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 urothelia normally maintains an impenetrable barrier to protect underlying tissues. However, during diabetes, barrier function is compromised, exposing the underlying tissue 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***. To investigate this hypothesis, we crossbred Akita diabetic mice with NLRP3 null mice and evaluated barrier function at two time points which we have previously shown are characterized by detrusor overactivity and detrusor underactivity.

Methods

Genetically Modified Diabetic Mouse Model

- Crossbred type I diabetic Akita mice with NLRP3 null mice
- Animal groups:
  - Non-diabetic NLRP3+/+
  - Diabetic NLRP3+/+
  - Non-diabetic NLRP3-/-
  - Diabetic NLRP3-/-

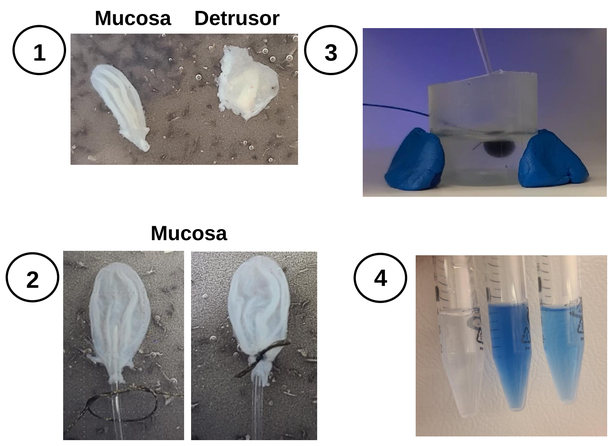
- Animals aged to **15 and 30 weeks** – time points which diabetic mice demonstrate detrusor **overactivity** and **underactivity**, respectively

In Vivo Barrier Permeability Assay

- 150 µl of PBS containing biotin (1 mg/ml) was intravesically incubated for 30 minutes
- Biotin was removed and bladders were then fixed in 4% paraformaldehyde before being processed for histology
- Sections were stained with zona occludin 1 to label urothelial cells (green) and streptavidin conjugated to texas red fluorophores 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
- Urothelia "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 solution 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.



