The enteric microbiota: Implications for IBD

Eugene B. Chang, M.D.  
University of Chicago
On a per cell basis, humans are mostly prokaryote
The microbial flora of the GI tract is the largest microbiome in the body.
Intestinal bacteria (microbiota)

- Protection of the epithelium
- Protection against enteric pathogens e.g. *C. difficile*
- Digestion of complex dietary polysaccharides
- Development and maintenance of gut immune system
- Stimulate intestinal angiogenesis

Challenges to studying the intestinal microbiome

Cultivable (<20%)

Non-cultivable
Why is the 16S rRNA gene used to classify bacteria?

Schematic of 16S ribosomal RNA gene

Constant regions (evolutionarily conserved)

Fewer phyla are represented in the human microbiome

Cell 124, 837–848, February 24, 2006
What is know about the human intestinal microbiota?

- Sampling from mucosal and fecal samples from 3 healthy subjects
- >11,000 prokaryotic 16S rDNA gene sequences
- 7 major divisions of bacteria, but Firmicutes and Bacteroidetes dominate (98% of all sequences)
- 395 bacterial phylotypes --> 62% novel

Variation in Bacterial Diversity within the Colonic Microbiota of Three Healthy Humans

Cell 124, 837–848, 2006
How would one study mucosal-associated bacterial communities of the human colon?
Etiopathogenesis of IBD

Genetics
- Nod2
- IBD5
- Autophagy
- IL23R

Environment
- MICROBES
- smoking
- diet
- carcinogens

Immune imbalance
Defective Host defense

IBD
**Influence of the Normal Luminal Bacterial Flora On Development of Inflammation in Animal Models**

<table>
<thead>
<tr>
<th>Model:</th>
<th>Species</th>
<th>Spec. Pathogen-free</th>
<th>Germ-free</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>Rat</td>
<td>Acute GI ulcers, Chronic SB ulcers</td>
<td>Attenuated acute, no chronic</td>
</tr>
<tr>
<td>CD45RB&lt;sup&gt;high&lt;/sup&gt; Tf</td>
<td>SCID</td>
<td>Colitis</td>
<td>No colitis</td>
</tr>
<tr>
<td>HLA-B27 transgenic</td>
<td>Rat</td>
<td>Gastritis, colitis, arthritis</td>
<td>No disease</td>
</tr>
<tr>
<td>IL-2 KO</td>
<td>Mouse</td>
<td>Colitis, gastritis, hepatitis</td>
<td>No or decreased disease</td>
</tr>
<tr>
<td>IL-10 KO</td>
<td>Mouse</td>
<td>Colitis, gastritis</td>
<td>No inflammation</td>
</tr>
<tr>
<td>TCR-α KO</td>
<td>Mouse</td>
<td>Colitis</td>
<td>No inflammation</td>
</tr>
</tbody>
</table>

* Modified from Sartor RB, in Inflammatory Bowel Disease, 5th Edition 1999*
Two potential causes of IBD

1. Bad intestinal bacteria induces IBD

2. Abnormal immune response to normal intestinal bacteria induces IBD

Insights into etiopathogenesis of IBD are provided by studies of NOD2

Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn’s disease


A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease


NOD2 stimulates and modifies activation of NFκB, but also is part of the inflammasome.

NOD2 mutations – Gain or Loss of Function?

Aberrations in autophagy are associated with increased CD risk

- ATG16L1 linked to Crohn’s disease
- ATG16L1 is expressed by intestinal epithelial cells
- Functional knock-down abrogates autophagy of Salmonella
- IRGM (Chr 5q33.1) also implicated

Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis

John D Rioux1,2, Ramnik J Xavier1, Kent D Taylor3, Mark S Silverberg2, Philippe Goyette1, Alan Haertl1, Todd Green2, Petrik Kabbani2, M Michael Bernatsky4, Lisa Wu Datta5, Yin Yao Shugart5, Anne M Griffiths6, Stephen R Targan1, Andrew F Hygien7, Edmund-Jean Bernard7, Ling Mei8, Dan L. Nicolae1,2, Miguel Regueiro9,10, I. Philip Schuman11, A. Hilary Steinhauser1, Jerome I Rotter1, Richard H Danner1,2, Judy H Cho1,14, Mark J Daly1,14,15, & Steven R Hand1,2,10,14,15

We present a genome-wide association study of IBD and two independent replication studies that identify several new regions of association to Crohn disease. Specifically, in addition to the previously established CARD15 and IL23R associations, we identified strong and significantly replicated association (combined P < 6.4×10⁻⁸) with an intergenic region on 14q12.1 and a coding variant in ATG16L1, the latter of which was also recently reported by another group. We also report strong associations with independent replication to variation in the genetic regions encoding PYHIN1, NOD2 and a predicted gene on 17q21 (CAND7). Finally, we demonstrate that ATG16L1 is expressed in intestinal epithelial cell lines and that functional knockdown of this gene abrogates autophagy of Salmonella replication. Together, these findings suggest that autophagy and host cell responses to intracellular microbes are involved in the pathogenesis of Crohn disease.
Autophagy is important for cell survival, metabolism, and host defense

- Described 50 years ago
- Evolutionary conserved
- Protein/organelle turnover
- Stress survival
- Defense against pathogens

* http://www.biken.osaka-u.ac.jp/act/act_yoshimori_e.php
Enteric bacteria within IBD gut epithelial cells

