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Microbiota Regulation of Bladder Toll-like Receptor Expression in Male and Female Mice

Introduction: The bladder urothelium exists at the interface between the bladder contents, the microbiota and the host. Critical at this interface are pattern recognition receptors, the best studied of which, Toll-like receptors (TLRs), recognisemolecular patterns displayed by microorganisms, including commensals and pathogens. Recent evidence, from peripheral blood mononuclear cells, suggests that altered TLR2 and TLR4 receptor-mediated responses are associated with bladder pain syndrome (BPS; 2). BPS comprises a large group of patients with bladder and/or urethral and/or pelvic pain and irritative storage symptoms with a female predominance of 5:1 (1). Emerging evidence also suggests that the bladder microbiome may be altered in patients with BPS (3). Despite this, however, the influence of the microbiota on bladder TLR expression has not been examined.

Aim: Our aim, therefore, was to assess TLR2, TLR4 and TLR11 receptor gene expression, the latter of which protects against uropathogenic infection in rodents, in male and female mouse bladder in the absence or presence of a microbial environment.

Methods: Bladder tissue was obtained from male and female Swiss Webster conventional (Conv) and germ-free (GF) mice, and GF mice subjected to microbiota colonisation in adulthood. Real-Time RT-PCR was carried out using probes designed by Integrated Life Technologies™ to mouse *Tlr2*, *Tlr4*and *Tlr11* genes. Data represent fold-change in expression levelsrelative to conventional (control) tissues and the data are expressed as mean ±SEM. Statistical differences were determined by two-way ANOVA and Bonferroni post-hoc test using GraphPad Prism 5.

Results: The expression of *Tlr2*was selectively influenced by microbiota colonisation in female mice only (Conv, 1.00 ± 0.11 (7); Colonised, 0.45 ± 0.11 (10), $P < 0.05$). An approximate 50% reduction in *Tlr11*expression was also observed in female colonised mice (Conv, 1.00 ± 0.27 (8); Colonised, 0.5 ± 0.10 (10)), though this did not reach statistical significance. No changes in the expression of *Tlr2*, *Tlr4*or *Tlr11*were observed in any of the male experimental groups nor was the expression of these *Tlr*s influenced by a germ-free environment in female mice. No significant interaction between microbiota status (Conv, GF or colonised) and sex with respect to *Tlr2*, *Tlr4*and *Tlr11*expression were identified.

Conclusion: These data indicate that a GF environment does not significantly influence TLR2, TLR4 or TLR11 receptor gene expression in urinary bladder. However, TLR2, and to a lesser degree TLR11, receptor expression display sensitivity to microbiota colonisation in a sex-dependent manner. These data suggest that the microbiota influences innate immune system signallingin female mouse bladder.

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(3) Siddiqui H et al. (2012). *BMC Microbiol*: **12**: 205.