Intestinal integrity in sepsis – cellular and molecular insights

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Conflicts

• My laboratory is funded by the NIH
The healthy gut

• An epithelium with a surface area of 30-300 square meters
  – Somewhere between half the size of a badminton court and a tennis court

• An adaptive immune system
  – More lymphocytes in the gut than any other place in the body

• A microbiome
  – More than 100 trillion microorganisms – more cells than the host has
The Gut in Critical Illness

- Plays a central role in the pathophysiology of critical illness
- Is often referred to as “the motor” of the systemic inflammatory response syndrome
- Our understanding of how (or if) the gut serves as the motor of MODS has evolved over the past 30 years
The gut epithelium

villus

Crypt of Lieberkühn
The gut epithelium

villus
Crypt of Lieberkühn
transit cells
stem cells
Paneth cells
goblet cells
enteroendocrine cells
enterocytes
Proliferation
Differentiation

Crypt of Lieberkühn
transit cells
stem cells
Paneth cells
The gut epithelium

- villus
- Crypt of Lieberkühn
- transit cells
- stem cells
- Paneth cells
- goblet cells
- enteroendocrine cells
- enterocytes

Proliferation → Differentiation → Exfoliation → Death
The gut epithelium

- Villus
- Crypt of Lieberkühn
- Transit cells
- Stem cells
- Paneth cells
- Goblet cells
- Enteroendocrine cells
- Enterocytes

- Proliferation
- Differentiation
- Exfoliation
- Death
- More death
Sepsis-induced apoptosis

- Preferentially increased in mouse models and human autopsy studies
  - Gut epithelium
  - Lymphocytes
  - Dendritic cells
CLP – Intestinal Epithelium

Hotchkiss et al Crit Care Med, 1997
Gut apoptosis in pneumonia

Sham  P. aeruginosa  S. pneumoniae
Human Autopsy Study

- Patient groups
  - 20 critically ill patients with sepsis and MODS
  - 16 critically ill patients who did not have sepsis
- Increased intestinal epithelial apoptosis in septic patients
- Low level apoptosis in only a single non-septic patient
Impact?

- CLP, pneumonia, and human autopsy data are descriptive in nature.
- However, gut epithelial apoptosis could be
  - Detrimental
  - Beneficial
  - Epiphenomenon
Preventing gut apoptosis

- Transgenic mice that overexpress the anti-apoptotic protein Bcl-2
- Transgene expressed only in the gut
  - all 4 cell lineages of small intestine along crypt-villus axis
  - in proximal colon
- Normal intestinal apoptosis, morphology, size under basal conditions

Coopersmith et al., AJP, 1999
Transgene Expression

Wild type

Bcl-2 Transgenic
CLP

Wild type

Bcl-2 Transgenic

Coopersmith et al., Crit Care Med, 2002
Survival -- CLP?

- Sepsis of intraabdominal origin
  - Bcl-2 overexpressors (n=23)
  - Wild type littermates (n=27)
- Ceftriaxone and metronidazole
  - q12h x 2 days after CLP

Coopersmith et al Crit Care Med, 2002
Survival -- CLP?

**p<0.005
Survival – *P. aeruginosa* pneumonia?

- Sepsis of extraabdominal origin
  - Bcl-2 overexpressors (n=25)
  - Wild type littermates (n=26)
- Animals followed 7 days for survival

Coopersmith et al. JAMA, 2002
Survival – *P. aeruginosa* pneumonia?

**p<0.005**
However, we cannot make a transgenic person
Is there any way to clinically impact gut integrity in sepsis?
Epidermal Growth Factor (EGF)

- Potent cytoprotective peptide that exhibits trophic and healing effects on the intestinal mucosa.
- Involved with regulation of cell proliferation and survival.
What is the role of systemic EGF in sepsis?
Study Design

- Using mice, the cecum was ligated and punctured twice with a 23-gauge needle
- Treated immediately following surgery with or without EGF (150 mg/kg/day)
  - Given every 12 hr
  - Intraperitoneal injection
- Antibiotic therapy initiated following surgery with a second dose given 12 hr later
- Mice sacrificed 24 hr after surgery
Villus Length

A)

B)

100 µm

Villus Length (µm)

Sham  Septic  Septic+EGF

* p<0.001 vs. Sham and Septic+EGF
# p<0.05 vs. Sham
Intestinal Proliferation

![Bar graph showing intestinal proliferation in different conditions.](image)

- **Sham**
- **Septic**
- **Septic+EGF**

* `p<0.001` vs. Sham and Septic+EGF
Intestinal Epithelial Apoptosis

**Active Caspase 3**

![Graph showing apoptotic cells/100 crypts for Sham, Septic, and Septic+EGF groups.](image)

* p<0.001 vs. Sham and Septic+EGF

**H&E**

![Graph showing apoptotic cells/100 crypts stained with H&E for Sham, Septic, and Septic+EGF groups.](image)

* p<0.001 vs. Sham and Septic+EGF
What is the Functional Significance of Giving EGF to Septic Mice?

