LIF Involvement in Uterine Decidualization and Early Implantation in the Golden Hamster

K. Egbert and W. Leavitt

Department of Biological Sciences, College of Liberal Arts and Sciences

1. Introduction

The aim of this research is to determine the role of leukemia inhibitory factor (LIF) in the decidualization response of the golden hamster uterus. The mechanism of LIF action in the implantation and decidualization process of mammals is unknown. LIF expression has been found to increase in several species during the implantation and decidualization process [1], but the mechanism controlling LIF expression during these events is not known. In the mouse, LIF appears to facilitate blastocyst implantation, which is associated with an estrogen surge [2]. There is no estrogen surge in the hamster, where progesterone, not estrogen, is the dominant hormone regulating embryo implantation and uterine decidualization [3]. The human is also a progesterone-dependent species and does not require estrogen for implantation [4,5]. Thus, we have tested the activity of LIF in the hamster, which is similar to the human in respect to the hormonal requirements for implantation, and this research is specifically designed to determine if LIF plays a role in the uterine decidualization response. The hypothesis is that treatment with LIF or an LIF antibody will affect the amount of uterine decidual tissue formed during the decidualization response following uterine traumatization in the progesterone-primed hamster.

2. Experimental Design and Results

Experiment 1: A time course study was first performed to determine the length of time required for an optimum decidual tissue response. Hamsters were treated with progesterone (P4) on day 1 and day 3 of the estrous cycle [6] and then decidualization was induced on day 4 while animals were under anesthesia. It was found that 72 hr was the optimum time for a maximal decidual response.

Experiment 2: This study was performed to compare the effect of 3 different traumatizing stimuli in the induction of the decidual response. Hamsters were P4 primed on day 1 and day 3, decidual induction performed on day 4, and all were autopsied after 72 hr of decidualization (day 7). The first stimulus was an intraluminal injection of either LIF or a saline vehicle delivered into the lumen of each uterine horn. This stimulus was not enough to produce a significant amount of decidualization. The second stimulus was insertion of silk suture into the uterine wall, plus the intraluminal injections as before. This method produced a small, localized amount of decidua at the insertion point of the suture. The third stimulus was the insertion of a Nylon monofilament into the uterine lumen [6], plus intraluminal injection of LIF or vehicle as before. This was the best method for production of a maximal decidual response.

Experiment 3: The optimal conditions determined from Expts. 1 and 2 were used to test the effect of LIF on decidualization in 6 hamsters. Hamsters were P4 primed on day 1 and day 3 of the estrous cycle, decidual induction was performed on day 4, and uterine decidualization measured on day 7. Decidual induction was as follows: hamsters were anesthetized on day 4; the dorsal area shaved; and an incision made in the skin followed by another in the muscle wall below the ribs on the right and left sides. The uterus was exposed; each horn traumatized by insertion of a Nylon monofilament; and injected with LIF (30ng/0.3ml saline) into the lumen of the experimental horn; and the contralateral horn was traumatized and injected with 0.3ml saline vehicle (control horn; Fig. 1). At autopsy, uterine horns were weighed and examined for the amount of decidual tissue. In this study, 5 of 6 hamsters had a decreased weight, comparing the experimental (LIF-treated) horn to the control (vehicle) horn.

Experiment 4: This study tested the effect of LIF injection on decidualization using a larger number of animals. The experimental design was identical to that in Expt.3, and 13 of 17 hamsters had a decreased uterine tissue weight in the experimental (LIF- treated) horn compared to the control (vehicle) horn.

Experiment 5: This study was done to determine the effect of an LIF antibody (goat polyclonal IgG) on uterine decidualization. Hamsters were treated for 24 hr with monofilament insertion plus either injection of LIF antibody in the experimental horn or PBS vehicle in the contralateral horn (control). There was evidence of decidual tissue in control and

experimental horns in all cases, but there was no significant weight difference, indicating that the LIF antibody had no effect under these conditions. This result raises the question of whether LIF is normally present in the hamster uterus.

