How Does Fecal Microbiota Transplantation Treat *Clostridium difficile* Infection?

Alexander Khoruts, MD
University of Minnesota

Human Microbiome Science 2013
Disclosures

- Research funding from CIPAC Limited, a start-up company commercializing Full-spectrum Microbiota for FMT

- NIH and UMN funding for studies of gut microbiota following FMT
Clinical Case

61 year old woman referred for evaluation of chronic diarrhea for 8 months. Symptoms originally started following treatment with cephalosporin and quinolone antibiotics for back surgery and pulmonary infection. During these 8 months she had several hospitalizations for intravenous hydration. A colonoscopy showed “ischemic colitis” on biopsies. Intermittently she was treated with variable success with Metronidazole and Vancomycin. She had bowel movements every 15 minutes with urgency and tenesmus. She lost 27 kg of weight and was confined to a wheelchair.
Recurrent *C. difficile* Infection Syndrome

Borody and Khoruts, 2011
Fecal Microbiota Transplantation: Mechanisms

WE NEED A DONOR!

THIS MAN HAS BAD POOP IN HIS BUTT

IT NEEDS TO BE GOOD POOP

AND FAST

WE NEED TO TAKE YOUR GOOD POOP OUT OF YOUR BUTT AND PUT THAT POOP INSIDE OF HIS BUTT

IN PLACE OF THE BAD POOP

THIS WILL SAVE HIS LIFE FOR SOME REASON

I'M A DOCTOR

Ryan Pequin, Three Word Phrase
Dr. Ben Eiseman (1917-2012)

- Chief of Surgery, Denver VA Hospital, 1953-61
- Founding Chairman of Surgery, University of Kentucky, 1961-67
- Founding Chairman of Surgery, Denver General Hospital, 1967-77
- > 450 scientific articles
- 7 books
- Active Military Duty in 4 wars
- Rear Admiral (MC) USNR – retired 1974
- Active academic through 2012
“In the early days of oral antibiotics we were plagued by frequent diarrhea in our patients due presumably to killing off intestinal bacteria. I was Chief of Surgery at the VA and simplistically considered merely reintroducing normal organisms to counter such absence. Those were days when if one had an idea, we simply tried it. It seemed to work and I wrote it up. It made a small splash…Best wishes. Ben Eiseman Emeritus Professor of Surgery – Now age 93” (2012).
Analysis of 16S clone libraries of the fecal microbiota in patients with antibiotic-associated diarrhea due to Clostridium difficile.

FMT Results in Restoration of Gut Microbial Diversity

Weingarden et al., Poster #45
FMT Results in Restoration of Gut Microbial Diversity

Weingarden et al., Poster #45
FMT Results in Restoration of Gut Microbial Diversity

Weingarden et al., Poster #45
FMT Results in Restoration of Gut Microbial Diversity

A

Shannon diversity index

Donor Pre-FMT Post-FMT

B

PC1 (44.5% variation explained)

PC2 (13.4% variation explained)

Donor Pre-FMT Post-FMT

Weingarden et al., Poster #45
What about the “Yuck” factor?
What about the “Yuck” factor?
What about the “Yuck” factor?

Standardized Full Spectrum Microbiota

- Rigorously tested volunteer donors
- Cryopreserved
- Virtually eliminated odor
- The same number of bacteria per dose
- Manufactured under GMP conditions at an FDA registered facility at the UMN
What about the “Yuck” factor?
Potential Mechanisms of FMT in R-CDI

Microbiota

Host

Bile Acids

SCFA

Innate and Adaptive Immunity

C. difficile

- Sporulation
- Spore germination
- Vegetative growth
- Adhesion to epithelial cells
- Toxin production

Illustration by Adam Alaniz
Competitive Niche Exclusion

Illustration by Adam Alaniz
Lymphopenia-inducing insult

Steady State:
1. Normal T cell population size
2. Great TCR Diversity

Recovery via LIP where all T cells proliferate equally:
1. Normal T cell population size
2. Limited TCR diversity

Recovery via LIP where some T cells have a selective advantage over others:
1. Normal T cell population size
2. Oligoclonal expansion
3. Greatly reduced TCR diversity
4. Potential autoimmunity

Recovery in presence of a functional thymus

Khoruts A and Fraser JM (2005)
• Thuricin CD inhibits vegetative growth of *C. difficile* in an ex vivo colon infection model
• Produced by *B. thuringiensis*
• Narrow activity spectrum

Rea et al., *PNAS* (2010)
Lactobacillus delbrueckii ssp. Bulgaricus B-30892 inhibits cytotoxicity and adhesion of C. difficile to Caco-2 cells

LDB releases bioactive components that inhibit C. difficile blocks toxin activity (mechanism?)

