

Reproductive hormones regulate human endometrial T-bet expression

Problem

The endometrial functions of reproduction, menstruation, and host defense are all regulated by locally-produced cytokines. The transcription factor *T-box expressed in T cell* (T-bet) regulates cytokine expression in T-cells and B-cells and has very recently been found to be expressed in endometrial epithelial cells. Thus, a better understanding of the mechanisms regulating endometrial cytokine expression may lead to new therapeutic approaches for reproductive, menstrual, and infectious disorders.

The proposed experiments are designed to generate data characterizing changes in endometrial T-bet RNA and protein expression during the normal menstrual cycle using qRT-PCR and immunohistochemistry.

Background

Cytokines Affect Reproduction

Cytokines play a major role in the regulation of three basic endometrial functions: host immunity, reproduction, and menstruation. Cytokines have been shown to regulate reproductive function during both pregnancy initiation (embryo implantation) and pregnancy maintenance (prevention of miscarriage and preterm delivery). For example, in female mice lacking the expression and production of the cytokine, leukemia inhibitory factor (LIF), implantation of blastocysts is completely prevented¹. Additionally, it has been shown that in vivo expression of LIF by endometrial epithelial cells may be regulated by female steroidal hormones². Although little is known about regulation of cytokine expression in endometrial epithelial cells, regulation of cytokine expression in T-cells is better understood.

As nascent T-cells develop in the thymus they are destined to become either CD4+ helper T-cells or CD8+ cytotoxic T-cells. The CD4+ T-cells further differentiate into T_{H1} helper T-cells, modulating a cellular immune response, or T_{H2} helper T-cells, modulating a humoral response. In general, T_{H1} T-cells produce pro-inflammatory cytokines while T_{H2} T-cells produce anti-inflammatory cytokines. The determination of T-cell phenotype as T_{H1} or T_{H2} is thought to be critical in control of infection as well as prevention of autoimmune disease³.

In regard to pregnancy, the polarization of T-cell phenotype appears to be critical for pregnancy maintenance. A T_{H2} pattern of cytokine expression is found in successful human pregnancies, while a T_{H1} pattern is found in women who have miscarried⁴⁻¹⁰. The data are especially compelling that suppression of T_{H1} cytokines is important to prevent miscarriage¹¹⁻¹³. Thus, regulation of T_{H1} versus T_{H2} cytokine expression pattern is crucial for both host defense and reproduction.

T-bet Regulation and Reproductive Hormones

T-box expressed in T-cells (T-bet) is a transcription factor that is responsible for the cellular programming maintaining the T_{H1} phenotype in T-cells. In immune cells, T-

bet expression is regulated by interferon gamma (IFN- γ) in a Stat1-dependent manner¹⁴ and by other cytokines in a Stat-1 independent manner¹⁵⁻¹⁷.

Until recently, it was thought that T-bet was only expressed in T-cells. However, recent unpublished reports suggest expression in endometrial epithelium¹⁸. Furthermore, non-immune cell expression of T-bet was found to be critical for the pathogenesis of the T_H1 mediated murine brain disease, *experimental autoimmune encephalomyelitis* (EAE), a model of Multiple Sclerosis. In *T-bet-/Stat1+* and *T-bet-/Stat1-* mice, the development of EAE was prevented. Interestingly, further experiments using adoptive transfer demonstrated that T-bet expression in bone-marrow derived immune cells was completely dispensable for development of EAE¹⁵. Thus, T-bet expression in epithelia or other non-lymphocytes are likely important for EAE induction.

Interestingly, low dose estrogen prevents induction of EAE in 2 different inbred mouse strains, suggesting modulation of T-bet expression or activity by reproductive steroid hormones¹⁹. Unpublished studies by our collaborator, Dr. Danny Schust (Boston University) also suggest regulation of T-bet by reproductive steroid hormones¹⁸. In these studies, immunohistochemistry on proliferative phase human endometrial epithelium was used to show that T-bet protein is localized to the endometrial epithelium. These studies have also that T-bet mRNA levels in an endometrial epithelial cell line (Ishikawa) fluctuate in a tri-phasic manner in response to progesterone and are slightly repressed in response to estrogen¹⁸. A simplified model is shown in figure 1.