OCTN1/2 mutations reported to increase risk of IBD


Functional variants of OCTN cation transporter genes are associated with Crohn disease

Vanya D Peltekova1,2, Richard E Wirthe3, Laurence A Rubin1,3,4, Christopher I Amos5, Qiqing Huang6, Xiangjun Gu7, Bill Newman1,2, Mark Van Oene8, David Cescon1,2, Gordon Greenberg1, Arne M Griffiths6, Peter H St George-Hyslop1,3,7 & Katherine A Siminovich1-3

Crohn disease is a chronic, inflammatory disease of the gastrointestinal tract. A locus of -250 kb at 5q31 (IBD5) was previously associated with susceptibility to Crohn disease, as indicated by increased prevalence of a risk haplotype of 11 single-nucleotide polymorphisms among individuals with Crohn disease, but the pathogenic lesion in the region has not yet been identified. We report here that two variants in the organic cation transporter cluster at 5q31 (a nonsense substitution in SLC22A4 and a G→C transversion in the SLC22A5 promoter) form a haplotype associated with susceptibility to Crohn disease. These variants alter transcription and transporter functions of the organic cation transporters and interact with variants in another gene associated with Crohn disease, CARD15, to increase risk of Crohn disease. These results suggest that SLC22A4, SLC22A5 and CARD15 act in a common pathogenic pathway to cause Crohn disease.

1672C→T and -207G→C are in strong linkage disequilibrium and create a two-allele risk haplotype (TC) enriched in individuals with Crohn disease (frequency = 0.24 in affected individuals versus 0.42 in controls, \( P = 0.0003 \); Table 1). Odds ratios conferred by allele 1672T, allele -207C or the TC haplotype were similar. Population
Gram positive bacteria produce bioactive factors that are transported by OCTN2

Preparation of conditioned media

Conditioned media (bacteria-free)

Bacterial incubation

Bacterial pellet

Cell Host & Microbe 1, 299–308, June 2007
Gram positive bacteria produce bioactive factors that are transported by OCTN2
Model of OCTN2 function and potential role in disease

Quorum-sensing CSF: EGRMT

- Luminal bacteria
- Mucosal-Associated Bacteria
- Colonocytes

Quorum-signaling profile

Hsp27/72
p-Akt*
p38 MAPK*

Enhanced survival, increased cytoprotection

Cell Host & Microbe 1, 299–308, June 2007
Hypothesis: Bad intestinal bacteria induces IBD

Several reports have implicated *E. coli* strains in the pathogenesis of IBD.

High Prevalence of Adherent-Invasive *Escherichia coli* Associated With Ileal Mucosa in Crohn’s Disease

_GASTROENTEROLOGY 2004;127:412–421_

ARLETTE DARFEUILLE–MICHAUD,* JÉRÔME BOUDEAU,* PHILIPPE BULOIS,† CHRISTEL NEUT,§ ANNE–LISE GLASSER,* NICOLAS BARNICH,* MARIE–AGNÈS BRINGER,* ALEXANDER SWIDSINSKI,† LAURENT BEAUGERIE,‖ and JEAN–FRÉDÉRIC COLOMBEL†

Invasive *Escherichia coli* are a feature of Crohn’s disease

Maiko Sasaki¹, Shanti V Sitaraman¹, Brian A Babbin², Peter Gerner-Smidt³, Efrain M Ribot³, Nancy Garrett³, Joel A Alpern⁴, Adil Akyildiz², Arianne L Theiss¹, Asma Nusrat² and Jan-Michael A Klapproth¹

_Laboratory Investigation (2007) 87, 1042–1054_

The role of *Escherichia coli* in inflammatory bowel disease

Jonathan M Rhodes

_Gut 2007;56;610-612_
Bacteria determine phenotype and course

- Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria.

  Kim SC, Et al. GASTROENTEROLOGY 2005;128:891–906

Different commensal bacterial species selectively initiate immune-mediated intestinal inflammation with distinctly different kinetics and anatomic distribution in the same host.
Difference in clinical presentation of microbe-induced colitis

Kim SC, Et al. GASTROENTEROLOGY 2005;128:891–906
Two potential causes of IBD

1. Bad intestinal bacteria induces IBD

2. Abnormal immune response to normal intestinal bacteria induces IBD

How enteric microbiota cause or contribute to IBD remains unknown

Crohn’s Disease

Ulcerative Colitis
Are reported changes in colonic microbiota a cause or effect of inflammation?

**INFLAMMATORY BOWEL DISEASE**

Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease

M P Conte, S Schippa, I Zamboni, M Penta, F Chiarini, L Seganti, J Osborn, P Falconieri, O Borrelli, S Cucchiara  
*Gut* 2006;55:1760-1767;

**Results:** A higher number of mucosa-associated aerobic and facultative-anaerobic bacteria were found in biopsy specimens of children with IBD than in controls. An overall decrease in some bacterial species or groups belonging to the normal anaerobic intestinal flora was suggested by molecular approaches; in particular, occurrence of *Bacteroides vulgatus* was low in Crohn’s disease, ulcerative colitis and indeterminate colitis specimens.
Many challenges remain in defining the role of enteric microbiota in IBD
Challenges ahead of us

• Prospective studies of individuals
• Improve sampling
• Increase whole genome database of enteric microbes
• In situ analysis
  – Metagenomics
  – Community organization and structure
• Concomitant studies of host response
• Advanced bioinformatic applications