3. Discussion

A nonparametric 1-way ANOVA was performed on the control uterine weights from Expts. 3 and 4, and there was no significant difference between the controls allowing the data from Expts. 3 and 4 to be pooled (N=23). In 18 of 23 (78%) hamsters, LIF decreased the amount of decidualization. When the weights of control and experimental horns were analyzed by t-test (Fig. 2), the control uterine horns were significantly larger than the experimental uterine horns when expressed relative to body weight ($t= 2.86$, $df= 44$, $p=0.0065$) or as total uterine weight ($t= 2.83$, $df= 44$, $p=0.007$). The highly significant difference between the control and experimental horns shows that LIF inhibits the decidualization response, perhaps suggesting that LIF may act to inhibit conversion of stromal cells into decidual cells.

To address the question of whether LIF is present in the hamster uterus (Expt.5), a western blot was done on day 4 uterine tissue, and no LIF was detected. In addition, day 4 uterine tissue was analyzed by ELISA assay for LIF in the cytosol fraction (results pending). Cell culture studies will be done next to test directly LIF activity on the growth and differentiation of uterine decidual cells in vitro [6].

4. Conclusions

I. It is concluded that LIF inhibits the uterine decidualization process in the golden hamster.
II. This is the first demonstration in any species that LIF inhibits the uterine decidual response under in vivo conditions.
III. These results are in contrast to previous work with the mouse [2] suggesting that LIF stimulates implantation and early pregnancy. Our results indicate that LIF may actually inhibit pregnancy by blocking formation of placental tissue.

7. Acknowledgments

Research supported by NIH grant P20 RR16475 from BRIN Program of the National Center for Research Resources.

8. References

[1] Arici et al. 1995. Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium. *Journal of Clinical Endocrinology and Metabolism*, 80(6): 1908-1915.
[2] Chen et al. 2000. Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. *Endocrinology*, 141(12): 4365-4372.
[3] Do YS and Leavitt WW. 1978. Characterization of a specific progesterone receptor in decidualized hamster uterus. *Endocrinology*, 102(2): 443-451.
[4] Graham JD and Clarke CL. 1997. Physiological action of progesterone in target tissues. *Endocrine Reviews*, 18(4): 502-519.
[5] Finn CA. 1986. Implantation, menstruation and inflammation. *Biological Reviews*, 61: 313-328.
[6] Leavitt WW, McDonald RG and Shwaery GT. 1985. Characterization of deciduoma marker proteins in hamster uterus: detection in decidual cell cultures. *Biology of Reproduction*, 32: 631-643.

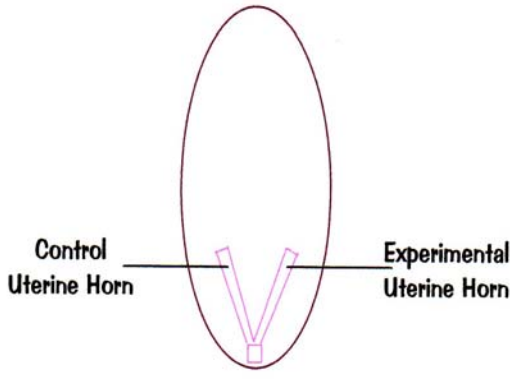

Figure 1. Experimental design using bilateral uterus.

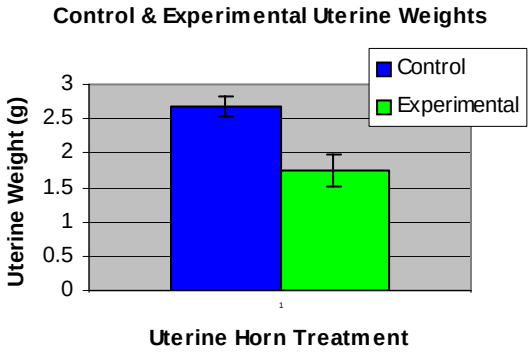

Figure 2. Uterine weight response following LIF treatment. (c=2.683±0.1555, e=1.748±0.2287; p=0.007)