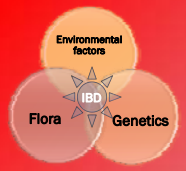
Estrogen Modulation of Innate Immunity in a Mouse Model of Inflammatory Bowel Disease

Nolen, N. Paul. Myles, Matthew; Franklin, Craig
Research Animal Diagnostic Laboratory (RADIL); University of Missouri; Columbia, Missouri

Background

Inflammatory Bowel Diseases (IBD), including Crohn's Disease and Ulcerative Colitis affect over one million people in the US. The pathogenesis of IBD is poorly understood but flora, genetics, and environmental factors all contribute to the development and severity of disease. IBD is a lifelong disease. Treatments are often ineffective and many people ultimately require a bowel resection.



Infection of the A strain of mice with *Helicobacter hepaticus* has emerged as an animal model of IBD that recapitulates many of the lesions seen in humans, most notably chronic inflammation of the large intestine. In this model, female mice develop more severe disease than males, and administration of estrogen (17-β-estradiol) markedly decreases disease severity, suggesting that gonadal sex hormones can modulate intestinal inflammation. Further analysis of infected mice lacking either the estrogen receptor alpha (ERα) or ERβ and mice treated with agonists that specifically target either ERα or ERβ suggests that estrogen may have either immunostimulatory or immunomodulatory properties that depend on which estrogen receptor is engaged. Specifically, ERβ agonists modulate disease whereas ERα agonists exacerbate or do not affect disease severity.

Goal

Our overall goal is to investigate the mechanism of *in vivo* estrogen effects in this mouse model by using *in vitro* studies to determine which specific cell types involved in the pathogenesis of IBD are modulated by estrogen. Several cellular targets are possible targets of estrogen including epithelial cells, dendritic cells and lymphocytes. In the study reported here, we have begun to assess whether dendritic cells responses to LPS are modulated by estrogen and/or ER agonists.

Hypothesis

The dendritic cell response to the TLR4 ligand, LPS, as assessed by expression of IL-12/23p40 and TNF-α, will be decreased in the presence of an ERβ agonist.

The dendritic cell response to LPS, as assessed by expression of IL-12/23p40 and TNF-α, will be increased in the presence of an ERα agonist.

Experimental Design

CD11c+ dendritic cell collection, culture and treatment:

Spleens were harvested from eight, 4-5 week old A/JCr mice. CD11c+ cells were isolated using anti-CD11c+ coated beads then plated at a concentration of $1.0x10^6$ cells/ well.

Twelve wells received serial dilutions of either estrogen, ERβ or ERα agonist treatment (1000 nM, 100 nM, 10 nM, 1 nM, 0.1 nM, 0.01 nM) and 2 wells received media alone.

One well from each group was designated as an experiment well and the other well served as a control. After ten hours of incubation with estrogen, an ER agonist or media, experimental wells were stimulated with 1 ng/mL LPS diluted in media containing the appropriate estrogen or agonist dilution. Control wells received media containing the appropriate estrogen or agonist dilution alone. After 6 hours of LPS stimulation, cells from all wells were lysed with RLT buffer.

Gene expression:

IL-12/23p40, TNF-α and HPRT gene expression was measured using Real Time-quantitative PCR. Cytokine gene expression was normalized to HPRT expression.

Previous Work

Cecal Lesions

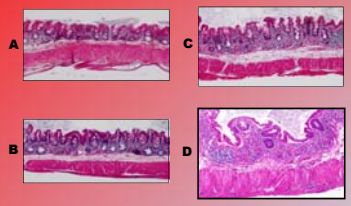


Figure 1. Cecal lesions were scored on a 10 point scale where severe disease is represented by a score greater than 6. **A.** Normal **B.** Cecal lesion score of 4 **C.** Cecal lesion score of 6 **D.** Cecal lesion score of 8

Lesion Scores of Estrogen / ER Agonist-Treated, *H. hepaticus*-Inoculated A/J Mice

