GROUP 5

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A. The Intestinal Epithelial Cell Barrier

B. IEC Tight Junctions

Modified from: Peterson & Artis, 2014. Intestinal epithelial cells: regulators of barrier function and immune homeostasis
Intestinal Barrier Function – Roles in GI Disease, Allergy, and other Diseases

1. What are the biggest research gaps?
   - a. What are the unanswered questions in relation to interactions between the microbiome and the barrier?
   - b. What are the limitations in translating in vitro and animal work to man?

2. What diseases/health conditions are associated with a compromised gut barriers?
   - a. In these disorders is gut barrier dysfunction a primary abnormality or a secondary phenomenon?

3. What novel techniques are being developed to measure or provide insight into gut barrier integrity?
   - a. Is the measurement of gut barrier function in man sufficiently robust and reproducible to detect defects in disease as well as the impact of therapeutic interventions?

4. Is the gut barrier a valid target for interventions that modify the microbiome?
Tissue barriers are required for survival of multicellular organisms


The intestinal epithelium provides a barrier between the external environment and the interior of the body.

The intercellular tight junction is the main determinant of epithelial barrier function.

Shen et al. Annu Rev Physiol (2011)

In vivo imaging of tight junction structure

Two distinct routes across the tight junction

- High capacity, high selectivity (charge and size): IL-13, claudin-2
- Low capacity, low selectivity: occludin, MLCK, TNF
Gut barrier

Obesity & metabolic syndrome

Alcohol dependence

Cancer cachexia

Probiotics

Prebiotics
Obesity & metabolic syndrome

Liver
- Lipogenesis
- Inflammation
- Oxidative stress
- Steatosis
- Insulin resistance

Fat
- Inflammation
- Macrophage infiltration
- Oxidative stress
- Insulin resistance

Muscle
- Inflammation
- Insulin resistance
Akkermansia muciniphila as a probiotic to improve the gut barrier function?

Everard et al, PNAS 2013
NAFLD AND MICROBIOTA

Intestinal microbiota determines development of NAFLD in mice

HFD 16 weeks

Non-Responder  Responder

Fecal transplantation

NR-Recipient  NRR  R-Recipient  RR

Germ Free mice

Responder: Hyperglycemia, high levels of pro-inflammatory cytokines

** NAFLD is transmissible through gut microbiota **

Le Roy et al. Gut. 2013
Gut microbiota from a patient with severe AH

Transplant

Germ Free Mice

Alcohol Feeding

Severe Liver Injury
Higher Disruption of mucosa

Clostridium bacteria

Gut microbiota from a patient with alcohol abuse and without AH

Less Liver Injury
Mild Disruption of mucosa

ALD AND MICROBIOTA

ALD is transmissible through microbiota
PROBIOTICS AND ALD IN ANIMAL STUDIES


- Alcohol + *Lactobacillus rhamnosus* GG-fed rats had less severe alcoholic steatohepatitis than alcohol-fed rats; LGG reduced alcohol-induced gut leakiness, oxidative stress and inflammation in both intestine and liver ([Forsyth et al., Alcohol, 2009](#)).

- In alcohol-fed rats, there was an “intestinal dysbiosis” that was not present in rats treated with probiotics (LGG) ([E. Mutlu et al., Alcohol Clin Exp Res 2009](#)).

- Probiotic supplementation attenuates alcohol-induced intestinal barrier dysfunction ([Wang et al., AJP 2011, AJP-GI&L 2013](#)).

- Probiotic supplementation positively modifies gut microbiome in ALD model ([Bull-Otterson L et al., PLoS One, 2013](#)).

- Probiotic supernatant is effective in preventing ALD ([Wang et al., AJP-GI&L, 2013, Zhang M. et al., J. Nutr Biochem 2014](#)).
METABOLOMICS APPROACH: BACTERIAL METABOLISM

Fecal Metabolomics

SCFA
LCFA
(Behatadecanoic acid)
BCAA

EtOH
EtOH+LGGs

Microbiota & the Blood Brain Barrier

Factors influencing barrier integrity

- Bile salts
- Nutriments (glutamin...)
- Allergens (Diet, gluten...)
- Enzymes
- Bacterial products (acetaldehyde, proteases, LPS...)
- Sepsis
- Stress
- Physical activity
- Inflammation (cytokines)
Genetic predisposition of epithelial barrier tightness

Intestinal permeability in Crohn’s disease first degree relatives without a CARD15 mutation (CD-R WT) and with 3020insC or 3020insC-R702W mutations (CD-R 3020insC)

Buhner S et al. Gut 2006
L:M test as a gold standard..