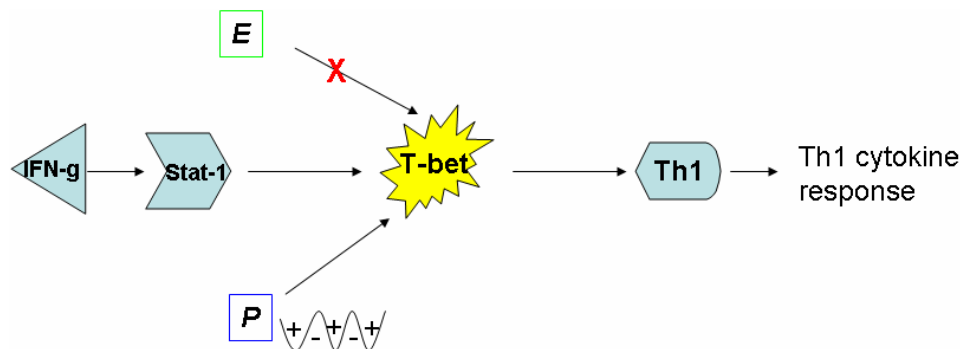


Figure 1: General relationship between T-bet, Stat-1, the Th1 cytokine response and female hormones.

These experiments suggest the possibility that T-bet is regulated by estrogen and progesterone. However it is unlikely that the in vitro model used will reflect in vivo changes associated with the normal menstrual cycle. The cell line used has been widely documented as unstable in steroid receptor expression and function²⁰. More importantly, in vivo effects of estrogen and progesterone depend on complex actions on multiple cell types resulting in local paracrine factors produced in one cell type acting on another²¹. Additionally, there is cyclic variation in one or more types of estrogen and progesterone receptors in endometrium²². In this proposal, we plan to directly examine the effects of the normal hormonal cycle on T-bet expression in human endometrial tissue.

Hypothesis

Recent studies have shown that endometrial epithelial cells express T-bet, suggesting a possible role for T-bet in regulating endometrial cytokine expression. This study will examine the expression of T-bet by endometrial tissue sampled throughout the normal menstrual cycle.

Predicted Results

Significant changes in T-bet expression in endometrial epithelial cells over the cycle are anticipated. A potential pitfall in our approach is the use of endometrial biopsy tissue RNA for RT-PCR. Whole endometrial biopsies contain a number of endometrial cell types. However, preliminary immunohistochemistry data¹⁸ as well as the relative paucity of T-cells and B-cells in the endometrium, suggest that this will not be a confounding problem. Furthermore immunohistochemical localization will be performed by us throughout the cycle allowing further assessment of expression outside of the epithelial layer.

Methods

In this project, endometrial tissue, sampled during each stage of the menstrual cycle from reproductive-aged women, will be analyzed for changes in T-bet mRNA and protein. RNA extracted from tissue samples will serve as a template for cDNA which will subsequently be subjected to qRT-PCR analysis to quantitate changes in T-bet RNA. Ishikawa endometrial cells will be used as the positive control during the probe for T-bet. The same tissue samples will also be analyzed by immunohistochemistry to evaluate location and changes in T-Bet protein expression.

Initially, 4 samples from each cycle phase (proliferative, early secretory, mid-secretory, and late secretory) will be utilized. Once pilot data are obtained the power of the experiment will be analyzed and additional samples obtained if needed and feasible to have an 80% power to detect a 3-fold change.

Future studies may include characterizing T-bet expression of endometrial epithelial cells in various disease states as well as further manipulation of in-vivo steroid levels to assess regulation of T-bet expression by specific steroid hormones.

This project has been approved by the UNC Institutional Board under protocol number 96-OBGYN-254.

Personal Objectives

1. To explore immunological expression patterns and understand how they affect human reproduction.
2. To become proficient in qRT-PCR and immunohistochemistry.
3. To understand the process of investigating a scientific problem that relates to disease in humans.
4. To immerse myself in the subject of reproductive endocrinology and infertility.

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