Diabetic Complications Consortium

Application Title: Toll-like receptor 4 mediates diabetic bladder dysfunction.
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1. Project Accomplishments:

In this project we investigated the role of TLR4 activation in diabetic bladder dysfunction pathogenesis in a mouse model of type 1 diabetes. Our hypothesis was that in diabetes, hyperglycemia and high mobility group box 1 (HMGB1) activate TLR4 in the bladder, leading to bladder smooth muscle hypertrophy and hypercontractility and mediating DBD. We used wild type and TLR4 knock-out mice either in basal conditions or following treatment with streptozotocin (STZ). Our results indicate that genetic lack of TLR4 expression protects mice from diabetic bladder dysfunction, manifested as bladder hypertrophy and hypercontractility, despite not protecting them from STZ-induced hyperglycemia. Our data also suggest that HMGB1 mediates bladder smooth muscle TLR4 activation in STZ-induced diabetes.

2. Specific Aims:

Aim 1 to test the hypothesis that HMGB1 release from dying cells during diabetes leads to DBD.

We observed that circulating HMGB1 levels are higher in STZ diabetic compared to control normoglycemic mice. When incubated ex vivo with urothelium-denunded mouse bladder strips, recombinant HMGB1 increased bladder TLR4 and MyD88 expression and enhanced contractile response to electrical field stimulation (figure 1).

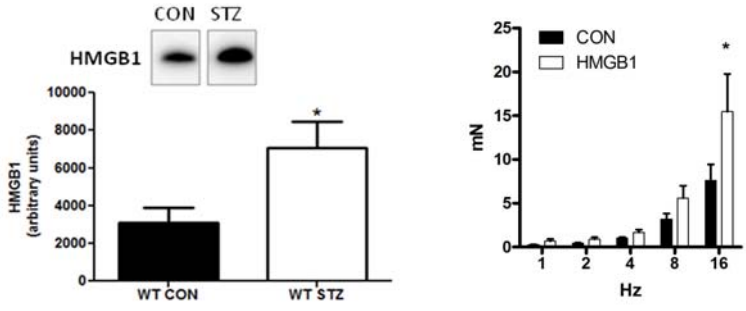

Figure 1: HMGB1 is increased in serum from STZ diabetic mice (left). HMGB1 incubation enhances contractile responses to electrical field stimulation (right).

In bladder from STZ diabetic mice, we observed increased expression of proteins downstream of TLR4 activation, including TLR4 and MyD88 (as presented in the preliminary data for this projet), as well as TRIF, IRAK and p65 (figure 2).

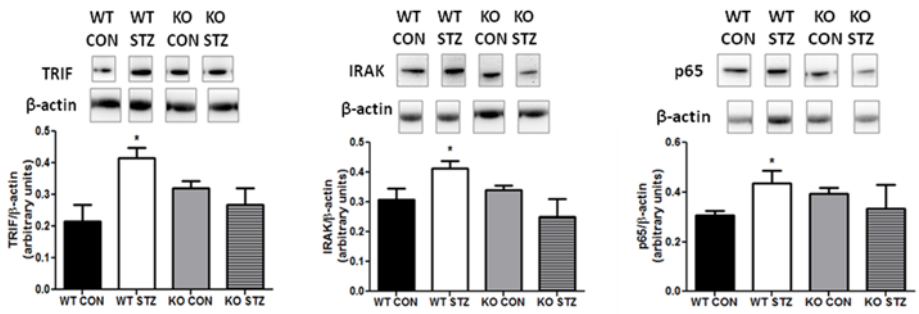

Figure 2: Expression of TRIF, IRAK and p65 is increased in bladders from STZ-induced diabetic compared to control wild type mice, while no significant change is observed in bladders from TLR4 knock-out mice.

Aim 2 to test the hypothesis that HMGB1-induced DBD is mediated by TLR4.

Immunohistochemistry confirmed expression of TLR4 in cross-sections of human bladder (figure 3). TLR4 was also expressed in the wild type mouse bladder, and TLR4 protein expression was absent from the TLR4 knock-out mouse bladder.

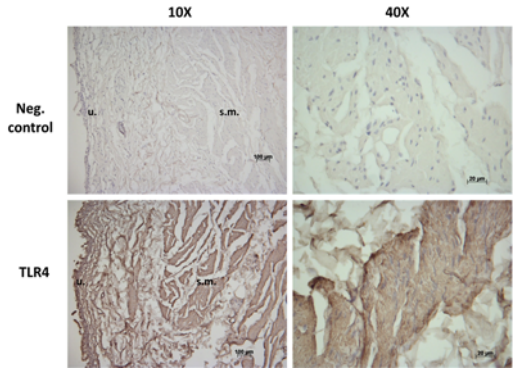

Figure 3: Expression of TLR4 was observed by immunohistochemistry in cross-sections of human bladder (both in the urothelium, marked as "u.", and in the smooth muscle, marked as "s.m.").

We observed increased bladder weight, as well as increased bladder wall thickness and smooth muscle layer thickness, in the wild type STZ diabetic mice, while this bladder hypertrophy was attenuated in TLR4KO mice (Figure 4).

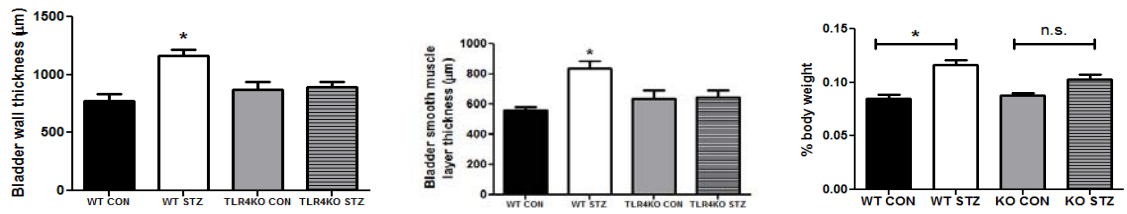

Figure 4: Bladder wall thickness (left), smooth muscle layer thickness (middle) and bladder weight (right) were increased in STZ diabetic compared to control wild type mice, however TLR4 knock-out mice were protected from bladder hypertrophy.

Genetic deletion of TLR4 also protected mice from increased bladder contraction to muscarinic agonists and to electrical field stimulation.

Our data suggest that HMGB1-induced TLR4 activation during diabetes mediates DBD-associated bladder hypertrophy and hypercontractility.

3. Publications:

Szasz T, Wenceslau CF, Burgess B, Nunes KP, Webb RC. Toll-Like Receptor 4 Activation Contributes to Diabetic Bladder Dysfunction in a Murine Model of Type I Diabetes. *Diabetes.* 2016 Sep 20. pii: db160480. [Epub ahead of print]