Diabetic Complications Consortium

Application Title: Toll-like receptor 4 mediates diabetic bladder dysfunction

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1. Project Accomplishments:

In this project, our goal was to investigate the role of TLR4 in diabetic bladder dysfunction. Our hypothesis was that TLR4 activation during diabetes leads to bladder hypertrophy and hypercontractility in the initial, overactive phase of diabetic bladder dysfunction. This idea was based partly on a wealth of recent evidence implicating the innate immune system in the development of diabetes-associated organ damage and partly on our own preliminary data demonstrating TLR4 activation in the bladder of streptozotocin (STZ)-induced diabetic mice. We addressed this hypothesis by using an STZ-induced diabetes model (low dose protocol - 50 mg/kg i.p. daily for 5 consecutive days) in adult male TLR4 genetically deficient (strain B6.B10ScN-Tlr4lps-del/Jth, #007227 Jackson) and wild type (strain C57Bl/6, #000664 Jackson) mice. Briefly, we demonstrated that TLR4 deletion, while not impacting the development of STZ-induced diabetes per se, partially protects against bladder hypertrophy and hypercontractility characteristic of diabetic bladder dysfunction. These data supporting our hypothesis will be used as a basis for a more thorough investigation of the involvement of TLR4 in the pathogenesis of diabetic bladder dysfunction.

2. Specific Aims:

Specific aim 1: to determine whether hyperglycemia activates TLR4 to increase cell proliferation and reactive oxygen species (ROS) production in mouse bladder smooth muscle cells.

In our preliminary data, high glucose (25 mM glucose) increased the expression of TLR4, MyD88 and TRIF in cultured smooth muscle cells, and the TLR4 inhibitor CLI-095 blocked this response. Also, previously, we had observed that reactive oxygen species (ROS) production was increased in response to high glucose conditions, and that treatment with the TLR4 inhibitor CLI-095 attenuated this increase, suggesting that TLR4 mediates hyperglycemia-induced ROS production in bladder smooth muscle cells. In this aim, we used a bladder smooth muscle cell culture system from wild type C57Bl/6 mice and knockout TLR4 mice to measure ROS production by dihydroethidium (DHE) fluorescence in response to a high glucose environment. Basal levels of ROS were similar between wild type and $TLR4^{-/-}$ bladder smooth muscle cells. While we still observed a robust increase in ROS levels in wild type cells exposed for 12h to high glucose, there was no significant increase in ROS levels in $TLR4^{-/-}$ cells (figure 1).

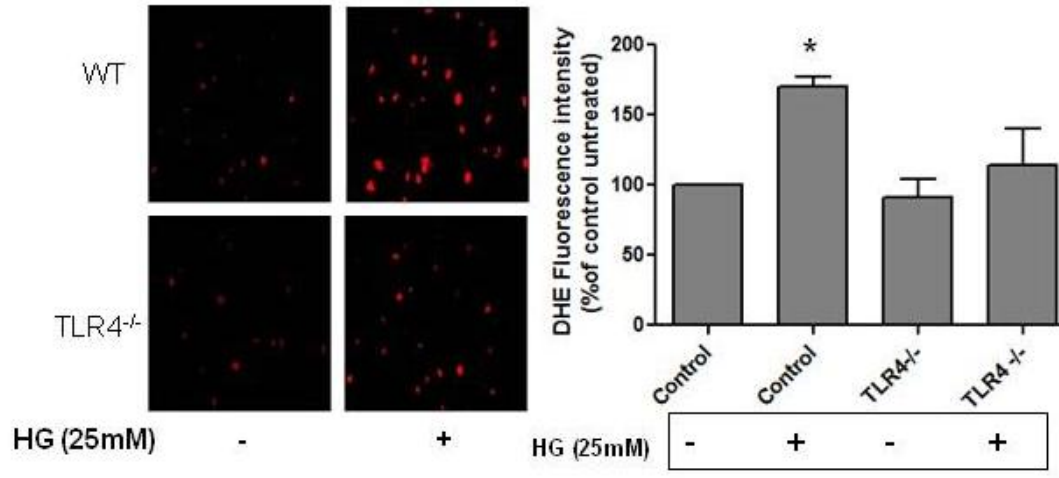

Fig. 1. Reactive oxygen species measured by DHE fluorescence in cultured bladder smooth muscle cells exposed to 25 mM glucose for 12h.

Specific aim 2: to determine whether bladder hypertrophy and hypercontractility in diabetes is mediated by TLR4.