Gene Expression

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

Results

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

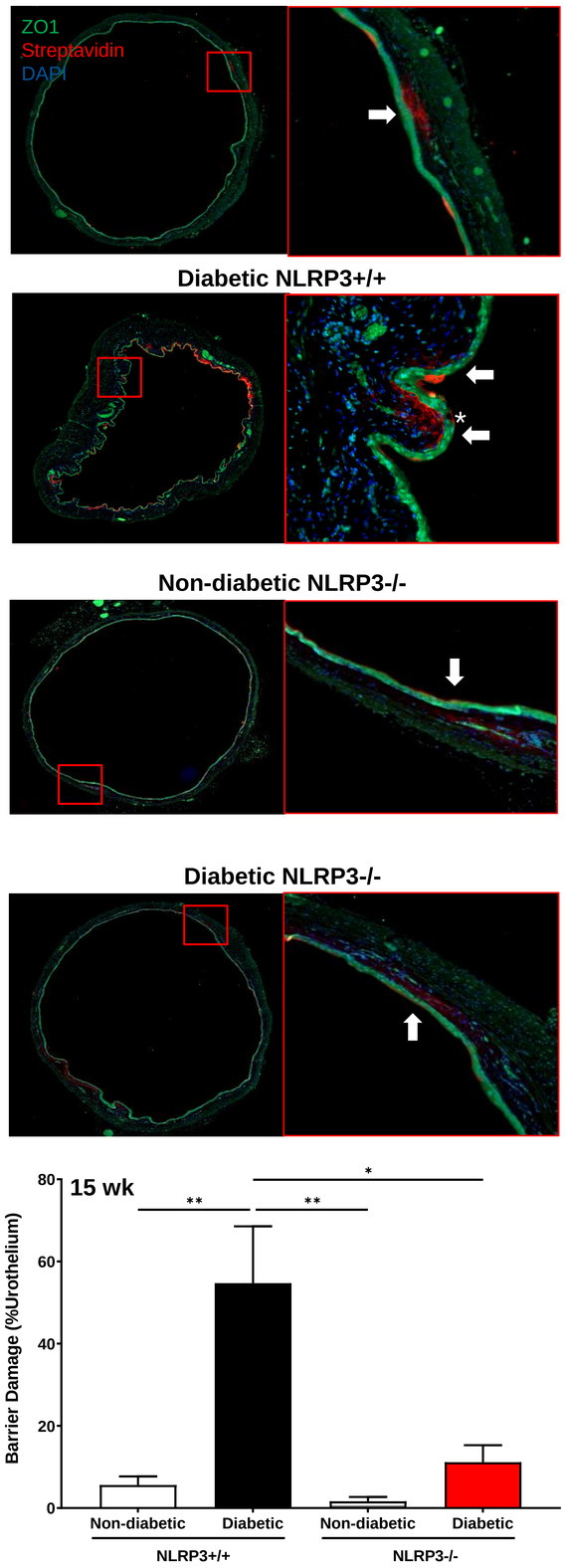


Figure 1: Upper panels: Arrows indicate regions where biotin has permeated through urothelia and into the submucosa. Asterisks show loss of apical urothelial cell. **Lower graph:** At 15 weeks, *in vivo* barrier function is damaged in diabetic mice. Conversely, without functional NLRP3, diabetics demonstrate 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.

At 15 weeks, *ex vivo* barrier permeability in diabetic mice is increased via NLRP3-dependent mechanisms

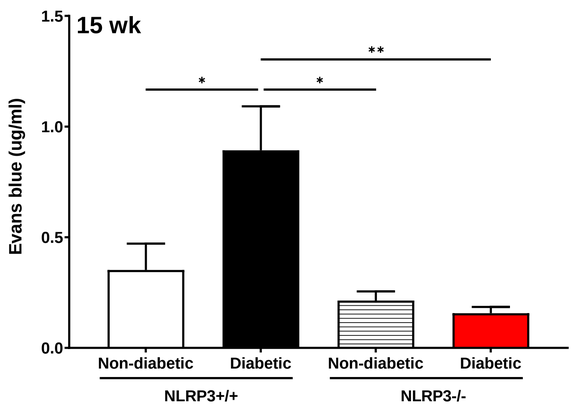


Figure 3: In 15 week diabetic mice, *ex vivo* barrier function is damaged in diabetic mice, but not in diabetics lacking NLRP3. Data are mean±SEM. *p<0.05, **p<0.01 vs Non-diabetic; n=4-8/grp.