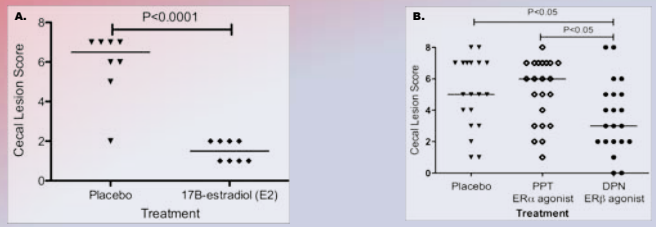


Figure 2. Both graphs depict the severity of disease in estrogen or ER agonist-treated, *Helicobacter hepaticus*- inoculated mice at 90 days post-inoculation/treatment. **A.** Treatment of mice with estrogen (17β estradiol) significantly decreased the median lesion score when compared to mice treated with a placebo. **B.** Administration of ERβ agonist (DPN) to infected mice significantly decreased the median lesion score when compared to mice treated with either placebo or ERα agonist (PPT). There was a trend towards increased disease severity in mice treated with PPT, however, this difference was not statistically significant. These findings lead us to hypothesize that signaling through ERβ is immunomodulatory while signaling through ERα is immunostimulatory.

Results

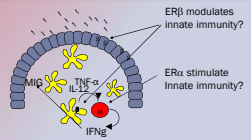
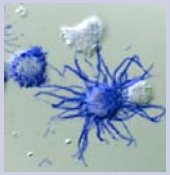


Figure 3. Proposed model for estrogen modulation of IBD pathogenesis . We hypothesize that in vivo ERβ agonist effects are mediated through epithelial cells and/or dendritic cells, while the ERα agonist effects are mediated through lymphocytes. The study reported here is focuses on the effects of estrogen conditioning of dendritic cells.



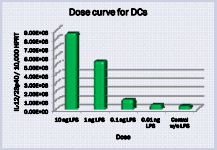


Figure 4. IL-12/23p40 gene expression of dendritic cells after stimulation with various doses of LPS for 6 hours. From this study, a dose of 1 ng/mL was selected for subsequent studies.

Gene Expression in Dendritic Cells Conditioned with Estrogen and Stimulated with LPS

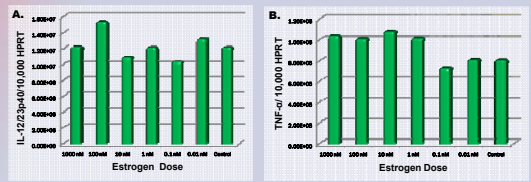


Figure 5. The expression of genes encoding for the pro-inflammatory cytokines, IL-12/23p40 and TNF-α, were measured to assess the response to LPS stimulation of estrogen-conditioned dendritic cells. The concentration of estrogen does not appear to influence the gene expression of IL-12/23p40 (A) or TNF-α (B) when the cells are stimulated with 1 ng/mL LPS.

Gene Expression in Dendritic Cells Conditioned with an ERβ Agonist and Stimulated with LPS

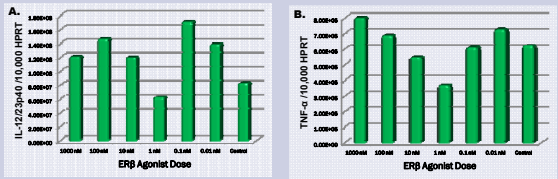


Figure 6. The expression of genes encoding for the pro-inflammatory cytokines, IL-12/23p40 and TNF-α, were measured to assess the response to LPS stimulation of ERβ agonist-conditioned dendritic cells. The trend evident suggests that signaling through ERβ may have a dose dependent effect on IL12/23p40 (A) or TNF-α (B) expression in LPS stimulated dendritic cells.

Future/Ongoing Research

Continue above experiments to confirm or refute trends observed in the response of estrogen-conditioned dendritic cells to LPS (TLR4 ligand).

Determine if estrogen conditioning of dendritic cells modulates their response to lipoteichoic acid (TLR2 ligand), flagellum (TLR 5 ligand) or *H. hepaticus*.

Determine if estrogen conditioning of epithelial cells modulates their response to LPS (TLR4 ligand), lipoteichoic acid (TLR2 ligand), flagellum (TLR 5 ligand) or *H. hepaticus*.

Conclusions

Estrogen does not modulate the response of dendritic cells to LPS, however, specific engagement of estrogen receptor beta may modulate LPS response in a dose dependent manner.

Special thanks to:

Aaron Ericsson, Andrew Hillhouse, & Beth Livingston

Acknowledgements

NIH-K26RR08811, NIH-K01RR018798, Merck/Merial
University of Missouri College of Veterinary Medicine and RADIL