- **Survival studies:**
  - Treated with or without EGF (150 ug/kg/day)
    - Given every 12 hr via i.p. injection for 7 days
  - Antibiotic therapy initiated after surgery and continued for 2 days
  - Followed for survival for 7 days
Survival?

![Graph showing survival rates over time for different groups: Sham (n=4), Sham+EGF (n=4), Septic (n=20), Septic+EGF (n=20). The graph indicates that Septic+EGF has a higher survival rate compared to the other groups.](image)

* p<0.05
Is the effect of exogenous EGF model dependent?

• Mice were given *Pseudomonas aeruginosa* pneumonia via intratracheal injection
• Treated immediately following surgery with or without EGF (150 ug/kg/day)
  – Given every 12 hr
• Antibiotic therapy
• Mice followed for survival
Survival

![Graph showing survival rates for different groups over days]

- Sham (n=4)
- Sham+EGF (n=4)
- Septic (n=20)
- Septic+EGF, 0hr (n=20)
- Septic+EGF, 24hr (n=22)

Annotations:
- *p<0.001
- p<0.05
Is the gut responsible for the improvement in survival with EGF or is it affected indirectly?

- EGF improves survival in sepsis and improves intestinal integrity
- However, EGF also acts on a wide variety of cell types, and EGF-R is present in multiple tissues and organs, including vascular smooth muscle, liver, and kidney.
- Clinicaltrials.gov currently lists 762 trials aimed at EGF or EGF-R on 8/21/15. The majority of these do NOT target the intestine.
To answer this question

• Similar studies were done as outlined above using IFABP-EGF mice that have enterocyte-specific overexpression of EGF.
• If gut-specific EGF induces similar outcomes as systemic EGF, the intestine is sufficient (and possibly necessary) to drive this response.
What is the Functional Significance of Overexpressing EGF in the Intestine in Septic Mice?

• Survival studies:
  – IFABP-EGF and WT mice
  – Antibiotic therapy initiated after surgery and continued for 2 days
  – Followed for survival for 7 days
Survival

- WT Sham (n=4)
- IF-EGF Sham (n=4)
- WT Septic (n=25)
- IF-EGF Septic (n=17)

*p=0.0003
How else might alterations in gut integrity be responsible for alterations in survival?
The Intestinal Barrier

• Tight junctions (TJ) create a regulated barrier to the paracellular movement of water, solutes, and immune cells

• Inflammatory mediators can modulate barrier function
  – TJ expression and localization

• TJ are compromised in critical illness
  – Increased permeability
  – Persistent activation of systemic inflammation
Measuring Intestinal Permeability

*In Vivo* Assay to Measure Functional Changes in Permeability

- **0 hr**
  - CLP or Sham

- **19 hr**
  - Gavaged with FITC-dextran (FD-4)

- **24 hr**
  - Plasma collected
  - Concentration of FITC measured by fluorometry
Permeability following CLP
Tight Junction Proteins

• Occludin important for tight junction formation

• Claudins (~24 members) regulate “tightness” of tight junction
  – Claudin-3
  – Claudin-2: pore-forming

• JAM

• Perijunctional actin-myosin complex
Tight junction proteins

<table>
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<th>Tight Junction Proteins</th>
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<tr>
<td>Claudin-1</td>
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<tr>
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How early are alterations in intestinal tight junction detectable?
Tight junctions 1 hour after CLP

Claudin-2

Claudin-5

Occludin

JAM-A
What if we target permeability?
Myosin light chain kinase (MLCK)

Myosin light chain (MLC) phosphorylation is mediated by MLC kinase (MLCK). This is critical to the regulation of intestinal barrier function as it results in contraction of actin-myosin ring which, in turn, increases permeability.
Intestinal Permeability in WT mice

![Bar chart showing FD-4 (ug/ml) at various time points: Baseline, 6 hours, 12 hours, 24 hours, 48 hours. The chart indicates significant increases at 12 and 24 hours compared to baseline with asterisks (*) and double asterisks (**) indicating statistical significance.](image)
Intestinal Permeability in MLCK\(^{-/-}\) mice
Survival MLCK knockout

![Graph showing percent survival over days after CLP for MLCK -/- (n=18) and B6 (n=18) with P<0.0001]
The ones who actually do the work present and past
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