The p75^{NTR} Antagonist Mediates Anti-Inflammatory Responses to LPS in Urothelial and Smooth Muscle Cells

Background: Current evidence links acute bacterial cystitis by E. coli to the progression of interstitial cystitis in aging women. Lipopolysaccharide found on these bacteria activates the TLR4 receptor to induce proinflammatory responses. Much as the TLR4, the p75NTR receptor also sets inflammation with proNGF to remodel bladder tissues and impair urodynamics, suggesting that it might be a key contributor of recurrences and onsets of chronic cystitis. As we showed previously that antagonism of p75^{NTR} can impede the development of diabetic voiding dysfunction, we aimed to demonstrate a molecular interplay in p75^{NTR} inflammatory signals through activation of TLR4.

Methods: We pre-treated rat primary urothelial (UTC) and detrusor smooth muscle (SM) cells with a specific p75^{NTR} antagonist (THX-B), followed by exposure to LPS. We evaluated the recruitment of the Traf6 ubiquitinase by p75^{NTR}, as well as its downstream activation of caspase-3/8, NF-κB and MAPKs pathways by immunoprecipitation, enzymatic assay and immunoblotting. Cytokinesis of TNF-α and its diffusion in cell medium with NO were examined by Western blotting and Griess' colorimetric method. Disruption of E-Cadherin, ZO-1 and Occludin on cell surface was identified using immunochemistry and immunoblotting.

Results: Both cell types showed expression of p75^{NTR} independently of TLR4 activity and absence of caspase activity under LPS or THX-B conditions. In UTC, LPS induced cytokinesis of TNF-α and NO release. Antagonizing p75^{NTR} before LPS exposure also decreased cytokinesis/release of TNF-α, without interfering with NO diffusion in these cells. While Traf6, Jnk and NF-κB were unchanged, increased levels of Erk were noted in presence of LPS, with no changes under p75^{NTR}antagonism. Expression of Occludin and ZO-1 on UTC surface greatly decreased with exposure to LPS, while this effect was prevented on occludin by THX-B. In SMC, LPS soared the recruitment of Traf6 by p75^{NTR}, which was then suppressed by pre-treatment. Similarly, pre-treating SMC with THX-B decreased Jnk and NF-κB activation by LPS. Expression of all tight junctions remained stable with LPS or p75^{NTR} antagonism.

Conclusions: Together, our results demonstrated cell-specific interplay between TLR4 and p75^{NTR} inflammatory pathways sensitizing hosts to recurrent bacterial cystitis and chronic interstitial cystitis. We emphasize the merit of characterizing the anti-inflammatory value of p75^{NTR} antagonism *in vivo*, as a potential therapeutic approach for women with cystitis.