Innate Immune Dysfunction Results in Altered Gut Microbiota, Colitis and Metabolic Syndrome

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TLRs sense microbial products
(Bar Code Readers)

TLR2
MyD88
NF-κB
Cytosol

TLR4
MyD88
NF-κB
Nucleus

TLR5
MyD88
NF-κB

Peptidoglycan
LPS
Flagellin

Proinflammatory Gene Expression
TNFα, IL-1β, IL-6, IL-8
Gut Microbiota: A Neglected Organ in Organ Lumenal Contents (50% bacteria by wt.)

1. Large area (200 m² ≈ tennis court)
2. $10^{14}$ bacteria, ≈ 500-1000 distinct species
3. Bacterial genes: 1 million
   Human genes: 25,000
4. Functions comparable to Liver
5. Gut bacteria: Yours BFF: Best Frenemy Forever

Goal of Mucosal Immune System:
1. Expediently detect/clear pathogens
2. Keep opportunists in-check.
3. This must be done while avoiding harm to beneficial microbes and host tissues.
I. TLR5 deficient mice develop spontaneous colitis

II. TLR5 deficiency results in metabolic syndrome
Innate Immunity

Acute Inflammation

IL-8

S. typhimurium
Flagellin

TLR5

NF-kB

IBD?

Commensal microbes

Epithelial cell

Innate Immunity
Acute Inflammation

Gewirtz AT 2001 J Immunol

Does this happen in vivo in IBD?
T5KO mice do not exhibit innate immune responses to flagellin.

TLR5KO=T5KO
Goal: Does loss of TLR5 reduce inflammation in models of IBD?

Prediction: Colitis driven by “immune dysregulation” might be reduced by loss of TLR5
T5KO mice develop spontaneous colitis

WT
500/500

T5KO
52/500 (10%)

Serum Amyloid A marks severity of T5KO colitis

Colitis

Rectal Prolapse

** WTWT T5KOT5KO--LL T5KOT5KO--HH

Colon [g/100 g body wt]

Spleen [% body wt]

Serum Amyloid A marks severity of T5KO colitis
T5KO mice have an increased bacterial burden
T5KO mice have an increased bacterial invasion
Antibiotic treatment ameliorates T5KO colitis
Embryo transfer changes environment but not genetics

“Rederived offspring”
Acquires microbiota of birth (recipient) mother but not genetic mother
Rederivation via embryonic transplant ameliorates colitis in T5KO mice

Rectal Prolapse: 0%
Potential mechanism of T5KO colitis

Loss of TLR5 Function
  ↓
Failure to Manage Microbiota
  ↓
Increased Activation of Hemopoietic TLRs
  ↓
Increased Expression of TH₁ Cytokines
    (IL-23, IL-12, IFNγ, TNFα)
  ↓
Persistent Inflammation of the Gut and Colitis
  ↓
Rectal Prolapse
  ↓
Decreased Epithelial Barrier Function
  ↓
Dissemination of Bacteria
  ↓
Acute Phase Response (SAA, Lipocalin)
  ↓
Anemia, Splenomegaly, Leukocytosis
I. TLR5 deficient mice develop spontaneous colitis

II. TLR5 deficiency results in metabolic syndrome
T5KO mice are overweight

(20-22%)

Vijay-Kumar et al (2010) Science
WT Leptin-KO (Ob/Ob) (12 week old)

I’m not obese. I’m robust!

WT TLR5-KO (18 week old)
Michel Angelo’s David ..... David after short stay in USA.....

