Commensal Bacteria, Toll-like Receptors and Intestinal Injury

Journal Club
December 16, 2004
Gut-Commensal Interactions

- Nutrient metabolism
- Tissue development
- Resistance to colonization with pathogens
- Maintenance of intestinal “homeostasis”
Gut-Commensal Interactions

• Healthy gut epithelium tolerates/sequesters commensals
  – Inflammatory response not triggered
• Inappropriate activation of immune system by commensals may lead to chronic inflammation and injury

How does the gut immune system distinguish between commensal and pathogenic bacteria?
Toll-like Receptors (TLRs)

- Cell surface receptors
  - On intestinal epithelial and resident immune cells in LP
  - Recognize specific conserved microbial molecules
  - Ligands produced by BOTH pathogens and commensals
- Critical for activation of innate inflammatory/immune responses
## Major TLRs and Ligands

<table>
<thead>
<tr>
<th>TLR</th>
<th>Ligand</th>
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<tbody>
<tr>
<td>TLR1</td>
<td>Tri-acyl lipopeptides</td>
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<tr>
<td>TLR2</td>
<td>LTA, peptidoglycans</td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA (viral)</td>
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<tr>
<td>TLR4</td>
<td>LPS</td>
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<tr>
<td>TLR5</td>
<td>Flagellin</td>
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<tr>
<td>TLR6</td>
<td>Di-acyl lipopeptides</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG DNA motifs</td>
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Commensal-TLR Interactions

• Gut epithelial cells are in constant contact with commensal microbes
• These cells have in response to repeated or prolonged exposure to commensal ligands
  – reduced responsiveness to TLR signaling\(^1\)
  – decreased TLR expression on cell surface
  – increased expression of a TLR signal inhibitor (Tollip)\(^2\)


**HYPOTHESIS**: Unresponsiveness to TLR signaling confers protection against inflammatory injury on gut epithelium *in vivo*
Methods

- Wild type mice
- MyD88-/-
- TLR4-/-
- TLR2-/-

- Oral dextran sulfate sodium (DSS) to induce colonic injury
- Deplete mice of intestinal microflora
- Reintroduce commensal-derived products

Key adaptor molecule for TLR signaling
TLR signaling protects against mortality and morbidity induced by colonic epithelial injury
Higher mortality in MyD88-deficient mice was due to severe colonic hemorrhage and anemia.
More epithelial injury in MyD88-/- mice

More severe erosions in MyD88-/- mice

NO differences in WBC infiltration
Gut Epithelial Protection from Injury

- Balance of cell proliferation and differentiation in the crypts
- Repopulation of the crypts after injury
- Production of cytoprotective mediators
Dysregulated proliferation and differentiation of gut epithelium in absence of TLR signaling

More sensitive to radiation injury

Deficient crypt repopulation following radiation injury

More proliferating cells per crypt at baseline

More cycling cells

Cells proliferating in middle and upper regions of crypt
TLR signaling regulates the expression of tissue-protective cytokines and Heat shock proteins.
Commensal microflora are required for protection from mortality due to colonic epithelial injury.

Colonic stool cultures: selective depletion of commensals by abx admin.

Depletion of all commensals: increased mortality.
- Commensal-depleted wt mice had mortality, greater weight loss, and colonic bleeding in response to DSS.
- Reintroduction of commensal-derived TLR ligands rescued them from this.

The rescue effect is specific to TLR and its ligand.
TLR ligands and signaling are crucial for the intestinal surface to **protect and repair** itself in the face of infectious or inflammatory insult.

Implications

• LPS and flagellin can have both beneficial and deleterious effects, depending on gut barrier function and mucosal immune response
• Surface inflammation is the exception rather than the rule
• Gut epithelium and immune system do not simply tolerate commensal microflora but are dependent on them
TLR signaling

Protection
Repair
HEALTH

Inflammation
Injury
DISEASE

Commensals

Pathogens

Epithelial integrity
Immune tolerance
Probiotics
Prebiotics
Judicious ABX

Genetic predispo.
Permeability defect
Ischemia
Surgery
Chemo/Radiation

Broad-spectrum ABX

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