytoFLEX flow cytometer at the Oregon State University Core Facility.

## RESULTS

- ❖ All three T-lymphoma cell lines (CLC, EMA, CLK) cell lines expressed LHR.
- ❖ The cell population that expressed LHR was smaller in size (forward scatter; FSC) and more granular (side scatter; SSC) (**FIGURE 2**).
- ❖ The CLC cell line had the most cells (45%) that expressed LHR compared to CLK (35%) and EMA (10%).
- ❖ There was no positive signal present in any of the negative controls.

## DISCUSSION & FUTURE RESEARCH

- ❖ This is the first study to show LHR expression in cultured canine lymphoma cells.
- ❖ Expression of LHR in normal and neoplastic lymphocytes may explain the higher incidence of lymphoma in dogs that have been spayed and neutered.
- ❖ Our laboratory is currently investigating the pro-neoplastic effects of LHR activation (cell proliferation, migration, and invasion) using these cell lines.

## ACKNOWLEDGMENTS

- ❖ T-lymphoma cell lines were generously donated by Dr. Takuya Mizuno from Yamaguchi University, Japan.
- ❖ Allison Ehrlich assisted with the immunocytochemistry protocol development and the flow cytometry.
- ❖ This research was funded by the Oregon State University Incentives Program.

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