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PATHOPHYSIOLOGY OF EXPERIMENTAL CYSTITIS: ROLE OF TOLL LIKE RECEPTOR 4 (TLR4)

Hypothesis/aims of study

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a multifactorial chronic inflammatory disease characterized by suprapubic pain, discomfort, urgency and excessive urinary frequency, which profoundly impairs patient's quality of life. Despite efforts to understand the etiology of IC/BPS, this condition remains poorly understood, and therefore no fully effective treatment has been developed to date[1]. Changes in innate immune function contribute to the onset and progression of numerous chronic inflammatory diseases, and toll-type receptors (particularly TLR4) are fundamental in this process[2]. However, there are still no studies in the literature addressing the contribution of TLR4 receptors in IC/BPS. The aim of the present study was to investigate the contribution of TLR4 signaling pathway in the development of cyclophosphamide (CYP)-induced cystitis.

Study design, materials and methods

Ten to twelve-week old C57BL/6 (wild-type, WT) and/or TLR4 knockout (TLR4$^{-/-}$) male mice (20-25 g) were treated with a single intraperitoneal injection of CYP (300 mg/kg) or saline (5 mL/kg, control), and studies were performed at 24 h after injection[3]. Measurements of TLR4, TRIF and MyD88 mRNA expression were determined by RT-PCR. For micturition pattern analysis, mice were placed in cages individually and urine output was collected for 3 h ~~ours~~ on filter paper covering the cage bottom, photographed under UV light and analyzed to identify the surface area of individual spots and volume, and calculated based on a calibration curve. Cystometry was performed in urethane-anesthetized mice. Briefly, bladders were filled at a constant rate (0.6 mL/h) and intravesical pressure was recorded for 45 min. Results are expressed as mean ± SEM of a number of experiments (*n*). Comparisons among the groups were evaluated using Student's *t* test and one-way ANOVA followed by Bonferroni's post hoc analysis (for multiple comparisons).

Results

As seen in figure 1, in isolated bladders of WT mice RT-PCR analysis for genes activated by TLR4 pathway revealed in CYP injected mice a significantly increase (P<0.05) of TLR4 itself and its downstream adaptor proteins MyD88 and TRIF (P < 0.05). In WT mice, micturition pattern analysis showed a marked increase in the number of spots and a reduction of micturition volume in CYP-injected mice (9 ± 1.7 spots and 328 ± 38 µL) in comparison to saline group (2.4 ± 0.5 spots and 779 ± 56 µL). TLR4 deletion significantly reduced the number of spots (3.5 ± 0.9 spots, P<0.05 in relation to CYP-injected WT mice) but had no effect in volume (262 ± 90 µL). Cystometric studies showed significant changes in several micturition patterns in CYP-injected WT mice compared to saline, including bladder capacity, micturition interval, voiding frequency and NVCs frequency, all of which were prevented by TLR4 deletion (Table 1).

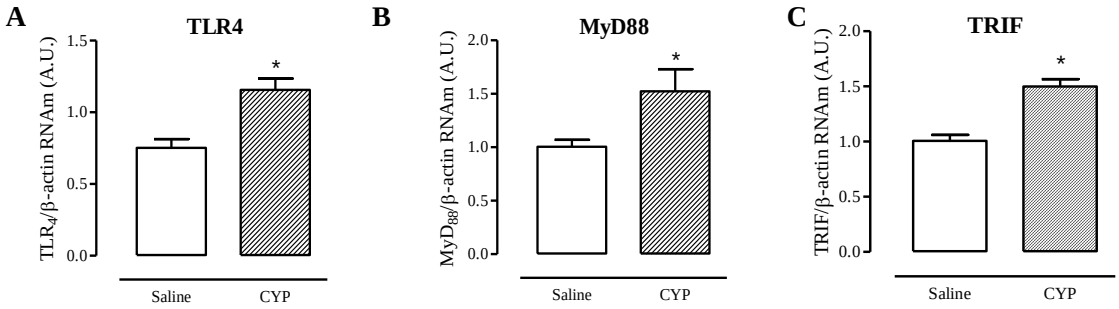

Figure 1. mRNA expression of Toll-like receptor (TLR4), MyD88, (Myeloid differentiation primary response gene 88) and TRIF (TIR-domain-containing adapter-inducing interferon-β) in isolated bladders from wild-type mice injected with saline or CYP (300 mg/kg, i.p., 24 h). The mRNA expression level of each gene was normalized to actin expression levels. Data represents means ± SEM, n = 5-8 animal/group. *P<0.05 in comparison to Saline-injected. Values are expressed as arbitrary units (A.U.).

Table 2. Changes in cystometric parameters in WT and TRL4$^{-/-}$ mice injected with saline or CYP (300 mg/kg, i.p., 24 h).

	WT		**TLR4$^{-/-}$**	
	Saline	**CYP**	**Saline**	**CYP**
Capacity (mL)	0.23 ± 0.02	0.05 ± 0.01*	0.16 ± 0.01	0.10 ± 0.02$^{\#}$
Voiding pressure (mmHg)	22.9 ± 2.0	7.9 ± 3.4*	14.6 ± 2.6	15.4 ± 1.3$^{\#}$
Voiding interval (min)	12.1 ± 1.4	2.7 ± 0.8*	9.0 ± 0.6	5.1 ± 0.6$^{\#}$
Voiding frequency (nº/min)	0.10 ± 0.01	0.42 ± 0.01*	0.11 ± 0.08	0.20 ± 0.03$^{\#}$
NVCs frequency (nº/min)	0.08 ± 0.03	1.3 ± 0.11*	0.55 ± 0.12	0.34 ± 0.20$^{\#}$

Values represents means ± SEM, n = 5-8 animals/group. * P<0.05 *vs* WT-Saline-injected.; $^{\#}$ P<0.05 *vs* WT-CYP-injected. NVCs, non-voiding contractions.

Interpretation of results

Administration of CYP caused pronounced urinary bladder inflammation and overactivity, which was accompanied by an up-regulation of TLR4 signaling pathway. TLR4 deletion prevents significantly these alterations and ameliorates bladder dysfunction.

Concluding message

TLR4 signaling pathway activation mediates inflammation and bladder dysfunction in CYP-induced cystitis and may provide a potential therapeutic target for IC/BPS.

References

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Disclosures

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