Dunlop, Am J Gastroenterol 2006
<table>
<thead>
<tr>
<th>Methods</th>
<th>In vivo</th>
<th>In vitro</th>
<th>Neuroimmunes functions</th>
<th>Junctions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACO-2/HT29</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ussing Chamber</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oral marker (L:M, CR\textsuperscript{51}-EDTA)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supernatants biopsies and faeces applied on cell line or tissus</td>
<td>+</td>
<td>-</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Immunohisto. Tight junction protein</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>mRNA expression of TJ protein</td>
<td>-</td>
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</tbody>
</table>
## Intestinal Permeability in IBS

### Table 2. Studies assessing intestinal permeability in IBS patients

<table>
<thead>
<tr>
<th>Reference</th>
<th>IBS Group(s)</th>
<th>Method of Assessment</th>
<th>Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiller et al., 2000 (113)</td>
<td>PI-IBS</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Marshall et al., 2004 (76)</td>
<td>PI-IBS</td>
<td>Sucrose/lactulose/mannitol excretion</td>
<td>Increased in 36% of pts</td>
</tr>
<tr>
<td>Dunlop et al., 2006 (37)</td>
<td>IBS-D (PI and nonPI)</td>
<td>( ^{51} \text{Cr-EDTA} ) excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Shulman et al., 2008 (110)</td>
<td>children with IBS or FAP</td>
<td>Lactulose/mannitol and sucralose/lactulose excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Gesce et al., 2008 (45)</td>
<td>all IBS subtypes</td>
<td>Fecal supernatants applied to murine colonic strips mounted in Ussing chambers; FITC-dextran transfer</td>
<td>Increased with IBS-D supernatants, no difference with IBS-C</td>
</tr>
<tr>
<td>Park et al., 2009 (89)</td>
<td>all IBS subtypes</td>
<td>PEG excretion</td>
<td>Increased in all IBS subtypes</td>
</tr>
<tr>
<td>Piche et al., 2009 (92)</td>
<td>all IBS subtypes</td>
<td>Colonic biopsies mounted in Ussing chambers; fluorescein-5.6 sulfonic acid</td>
<td>Increased in all IBS subtypes</td>
</tr>
<tr>
<td>Zhou et al., 2009 (134)</td>
<td>IBS-D</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased in 39% of patients</td>
</tr>
<tr>
<td>Kerckhoffs et al., 2010 (59)</td>
<td>all IBS subtypes</td>
<td>PEG excretion</td>
<td>No difference</td>
</tr>
<tr>
<td>Lee et al., 2010 (68)</td>
<td>IBS-D</td>
<td>Colonic biopsies mounted in Ussing chambers; horseradish peroxidase</td>
<td>Increased</td>
</tr>
<tr>
<td>Zhou et al., 2010 (133)</td>
<td>IBS-D</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased in 42% of patients</td>
</tr>
<tr>
<td>Rao et al., 2011 (99)</td>
<td>IBS-D</td>
<td>Lactulose/mannitol excretion</td>
<td>Increased</td>
</tr>
<tr>
<td>Gece et al., 2012 (46)</td>
<td>IBS-D and IBS-C</td>
<td>( ^{51} \text{Cr-EDTA} ) excretion</td>
<td>Decreased in small proximal small intestine of IBS-C patients; increased in colon of IBS-D patients</td>
</tr>
<tr>
<td>Vivinus-Nébot et al., 2012</td>
<td>all IBS subtypes</td>
<td>Colonic biopsies mounted in Ussing chambers; fluorescein-5.6 sulfonic acid</td>
<td>Increased in all IBS subtypes</td>
</tr>
</tbody>
</table>

Table from Rao et al. (100) updated. Permeability is compared with healthy controls. FAP, functional abdominal pain; FITC, fluorescein isothiocyanatelabeled; IBS, irritable bowel syndrome; D, diarrhea predominant; C, constipation predominant; PI, postinfectious; PEG, polyethylene glycol.

Increased intestinal permeability and miR-29 expression in humans

Dietary Fat (PUFA) and EtOH Altered Bacterial Diversity and Metabolic Activity

- **USF+EtOH-8 weeks**
- **USF+EtOH-6 weeks**
- **USF+EtOH-1 week**
- **SF+EtOH-8 weeks**
- **SF+EtOH-6 weeks**
- **SF+EtOH-1 week**

% Relative Abundance

![Bar charts showing relative abundance of bacterial groups across different conditions and time points.](chart)

- **Feces Octanoic Acid**
- **Feces Butyric Acid**

Kirpich et al., AJP. 2015; submitted
EtOH+USF Promoted Hepatic Steatosis, Inflammation and Injury

Kirpich et al., ACER. 2012; 36(5):835-46
NAFLD patients had increased intestinal permeability and altered tight junctions.
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  Microbial function - e.g. metabolomics

  Variability - probiotic cultures, microbial species, animal models, human studies

  Association vs causation
2. What diseases/health conditions are associated with a compromised gut barriers?
   a. In these disorders is gut barrier dysfunction a primary abnormality or a secondary phenomenon?

Celiac disease
IBD
IBS-D, PI-IBS
Liver disease

Primary abnormality - possibly a subset of IBS-D. Gene polymorphisms in IBD related to permeability

Many disorders may reflect the interaction between genetic predisposition and environmental stressors
3. What novel techniques are being developed to measure or provide insight into gut barrier integrity?
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Refinements of Lactulose:Mannitol assays

Suite of assays

Mir 29

A normal range has been defined, but the variability is substantial

Ability to detect change in response to an intervention is not adequately defined
4. Is the gut barrier a valid target for interventions that modify the microbiome?

Absolutely, but there are still many confounders (diet, saturated fatty acids, alcohol, NSAIDs)

Limitation is reproducibility and the sensitivity of available tools