National Health and Nutrition Examination Study
By 2030, 86% US adults will be overweight/Obese

9.1% of Total Medical Expenditure in 2010 (168 Billions)

Wang et al Obesity 2008
Metabolic examination of T5KO mice

I. Gross Phenotype
1. Body Weight
2. Abdominal Fat Pad

II. Blood Chemistry
1. 15h Fasting Blood Glucose
2. Serum Triglycerides
3. Serum Cholesterol
4. Serum Insulin
5. Serum Leptin
6. Serum NPY

III. Functional Tests
1. Glucose Tolerance Test
2. Insulin Sensitivity Test
3. Food Intake
4. Stool Output
5. Blood Pressure

IV. Cecal Microbiota
1. 16s rRNA Sequencing
T5KO mice develop obesity

**Body Weight (g) vs. Week**
- **Males**
- **Females**

**Serum Cholesterol [mg/dL]**
- **WT**
- **T5KO**

**Blood Pressure [mmHg]**
- Systole
- Diastole

**Serum Triglycerides [mg/dL]**
- **WT**
- **T5KO**

**Fat Pad [g]**
- **Males**
- **Females**
T5KO exhibit hyperglycemia/insulin resistance

[Images of tissue samples comparing WT and T5KO islets with insulin stain]
Interventional examination of T5KO mice

I. Diet
High Fat Diet for 8 weeks

II. Calorie Intake
Calorie Restriction (12 weeks)

III. Microbiota Ablation
Ampicillin and Neomycin (12 weeks)

VI. Gnotobiotic T5KO mice
Studying MetS in germ-free T5KO in Comparison to germ-free WT mice

V. Microbiota Transfer
Transfer Cecal Microbiota to Germfree Mice
High fat diet aggravates metabolic syndrome in T5KO mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>T5KO</th>
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<tbody>
<tr>
<td>Fat Pad [g]</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Body Weight [g]</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>15h Fasting Glucose [mg/dL]</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>Serum Insulin [ng/mL]</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Serum Triglycerides [mg/dL]</td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>Serum Cholesterol [mg/dL]</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Serum Leptin [ng/mL]</td>
<td>353</td>
<td>500</td>
</tr>
</tbody>
</table>

Pancreas (Insulitis)
Liver (Steatosis)
T5KO mice exhibit hyperphagia

Food Intake [g/mouse/day]

WT  T5KO

Wet stool output [mg/h]

WT  T5KO

Dry stool output [mg/h]

WT  T5KO
Calorie restriction improves metabolic syndrome in T5KO mice

- **% Weight Gain**
  - Days: 0, 20, 40, 60
  - WT vs. T5KO

- **Serum Leptin [ng/mL]**
  - WT vs. T5KO

- **Fat Pad [g]**
  - Males: WT, T5KO
  - Females: WT, T5KO

- **Serum Cholesterol [mg/dL]**
  - WT vs. T5KO

- **15h Fasting Glucose [mg/dL]**
  - WT vs. T5KO

- **Blood Glucose [% starting value]**
  - Time: 0, 30, 60, 90, 120
  - WT, T5KO, + Insulin
T5KO colons have elevated innate immune gene expression

**Pattern Recognition Receptors**
- TLR4
- CD14
- LBP
- Lumican

**Stress proteins**
- HSP70
- HIF1α
- HSP12
- HSP1

**Antimicrobials**
- RegIIIγ
- SLPI
- iNOS
- IDO
- Lipocalins
- Defensins
- FcRn

**Cytokines**
- S100A9
- IL-1β
- TNFα
- IL-10
- IL-12
- CSF-1

**Acute phase proteins**
- Haptoglobin
- SAA
- Hepcidin
- Lipocalin

**ROS enzymes**
- NOX1
- NOX3
- NOA-1
- NOO-1
- MPO

**Antioxidant enzymes**
- GPx
- GST
- GR
- SOD

**Adhesion molecules**
- CEACAM-10
- CEACAM-13
- P-Selectin
- VCAM-1
- ICAM-2

Vijay-Kumar et al Infec. Immun. 2008
Does loss of TLR5 alter composition of the gut microbiota?

WT  T5KO

Extract cecal contents (n= 5 mice/genotype)

Subject to 454 sequencing of 16S rRNA (approx 2500 sequences/mouse)

UNIFRAC

Survey says...

