Diabetes Damages Urothelial Barrier Function in Mice via NLRP3-Dependent Mechanisms

Michael R. Odom, Francis M. Hughes Jr., Huixia Jin, J. Todd Purves

Division of Urology
Department of Surgery
Duke University Medical Center
Durham, North Carolina

Michael R. Odom
Michael.R.Odom@duke.edu

Introduction

Diabetic bladder dysfunction (DBD) is a progressive deterioration of urinary function common among diabetic patients. It has been proposed that inflammation is a major culprit underlying this complication. Bladder urothelium normally maintains an impermeable barrier to protect underlying tissues. However, during diabetes, barrier function is compromised, exposing the underlying tissues to pro-inflammatory diabetic metabolites in the urine that are proposed catalysts of further inflammation. We have previously shown that activation of the NLRP3 inflammasome is responsible for inflammation and DBD in the Akita type 1 diabetic mouse model. In this study, we hypothesize NLRP3 activation during diabetes mediates a loss of urothelial barrier function.

Methods

Genetically Modified Diabetic Mouse Model
- Crossbred type 1 diabetic Akita mice with NLRP3 null mice
- Animal groups:
  - Non-diabetic NLRP3+/+
  - Diabetic NLRP3+/+
  - Non-diabetic NLRP3-/-
  - Diabetic NLRP3-/-
- Animals aged to 15 and 30 weeks - time points which diabetic mice demonstrate detrusor overactivity and underactivity, respectively

In Vivo Barrier Permeability Assay
- 150 μl of PBS containing biotin (1 mg/ml) was intravesically instilled to 30 mice
- Biotin was removed and bladders were then fixed in 4% paraformaldehyde before being processed for histology
- Sections were stained with zoon occludin 1 to label urothelial barrier. Green and streptavidin conjugated to Texas red fluorescent to identify biotin
- The urothelial circumference was calculated and regions containing biotin in submucosa were measured

Ex Vivo Barrier Permeability Assay
- Detrusors were removed from intact urothelial layers
- Urothelial "balloons" were cannulated with flexible catheters and tied proximal to ureters
- Preps were maintained in aerated (95% oxygen / 5% CO2) Krebs solution at 37 degrees
- Evans blue was intravesically administered at a rate of 15 μl/min for 10 minutes.
- 30 minutes after infusion was complete, the amount of Evans blue which permeated into the bathing solution was measured.

Results

At 15 weeks, in vivo barrier function is damaged in diabetic mice via NLRP3-dependent mechanisms

At 30 weeks, diabetic mice no longer demonstrate any changes in barrier function in vivo

At 15 weeks, barrier expression is downregulated in diabetic mice but preserved in diabetic mice without NLRP3

Conclusions

Type 1 diabetic Akita mice demonstrate a temporary period of decreased barrier function associated with detrusor overactivity, but not at the later detrusor underactivity phase. Genetic ablation of NLRP3 protects diabetic mice from both degradation of the urothelial barrier and urinary voiding dysfunction, supporting the causative role of NLRP3 in mediating DBD.

Future Directions

Urothelial barrier damage permits harsh diabetic metabolites found in urine to pass through urothelium and come in direct contact with bladder smooth muscle, thereby exacerbating inflammation. The next steps will be to determine how NLRP3 impacts diabetic bladder smooth muscle function. Emphasis will be placed on elucidating NLRP3-dependent signaling pathways dysregulated in the progression of DBD.

Gene Expression

- Urothelium from each group were excised
- Used qPCR to measure gene expression of:
  - Tight junctions:
    - Zona occludin 1 (ZO1)
    - Claudin 4 (CL4)
    - Claudin 8 (CL8)
  - Adherin junctions:
    - Beta Catentin (BCT)
  - Uroplakins:
    - Uroplakin 1 (UP1)
    - Uroplakin 2 (UP2)

By 30 weeks, no changes in ex vivo barrier function are evident in diabetic mice

Figure 1: Upper panels: Arrows indicate regions where biotin has permeated through urothelium and into the submucosa. Asterisks show loss of apical urothelial cell. Lower graph: At 15 weeks, in vivo barrier function is damaged in diabetic mice. Conversely, without functional NLRP3, diabetic mice demonstrate no difference in barrier function compared to non-diabetics. Data are mean±SEM. *p<0.05, **p<0.01 vs Non-diabetic; n=3-5.

Figure 2: Upper panels: Arrows indicate regions where biotin has permeated through urothelial layers and into the submucosa. Lower graph: In 30 week diabetic mice, in vivo barrier function is comparable among all groups. Data are mean±SEM. n=3-6/group.

Figure 3: In 15 week diabetic mice, ex vivo barrier function is damaged in diabetic mice, but not in diabetics lacking NLRP3. Data are mean±SEM. *p<0.05, **p<0.01 vs Non-diabetic; n=4-6/group.

Figure 4: In 30 week mice, ex vivo barrier function is restored in diabetic mice compared to all groups. Data are mean±SEM. n=4-6/group.

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