The influence of the mucosal immune system and gut microbiota on inflammatory bowel disease (IBD)

Microbial Ecology in States of Health and Disease
Institute of Medicine – Forum on Microbial Threats
March 18, 2013

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IBD: a central problem in the tridirectional relationship between the commensal microbiota, epithelium and immune system
Altered bacterial phyla identified in the human gut microbiota of IBD

- Decreased abundance and diversity of Bacteroidetes in IBD
- Altered composition of Firmicutes and Maintenance or even bloom of Proteobacteria in IBD
- Decrease in protective microbes (e.g. Faecalibacterium prausnitzii)
- Some Proteobacteria (AIEC) are adherent to the epithelium via binding to CEACAM6 and inflammatory

*Frank, PNAS 2007; Peterson, Cell Host & Microbe 2008; Barnich, J Clin Invest 2007*
Chemical, enteropathogenic or genetically induced inflammation in mice causes dysbiosis similar to IBD

• Host-mediated inflammation disrupts intestinal microbial composition
• Decreased Firmicutes and Bacteroidetes with overgrowth of Proteobacteria (homology with bloom of AIEC in IBD)
• Similar microbial changes secondary to
  – pathogen-induced inflammation (e.g. *Citrobacter rodentium*)
  – Chemically-induced inflammation (e.g. dextran sodium sulphate)
  – Genetically-induced inflammation (e.g. *Il10*−/− mice)

*Lupp, Cell Host Microbe 2007*
When in life does IBD originate as a disease process?

- **Newborn**
  - Initial gut bacteria (founder species) depends upon delivery mode
  - Vaginal delivery: *Lactobacillus, Prevotella spp.*
  - C-section: *Staphylococcus, Corynebacterium, Propionibacterium spp.*
  - Vertical inheritance from mother
  - Higher susceptibility to certain pathogens
  - Higher risk of atopic diseases

- **Early childhood**
  - New strains (less certain in origin) outcompete old ones
  - Rapid increase in diversity
  - Early microbiota development = high instability
  - Shifts in response to diet, illness

- **Adult**
  - Highly distinct, differentiated microbiota
  - Microbial community may continue to change, but at a slower rate than in childhood

- **Elderly**
  - Substantially different gut communities than in younger adults

*Domínguez-Bello, Gastroenterology 2011*
Natural killer T (NKT) cells

- Natural killer T (NKT) cells recognize lipid antigens presented by the atypical MHC class I molecule CD1d
- NKT cells are early innate-like responders that activate other innate and adaptive immune cells

van Kaer
2004
CD1d and NKT expression in intestines

Professional antigen presenting cells (dendritic cells, macrophages, B-cells)

Intestinal epithelial cells

1B1/IgG2b

phalloidin

DAPI

Cd1d\(^{+/+}\)

Cd1d\(^{-/-}\)

Balk, Science 1994; Wingender, Gastroenterology 2012

Dougan, Curr Topic Micro Immunol 2009; Mayer, IBD 2008
CD1d and NKT cells are involved in the pathogenesis of IBD

• Mouse:
  – Oxazolone-induced colitis is CD1d-dependent (*Cd1d*<sup>-/-</sup>) and NKT-mediated (*Ja18*<sup>-/-</sup>) ([Heller, Immunity 2002](#))
  – Involves innate (IL-1β), Th1 (IFNγ) and Th2 (IL-4, IL-13) cytokines ([Boirivant, JEM 1998; Iijima JEM 2004](#))

• Human:
  – Increased NKT cells in ulcerative colitis that exhibit CD1d-restricted IL-13 production ([Fuss, J Clin Invest 2005](#))
  – NKT cells are enriched in expression of genes associated with IBD risk loci ([Jostins, Nature 2012](#))
CD1d dependent macrophage-mediated clearance of *Pseudomonas aeruginosa* from lung

*Nieuwenhuis EES, Nature Med 2002*
CD1d dependent regulation of bacterial colonization in the intestine of mice

(also *L. gasseri*, *S. aureus*, *P. aeruginosa*)
NKT cells – an early warning system for viral infection

• These studies demonstrate that CD1d – iNKT cell pathways regulate commensalism and pathogenic infection at the mucosal interface

• Can the microbiota regulate CD1d – iNKT cell pathways and what are the long-term consequences for the host?
Increased colonic iNKT cells in GF mice

Colonic iNKT cell population increases during lifetime

Olszak, An Science 2012
Prevailing dogma: “no microbiota, no colitis”

- Germ-reduced mice exhibit more severe DSS-induced colitis (Rakof-Nahoum S, Cell 2004)

- Germ-free mice exhibit more severe DSS-induced colitis as a consequence of decreased short chain fatty acid production and engagement of GPCR43 receptor (Maslowski KM, Nature 2009)
Oxazolone colitis protocol

7 week old mice*

6 days

Skin oxazolone (3%)

6 days

Rectal oxazolone (1%)

5 days

Sacrifice

*Swiss-Webster
GF mice develop severe morbidity in oxazolone colitis

Survival (%)

Body weight loss

Cytokine (ng/ml)

SPF (ox)
SPF (EtOH)
GF (ox)
GF (EtOH)

IL-4
IL-13
IL-1β

* p < 0.05
** p < 0.01
The high mortality of GF mice in oxazolone colitis is CD1d-restricted.
Early lifetime exposure to microbiota protects GF mice from high colonic iNKT cell infiltration

GF/a: microbial exposure in adulthood
GF/n: microbial exposure as neonate

* p < 0.001
...and colitis as a consequence of oxazolone administration

![Graphs showing survival, body weight loss, and mean pathology score.](image)