Ruth Ley
Cornell Univ
T5KO mice exhibit taxonomical alterations in the gut microbiota

Enriched Phylotypes (in KO): 35
- Bacteroidetes: 18
- Firmicutes: 16
- Proteobacteria: 1

Depleted Phylotypes (in KO): 77
- Bacteroidetes: 51
- Firmicutes: 24
- Proteobacteria: 2

No major shift in Bacteroidetes and Firmicutes
Antibiotics Normalizes Gut Bacteria in WT and in T5KO mice

Antibiotics Normalizes Gut Bacteria in WT and in T5KO mice

Antibiotics: - + - +

Cecal Bacteria/g [x10^12]

WT

T5KO

Antibiotics: - + - +

Cecal Bacteria/g [x10^12]

WT

T5KO
Antibiotics prevent metabolic syndrome in T5KO mice

Food Intake [g/mouse/day]

Fat Pad [g]

15h Fasting Glucose [mg/dL]

Serum Cholesterol [mg/dL]

Weaning

Post-Abx
Germ free T5KO mice do not exhibit metabolic syndrome
T5KO microbiota is necessary and sufficient to transfer metabolic syndrome to WT germfree mice.

- **Food Intake (g/mouse/day)**: WT donor and T5KO donor showed a difference in food intake over time.
- **% Weight Gain**: WT donor and T5KO donor showed a consistent increase in weight gain over days post transplant.
- **15h Fasting Glucose (mg/dL)**: WT donor and T5KO donor showed differences in glucose levels.
- **Serum Insulin (ng/mL)**: WT donor and T5KO donor showed differences in serum insulin levels.
- **Blood Glucose (% starting value)**: WT and T5KO donor showed a decrease in blood glucose levels with insulin injection.
- **Colon Cytokine (pg/mL)**: WT and T5KO donor showed differences in colon cytokine levels.

* represents statistical significance.
Summary

Metabolic Syndrome in T5KO mice is

1. Hyperphagia

2. Aggravated by High fat diet

3. Treatable by Calorie Restriction and Antibiotics

4. Transferable to Germ-free mice by Cecal Microbota

5. GF-T5KO mice do not suffer from Metabolic Syndrome
## Relevance in humans?

About 1 in 250 humans lacks TLR5 function due to the “TLR5-stop” SNP (T.R. Hawn, Aderem and colleagues)

Karen Mohlke (UNC)

<table>
<thead>
<tr>
<th>Trait Penetrance (%)</th>
<th>TLR5-stop (null) Genotype</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WT (n=13,000)</td>
<td>Heterozygous (n=1400)</td>
<td>Homozygous (n=52)</td>
<td>P-value WT vs. Homozyg.</td>
</tr>
<tr>
<td>Hyperglycemia (FG&gt;110)</td>
<td>32.4</td>
<td>34.6</td>
<td>42.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Large waist (&gt;102 or 88 cm for M or F)</td>
<td>35.4</td>
<td>36.1</td>
<td>44.9</td>
<td>0.08</td>
</tr>
<tr>
<td>High TG (&gt;149)</td>
<td>36.1</td>
<td>38.1</td>
<td>38.0</td>
<td>0.39</td>
</tr>
<tr>
<td>Low HDL (&lt; 41 or 51 for M or F)</td>
<td>23.4</td>
<td>24.9</td>
<td>34.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypertension (&gt;130 or 85 for S or D)</td>
<td>76.2</td>
<td>78.7</td>
<td>84.0</td>
<td>0.09</td>
</tr>
</tbody>
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Proposed Mechanism

- Loss of TLR5
  - Altered host-microbiota interactions
    - IL-1β and other pro-inflammatory cytokines
      (Low-grade chronic or sub-clinical inflammation)
      - Insulin resistance ↔ Hyperphagia
        - Hyperglycemia
        - Hyperinsulinemia
        - Hyperinsulinemia
        - Hyperlipidemia, Hypertension
        - Diabesity
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