We had previously observed that the expression of TLR4 and MyD88 was increased in bladder from STZ-induced diabetic mice, and that pharmacological inhibition of TLR4 with CLI-095 decreased the augmented carbacholine (CCh)-induced contraction of bladder strips from STZ diabetic mice. In this aim, we induced diabetes using STZ in wild type and TLR4 knockout mice and examined the contractile function of the bladder. After 4 weeks of treatment, the body weight, glycemia and overall health status of TLR4 knockout mice upon STZ treatment was not different from those of wild type mice. STZ induced an increase in bladder weight in the wild type mice, which was slightly attenuated in TLR4 knockout mice (figure 2), suggesting that TLR4 may contribute to diabetic bladder hypertrophy.

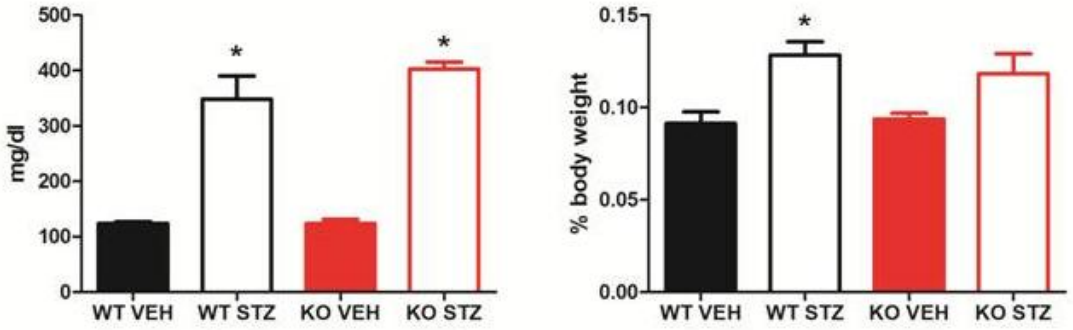

Fig. 2. Glycemia levels after 4 weeks of STZ-induced diabetes development in wild type (black) and TLR4 knockout (red) mice (left). Relative bladder weight of control and STZ-induced diabetic wild type and TLR4 knockout mice (right).

Urothelium-denuded bladder strips from STZ diabetic wild type mice displayed increased contractile responses to electrical field stimulation (EFS) and CCh, compared to their non-diabetic counterparts. This increase in CCh and EFS-induced contractile responses was normalized in urothelium-denuded bladder strips from TLR4 knockout mice (figure 3), suggesting that TLR4 mediates hypercontractility characteristic of overactive bladder dysfunction, the initial stage of diabetic bladder dysfunction.

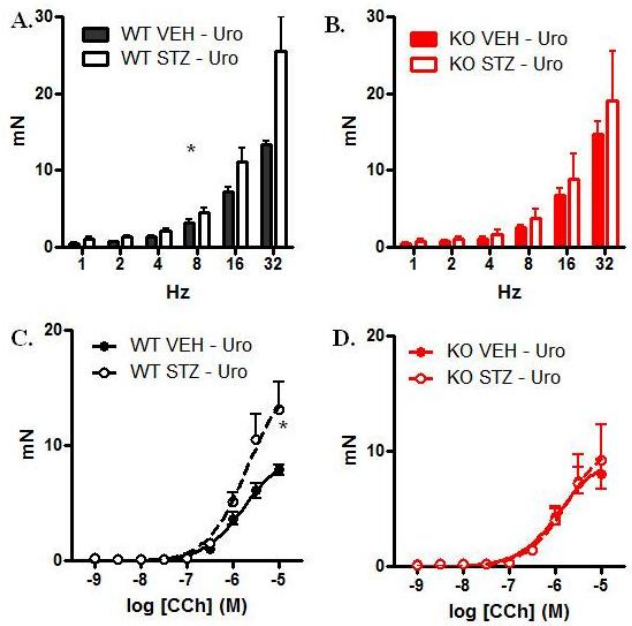

Fig. 3. Electrical field stimulation (EFS, A-B) and carbacholine (CCh, C-D)-induced contractile responses in urothelium-denuded bladder strips from vehicle (full symbols) and STZ (empty symbol)-treated wild type (black, A, C) and TLR4 knockout (red, B, D) mice.

3. Publications:

Szasz T, Nunes KP, Webb RC. Activation of Toll-like receptor 4 mediates bladder hypercontractility in diabetes. *FASEB J* April 2014 28:865.10 (abstract, Experimental Biology 2014).