- Survival (%): GF, GF/a, GF/n
- Body weight loss (% of initial): GF, GF/a, GF/n
- Mean pathology score: GF, GF/a, GF/n

*P, <0.01  **P, <0.001
Early-life microbial exposures determine later-life susceptibility to immune-mediated diseases: “microbial (hygiene) hypothesis”

• First suggested by David P. Strachan (*Br Med J* 1989) to explain the relationship between household size, birth order and hay fever in a birth cohort during one week in 1958 and followed for 23 years

• Extended by J.-F. Bach to include autoimmune diseases (*N Engl J Med* 2002)

• Association between antibiotic use in first year of life and pediatric IBD (*Shaw, Am J Gastroenterol* 2010)

• Inverse relation between asthma and growing up on a farm correlated with exposure to a wider range of microbes than children in the reference group (*Ege, N Engl J Med* 2011)
Emergence of IBD as a global disease: increasing incidence and prevalence over time and in different regions of the world

- Systematic literature review of 262 studies (1950-2010)
- 75% of CD studies and 60% of UC studies showed an increasing incidence ($P < 0.05$)
- Emergence in Asia, Africa, South America, Middle East

Molodecky, Gastroenterology 2012
Greatest increase in IBD incidence found in early onset subgroup

<table>
<thead>
<tr>
<th>AGE</th>
<th>Change in Incidence Rate</th>
<th>95% CI</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>+5.0% / year</td>
<td>0.5% - 10.5%</td>
<td>0.032</td>
</tr>
<tr>
<td>5-9</td>
<td>+7.6% / year</td>
<td>4.4% - 10.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10-14</td>
<td>+0.63% / year</td>
<td>-0.9% – 2%</td>
<td>0.407</td>
</tr>
<tr>
<td>15-17</td>
<td>-0.21% / year</td>
<td>-1.3% – 0.9%</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Benchimol, Gut 2009
Asthma, eczema and hay fever exhibit increasing prevalence and possess an iNKT cell component.
Early exposure to microbiota protects GF mice from allergic asthma

GF/a: microbial exposure in adulthood
GF/n: microbial exposure as neonate

★ p < 0.05
★★ p < 0.01
Chemokine (C-X-C motif) ligand 16

- CXCL16 has some unusual features for chemokines:
  - it is composed of:
    - 254 amino acids
    - a CXC chemokine domain
    - a mucin-like stalk
    - a transmembrane domain
    - a cytoplasmic tail containing a potential tyrosine phosphorylation site
  - can therefore be expressed as a cell surface bound molecule as well as a soluble chemokine

- NKT cells bind and migrate in response to CXCL16 through expression of CXCR6
- CXCL16 a marker for human IBD

Matloubian, Nat Immunol 2000
Abel, J Immunol 2004
Uza, Gut 2011
Lehrke, Scand J Gastro 2008
CXCL16 expression in colon and lung are similar at birth in GF and SPF mice but diverge thereafter.
GF mice show increased levels of the chemokine ligand CXCL16.

**serum CXCL16**

- GF: 400 pg/ml
- GF/a: 600 pg/ml
- GF/n: 200 pg/ml
- SPF: 0 pg/ml

**colonic CXCL16**

- GF: 8 (fold increase)
- GF/a: 6 (fold increase)
- GF/n: 4 (fold increase)
- SPF: 0 (fold increase)

**lung CXCL16**

- GF: 2.5 (fold increase)
- GF/a: 2 (fold increase)
- GF/n: 1.5 (fold increase)
- SPF: 1 (fold increase)

CXCL16 expression in GF and SPF mice.
Age dependent microbial exposure regulates CXCL16 methylation
CXCL16 is characterized by 5-hydroxymethylcytosine hypermethylation: an activating signature.
Forced methylation by folinic acid treatment increases CXCL16 and iNKT cells in SPF mice in early-life

* \( P < 0.05 \), ** \( P < 0.01 \)
Inhibition of methylation in GF mice with 5-azacytidine during early-life decreases CXCL16 and iNKT cells

*, $P < 0.05$, **, $P < 0.01$
Human microbiota is acquired in early life by mother-to-child vertical transmission and modified by environmental factors.
Germ-reduced B6 SPF mice exhibit increased colonic iNKT cells and CXCL16 and colitis

- B6 neonate
- B6 (antibiotic)
- CD1dKO (antibiotic)
- Ja18KO (antibiotic)

CXCL16 (pg/ml)

Survival (%)

Days

P<.05
Potential mechanisms of iNKT cell control in mucosal tissues by commensal microbiota

- Increased recruitment of iNKT cells
- Absence of CD1d-restricted microbial antigen induced maturation or suppression of expansion & autoreactivity of iNKT cells

adapted from Coppieters & Elewaut, Gastroenterology 2012
Acknowledgments

Torsten Olszak
Miguel Pinilla Vera
Rebecca Baron
Jennifer Cusick
Joana Pereira das Neves
(Brigham and Women's Hospital,
Harvard Medical School)

Sebastian Zeissig
(University Medical Center Kiel)

Julia Richter
Andre Franke
(Christian-Albrechts University Kiel)

Dennis Kasper
Dingding An
(Channing Laboratory,
Harvard Medical School)

Jonathan Glickman
(Caris Diagnostics)

Deutsche Forschungsgemeinschaft
(DFG)

NATIONAL INSTITUTES OF HEALTH

CROHN'S & COLITIS FOUNDATION OF AMERICA