Results

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

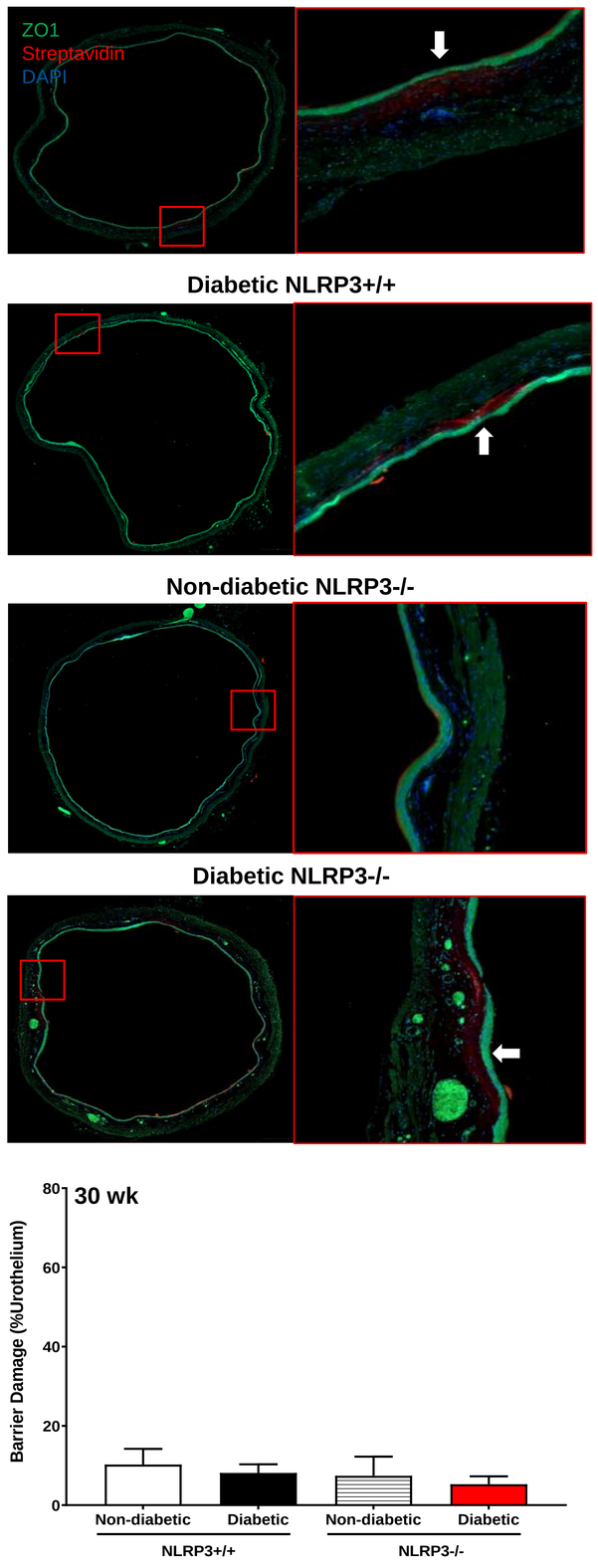


Figure 2: Upper panels: Arrows indicate regions where biotin has permeated through the urothelial layers and into the submucosa. **Lower graph:** In 30 week diabetic mice, *in vivo* barrier function is comparable among all groups. Data are mean±SEM. n=3-5/grp.

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

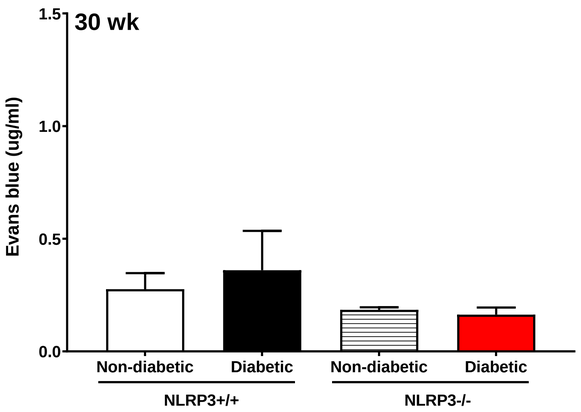


Figure 4: In 30 week mice, *ex vivo* barrier function is restored in diabetics compared to all groups. Data are mean±SEM; n=4-8/grp.

Results

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

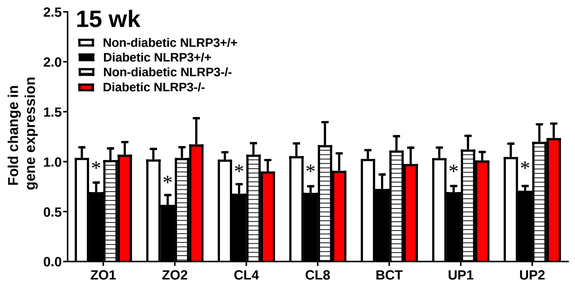


Figure 5: In 15 week diabetic mice, barrier genes ZO1, ZO2, CL4, CL8 and UP1 are downregulated. Diabetic mice without functional NLRP3 demonstrate no changes in gene expression compared to non-diabetic mice and non-diabetic mice with functional NLRP3. Data are mean±SEM. *p<0.05 vs Non-diabetic; n=6-12/grp.

30 week diabetic mice exhibit either no change or increases in barrier gene expression

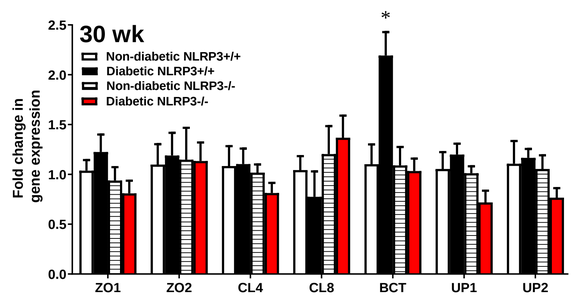


Figure 6: In 30 week diabetic mice, BCT gene expression is upregulated while all other gene expression is comparable to non-diabetic controls and diabetic mice without functional NLRP3. Data are mean±SEM. *p<0.05 vs Non-diabetic; n=6-12/grp.

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 urothelia 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.

Funding

- NIH K12-DK100024: PI: Cindy Amundsen.
- NIH RO1-DK117890 PI: J. Todd Purves

Acknowledgements

Duke University Urinary Dysfunction Lab

- J. Todd Purves
- Francis "Monty" Hughes, Jr.
- Huixia Jin
- Michael R. Odom
- Anissa Cervantes
- Margaret Lim
- Adam Lin
- Jack Mulcrone

NIH-NIDDK
CAIRIBU

Department of Surgery
Duke University School of Medicine