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

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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 recapitates 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 (17β-estradiol) markedly decreases disease severity, suggesting that gonadal sex hormones can modulate intestinal inflammation. Further analysis of infected mice lacking either α or mice treated with agonists that β modulate signaling through ER α or ER β 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.

Hypothesis

The dendritic cell response to theTLR4 ligand, LPS, as assessed by expression of IL-12p35 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-12p35 and TNF-α, will be increased in the presence of an ERα agonist.

Experimental Design

CD11c+ dendritic cell collection, culture and treatment: Splines were harvested from 8-4 week old A/JCr mice. CD11c+ cells were isolated using anti-CD11c+ coated beads then plated at a concentration of 1.0 10^6 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 ER antagonist, 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-12p35, TNF-α and HPRT gene expression was measured using Real Time-quantitative PCR. Cytokine gene expression was normalized to HPRT expression.

Future/Ongoing Research

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

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.

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