Toll-like receptor 2 signaling contributes to cerebrovascular dysfunction and decreased cerebral blood flow in diabetes mellitus

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Conflicts of interest

- Conflicts of Interest: None to Disclose
Hypertension causes and risk factors

• Chronic Kidney Disease
• Age
• **Diabetes Mellitus**
• Elevated LDL
• Obesity
• Cardiovascular disease
• Renovascular disease
• Cushing's Syndrome (glucocorticoid excess)
• Primary aldosteronism
• Renovascular disease
• Sleep Apnea
• Thyroid/Parathyroid Disease
• Coarctation of the aorta
• Drug induced
• Microalbuminuria
As of 2012, there were 29.1 million Americans living with diabetes.

Macrovascular and microvascular changes lead to hypertension, stroke, and cognitive impairment.

- **Diabetes**
  - Resistance arteries & arterioles
  - Chronic moderate hyperglycemia
  - $\uparrow$ Vascular tone, $\downarrow$ CBF
Diabetes and cerebrovascular dysfunction

Data from our lab has shown vascular dysfunction, decreased cerebral blood flow, and cognitive impairment in diabetic GK rats.

*Harris et al., American Journal of Physiology, 2007

Kelly-Cobbs et al., Journal of Pharmacology and Experimental Therapeutics, 2012

Prakash, R. et al. Stroke, 2013

Statistical significance:

- *p ≤ 0.05 vs. Control

Mean ± SEM, n=6-8
Diabetic microvascular dysfunction

Diabetes

→ Microvascular dysfunction

→ Hypoperfusion/hypoxia

→ Neuro/inflammation

→ White matter damage
  BBB disruption

→ Cognitive impairment
What role does inflammation play in the development of diabetic cerebrovascular dysfunction?
The effect of inflammation on the pathological development of diabetic cerebrovascular disease has implicated a role of TLRs.

Propagation of signaling response leading to activation of NFκB and IFN-β → release of pro-inflammatory cytokines and chemokines.
Innate immune system, TLRs, and diabetes

- TLRs 2 and 4 involved in pathogenesis of cardiovascular disease and diabetes


Hypothesis

Diabetes

Microvascular dysfunction

Hypoperfusion/hypoxia

Neuro/inflammation

Cerebrovascular Dysfunction

↓ Cerebral Blood Flow

White matter damage

BBB disruption

Cognitive impairment

TLR2?
Experimental design

1. KCl max response
2. Endothelial integrity
3. 40’ incubation with antiTLR2 or vehicle
4. Serotonin dose response curve
5. Acetylcholine dose response curve
6. Repeat incubation
7. Endothelin-1 dose response curve
Anti-TLR2 treatment increases Ach-induced relaxation in GK basilar arteries

A) % Relaxation vs Acetylcholine Log [M]

- GK
- GK+antiTLR2

B) Total Relaxation

ACh Relaxation

C) Sensitivity

EC50 (nM concentration)

* = p ≤ 0.05 vs GK, n= 6/group
Anti-TLR2 treatment does not affect vascular contraction to serotonin or ET-1 in GK basilar arteries.
TLR2 downstream signaling is upregulated in GKs

Brain MVEC

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>GK</th>
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<tbody>
<tr>
<td>TLR2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>92 kDa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-actin</td>
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<td></td>
</tr>
<tr>
<td>MyD88</td>
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</tr>
<tr>
<td>33 kDa</td>
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<td></td>
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<tr>
<td>B-actin</td>
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Cerebral arteries

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>GK</th>
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<tbody>
<tr>
<td>pIRAK4</td>
<td></td>
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<tr>
<td>IRAK4</td>
<td></td>
<td></td>
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<tr>
<td>β-actin</td>
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Fold of control/Beta-actin

* = p ≤ 0.05 vs Wistar, n= 4/group
What is the effect of TLR2 signaling on cerebral blood flow in diabetes?
**TLR2 knockout in STZ-induced diabetes**

<table>
<thead>
<tr>
<th>5 days</th>
<th>28 Day Diabetic Period</th>
<th>CBF Measurement</th>
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<tbody>
<tr>
<td>STZ injection i.p., 50 mg/kg/day</td>
<td>Blood Glucose Measurement 1x/week</td>
<td></td>
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- 10 week-old male C57Bl:6 and TLR2 knockout mice from Jackson Labs
- Streptozotocin (STZ)-induced diabetes
- Follow for 4 weeks post induction of diabetes and assess CBF using high resolution Laser Speckle Contrast Imaging (LSCI; Perimed)
TLR2 knockout in STZ-induced diabetes

Graph showing blood glucose levels over weeks for WT and TLR2 KO STZ.
TLR2 knockout attenuates decreased cerebral blood flow in Type 1 diabetes

* = p≤ 0.05 vs. wild type
# = p≤ 0.05 vs. wild type STZ, n=4-6/group
Conclusions

• TLR2 signaling is involved in vascular dysfunction in both type 1 and type 2 diabetes.
• TLR2 likely contributes to decreased vascular function through an endothelium dependent mechanism.
• TLR2 knockout protects against decreased cerebral blood flow in diabetes.
Future directions

• The role of TLR2 in the development of cognitive impairment in diabetes using knockout mice.

• Role of glycemic control in the prevention of TLR2-mediated cerebrovascular dysfunction and cognitive impairment.
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