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HORMONE MODULATION OF TOLL-LIKE RECEPTOR 5 IN CULTURED HUMAN BLADDER EPITHELIAL CELLS

Hypothesis / aims of study

Bacteria stimulate the innate immune response by binding to highly conserved pattern recognition receptors (PRR). Toll-like receptor 5 (TLR5) is a PRR that specifically responds to flagellin, a component of gram negative bacteria such as *E coli* [1]. TLR5 has been identified in the human bladder [1]. Clinical studies have observed that urinary tract infection (UTI) frequency and attachment of *E coli* are correlated with the menstrual cycle [2, 3]. The effect of hormone levels on the stimulation of TLR5 receptors in the bladder is unknown.

We hypothesized bladder epithelial cells express functionally active TLR5 whose signaling pathway is modulated by sex steroids at physiologic concentrations.

Study design, materials and methods

These studies were performed using the T24 human urinary bladder (HUB) cell line (ATCC HTB-4). After growing to near confluence, cells were incubated in hormone-free media for 72 hours. The HUB cells were subsequently incubated in 1) hormone free media, 2) estradiol containing media at physiologic concentrations (10^{-9} to 10^{-11} M), 3) progesterone containing media at physiological concentrations (10^{-7} to 10^{-9} M), or 4) media containing estradiol and progesterone for 72 hours. Flagellin was added for 0, 6, and 24 hours. Cells and media were collected. TLR5 expression in the homogenized cell pellet and stimulated IL-6 production in the culture media were analyzed with ELISA. Cellular protein assays were performed to normalize results. Additional studies were performed utilizing a TLR5 antagonist (added to the media for 24 hours prior to flagellin exposure) to confirm that stimulation from flagellin was mediated by TLR5 signaling.

Results

Cultured HUB cells were observed to express TLR5 protein in hormone free media at an average level of 41 ng/mg protein after 24 hours exposure to 100 ng/ml flagellin. All hormone environments decreased TLR5 expression at least three-fold. The combined estradiol-progesterone environment had the least suppressive effect. Progesterone-only environment had the most suppressive effect on TLR5 expression; i.e. a six-fold decrease compared to hormone-free media. [Figure]

Function of TLR5 was measured by IL-6 production after flagellin exposure. IL-6 production was 75% higher in the estradiol than progesterone environment. The progesterone environment produced IL-6 levels twice that observed in hormone-free and combined estrogen-progesterone. Interestingly, higher TLR5 expression was associated with lower IL-6 production. [Figure]

Following 24 hours pre-treatment with the flagellin antagonist (i.e.100 ng/ml hTLR-5 Fc), there was a 37% decrease in IL-6 production in response to flagellin in estradiol (p=.022) and a 47% decrease in hormone-free environments (p=.017).

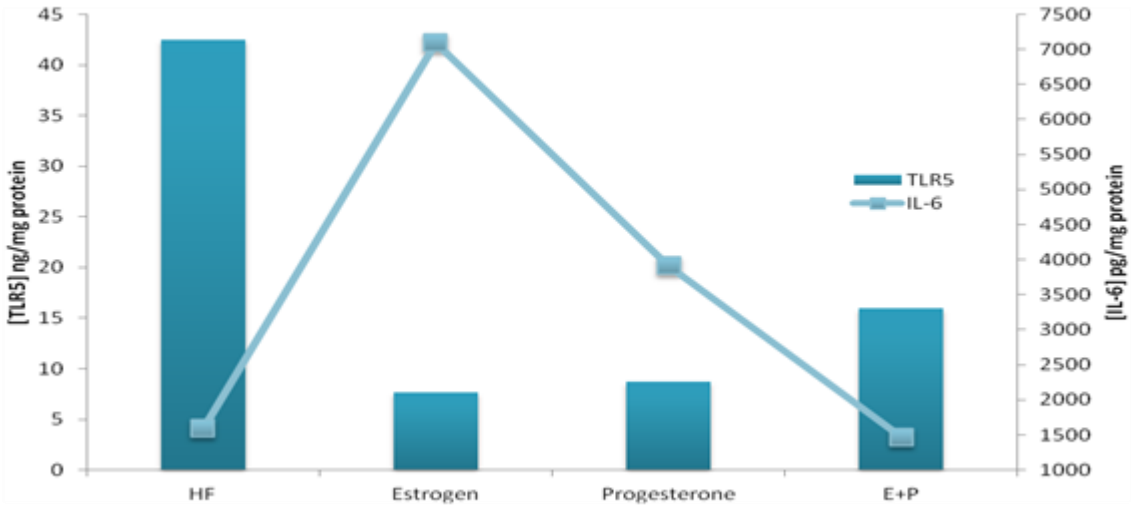

Figure TLR5 and IL-6 response to steroid hormones. Human urinary bladder cell culture exposed to varying physiologic concentrations of estradiol, progesterone, and combined estradiol and progesterone for 72 hours followed by flagellin stimulation for 24 hours. TLR5 ELISA and protein assay performed on cell culture, IL-6 ELISA performed on media supernatant.

Interpretation of results

Our study demonstrated that TLR5 expression and its functional activity as measured by cytokine IL-6 production are modulated by estradiol and progesterone.

Concluding message

The functional increase in TLR5-associated IL-6 may play a role in increasing the rate of symptomatic UTI. Likewise, low TLR5 functional activity may dampen the response of the innate immune system thereby lessening the likelihood of a symptomatic UTI. These findings are consistent with clinical studies [2,3] which show increased risk of symptomatic UTI and *E coli* binding during the follicular phase (consistent with our estradiol-only experiment), moderate prevalence of UTI and *E coli* binding during the luteal phase (consistent with our combined estradiol-progesterone experiments), and lower prevalence of UTI and *E coli* binding during menses (consistent with our hormone-free studies).

References

1. J Urol. 2011;186:1084-1092.
2. Clin Infect Dis 1996;23:635-6.
3. J Infec Dis 1983;148(3):412-21.

Disclosures

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