Lactobacillus delbrueckii ssp. Bulgaricus B-30892 inhibits cytotoxicity and adhesion of C. difficile to Caco-2 cells

LDB releases bioactive components that inhibit C. difficile blocks toxin activity (mechanism?) and adhesion to epithelial cells

Lactobacillus delbrueckii ssp. Bulgaricus B-30892 inhibits cytotoxicity and adhesion of \textit{C. difficile} to Caco-2 cells


LDB releases bioactive components that inhibit \textit{C. difficile} blocks toxin activity (mechanism?) and adhesion to epithelial cells, but vegetative growth of \textit{C. difficile} is not impaired.

Immune-mediated colonization resistance

Microbiota

Innate and Adaptive Immunity

Illustration by Adam Alaniz
Immunity against *C. difficile*

- Antimicrobial peptides

α-Defensins neutralize *C. difficile* toxin B

Giesemann T, et al. (2008)
Immunity against *C. difficile*

- Antimicrobial peptides
- NOD1
- MyD88
- IL-1β
- CXCL1
- TLR5
- Anti-*C. difficile* toxin IgG

Giesemann T, et al. (20008)
Hasegawa M, et al. (2011)
Jarchum I, et al. (2012)
Hasegawa M, et al. (2012)
Kyne L, et al. (2000)
Lowy I, et al. (2010)

$\alpha$-Defensins neutralize *C. difficile* toxin B
Bile Acids and Salts

Cholic Acid

CA = cholic acid
DxCA = deoxycholic acid
LiCA = lithocholic acid
ChDxCA = chenodeoxycholic acid
Bile Acids and Salts

Hofmann, AF (2008)
Bile Acids and Salts

Hofmann, AF (2008)
Microbiota Alter Bile Acid Composition

Sayin et al., *Cell Metab* (2013)
Metabolism of Bile Salts in Mice Influences Spore Germination in *Clostridium difficile*

Jennifer L. Giel¹, Joseph A. Sorg², Abraham L. Sonenshein², Jun Zhu¹*

¹ Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, United States of America, ² Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, Massachusetts, United States of America
Bile salts in *C. difficile* spore germination

![Graph showing CFU recovery (%) for various treatments.](image)
Bile salts in *C. difficile* spore germination

Germination Factor is:
- small
- heat-stable
- water-soluble
- sensitive to cholestyramine

Giel, JL (2010)
### Bile salts in C. difficile spore germination

<table>
<thead>
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<tr>
<td>• sensitive to cholestyramine</td>
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</tbody>
</table>

A. Clindamycin eliminates secondary bile salts in feces
B. Fecal bacteria block pro-germinant activity of TA

Giel, JL (2010)
Fecal bile acids pre- and post-FMT

Weingarden et al., Poster #45
Pre- and post-FMT untargeted metabolomics

Weingarden et al., Poster #45
<table>
<thead>
<tr>
<th>ID</th>
<th>[M-H]⁻</th>
<th>Formula</th>
<th>Identity</th>
<th>Effect of FMT</th>
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<tbody>
<tr>
<td>I</td>
<td>407.2798</td>
<td>C₂₄H₄₀O₅</td>
<td>cholic acid (CA)</td>
<td>↓</td>
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<tr>
<td>II</td>
<td>391.2848</td>
<td>C₂₄H₄₀O₄</td>
<td>chenodeoxycholic acid (CDCA)</td>
<td>↓</td>
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<tr>
<td>III</td>
<td>391.2848</td>
<td>C₂₄H₄₀O₄</td>
<td>deoxycholic acid (DCA)</td>
<td>↑</td>
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<tr>
<td>IV</td>
<td>375.2899</td>
<td>C₂₄H₄₀O₃</td>
<td>lithocholic acid (LCA)</td>
<td>↑</td>
</tr>
<tr>
<td>V</td>
<td>391.2848</td>
<td>C₂₄H₄₀O₄</td>
<td>isodeoxycholic acid (isoDCA)</td>
<td>↑</td>
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<tr>
<td>VI</td>
<td>514.2839</td>
<td>C₂₆H₄₅NO₇S</td>
<td>taurocholic acid (TCA)</td>
<td>↓</td>
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<tr>
<td>VII</td>
<td>498.2889</td>
<td>C₂₆H₄₅NO₆S</td>
<td>taurochenodeoxycholic acid (TCDCA)</td>
<td>↓</td>
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<tr>
<td>VIII</td>
<td>464.2817</td>
<td>C₂₆H₄₃NO₆</td>
<td>glycocholic acid (GCA)</td>
<td>↓</td>
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<tr>
<td>IX</td>
<td>448.3063</td>
<td>C₂₆H₄₃NO₅</td>
<td>glycochenodeoxycholic acid (GCDCA)</td>
<td>↓</td>
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Current Directions Include:

- Further Development of Standardized Full-Spectrum Microbiota for Therapeutic Transplantation
- Expansion of GMP Manufacturing of Full-Spectrum Microbiota
- Mechanism-based Development of Disease Targeted Microbiota Therapeutics
Gaps and Challenges
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