Clostridium difficile Infection: The Tangled Web of Epithelium, Microbiota and Pathogen

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Take everything I say with a grain of salt: …and I will supply the salt shaker
**C. difficile** and the indigenous gastrointestinal microbiota

- **Theory**: the indigenous microbiota can prevent colonization by *C. difficile* or it can control the population size in colonized patients.

- **Corollary**: antibiotics disturb the indigenous microbiota, allowing colonization or overgrowth and toxin production.
Britton and Young (2014)
Gastroenterology 146:1547-1553
Point #1: The microbiome is the latest and greatest new field of scientific discovery.
THE FATAL ENTERIC CHOLERA INFECTION IN THE GUINEA PIG, ACHIEVED BY INHIBITION OF NORMAL ENTERIC FLORA

ROLF FRETER*

From the Department of Microbiology, The University of Chicago, Chicago 37, Illinois

In this respect it might be worthwhile to consider the possibility of inhibitory action on the part of the normal human enteric flora as a factor in the resistance of humans to enteric diseases. This theory has—to the knowledge of the author—first been discussed by Nissle (1916).
Murine models of *C. difficile* infection

Gut Microbes (2011) 2:326

The interplay between microbiome dynamics and pathogen dynamics in a murine model of *Clostridium difficile* infection

Gut Microbes (2011) 2:145
Antibiotic treatment to create susceptibility to *C. difficile*
Point #2: 16S analysis is anatomy of a microbial community, not the physiology.
Metabolic status of the gut and C. difficile infection

Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection

Casey M. Theriot¹,², Mark J. Koenigsknecht¹,³, Paul E. Carlson Jr³, Gabrielle E. Hatton¹, Adam M. Nelson¹,², Bo Li⁴, Gary B. Huffnagle², Jun Z. Li⁴ & Vincent B. Young¹,³
State transitions of the gut microbiota after antibiotics

Theriot et al., Nat Commun 5, 3114 (2014)

Cefoperazone-treated mice as an experimental platform to assess differential virulence of Clostridium difficile strains

Reproducible Community Dynamics of the Gastrointestinal Microbiota following Antibiotic Perturbation

Theriot et al., Gut Microbes 2:1
Antonopoulos et al., IAI 77:2367
Theriot et al., Nat Commun 5, 3114 (2014)
<table>
<thead>
<tr>
<th>Lipid/Bile Acid</th>
<th>Resistant</th>
<th>Susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>beta-hydroxyliothocholate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lithocholate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beta-muricholate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholate [6-oxo or 7-keto]</td>
<td></td>
<td></td>
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<tr>
<td>deoxycholate</td>
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<tr>
<td>gamma-muricholate</td>
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<tr>
<td>3-dehydrocholate</td>
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<td>cholate</td>
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<td>7,12-diketolithocholate</td>
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<td>12-dehydrocholate</td>
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<td>hyodeoxycholate</td>
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<td>alpha-muricholate</td>
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<tr>
<td>taurocholate</td>
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</tbody>
</table>

**Treatment Group**

- S1
- R1
- R2
- R3
ex vivo growth of *C. difficile* in cecal contents
Bile acids & the microbiota

Britton & Young (2012)
Trends Microbiol 20:313-9
Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*

Charlie G. Buffie¹,², Vanni Bucci³,⁴, Richard R. Stein³, Peter T. McKenney¹,², Lilan Ling², Asia Gobourne², Daniel No², Hui Liu⁵, Melissa Kinnebrew¹,², Agnes Viale⁶, Eric Littmann², Marcel R. M. van den Brink⁷,⁸, Robert R. Jenq¹, Ying Taur¹,², Chris Sander³, Justin Cross⁸, Nora C. Toussaint²,³, João B. Xavier²,³ & Eric G. Pamer¹,²,⁸

Inhibiting the Initiation of *Clostridium difficile* Spore Germination using Analogs of Chenodeoxycholic Acid, a Bile Acid⁷

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Received 28 May 2010/Accepted 19 July 2010

To cause disease, *Clostridium difficile* spores must germinate in the host gastrointestinal tract. Germination is initiated upon exposure to glycine and certain bile acids, e.g., taurocholate. Chenodeoxycholate, another bile acid, inhibits taurocholate-mediated germination. By applying Michaelis-Menten kinetic analysis to *C. difficile* spore germination, we found that chenodeoxycholate is a competitive inhibitor of taurocholate-mediated germination and appears to interact with the spores with greater apparent affinity than does taurocholate. We also report that several analogs of chenodeoxycholate are even more effective inhibitors. Some of these compounds resist 7α-dehydroxylation by *Clostridium scindens*, a core member of the normal human colonic microbiota, suggesting that they are more stable than chenodeoxycholate in the colonic environment.
Points #3 & 4

The dynamics of the system are important. Where you look is important (the answer isn’t always in the feces)
What happens during establishment of infection?

- Timecourse of murine CDI
- Infection with 100 spores of *C. difficile* VPI 10463 (cefoperazone model)
- Sacrifice animals every 6 hours up for 36 hours (all animals moribund by 36 hours)
- Follow vegetative CFU, spores, toxin

Koenigsknecht et al. I&I Mar;83(3):934-4
Colonization and growth
ex vivo germination and growth
Point #5

What happens in vitro may not tell you what happens in vivo
Alteration of the Murine Gastrointestinal Microbiota by Tigecycline Leads to Increased Susceptibility to Clostridium difficile Infection

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Humans make a lousy model of murine physiology

(But if you are careful, you can find useful information)
Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*

Els van Nood, M.D., Anne Vrieze, M.D., Max Nieuwdorp, M.D., Ph.D., Susana Fuentes, Ph.D., Erwin G. Zoetendal, Ph.D., Willem M. de Vos, Ph.D., Caroline E. Visser, M.D., Ph.D., Ed J. Kuijper, M.D., Ph.D., Joep F.W.M. Bartelsman, M.D., Jan G.P. Tijssen, Ph.D., Peter Speelman, M.D., Ph.D., Marcel G.W. Dijkgraaf, Ph.D., and Josbert J. Keller, M.D., Ph.D.

Recovery of the Gut Microbiome following Fecal Microbiota Transplantation

A. C. difficile infection

- Vanco
- Monitor relapse
- i.p. clindamycin

B. CFU g sample

- Log_{10} (reciprocal dilution toxin per g of sample)

- day (p.i.)
Point #7

*C. difficile* infection represents a complex system with the host, microbiota and pathogen

Sometimes you can’t study everything, but when you don’t, remember that there is something you aren’t looking at
Jhansi Leslie
unpublished data
How to get rid of the microbiome: Organoids

Jason Spence
Jhansi Leslie
Sha Huang

Leslie et al. (2015) Infect Immun
2015 Jan;83(1):138-45
De novo differentiation

iPS reprogramming
Yamanaka Factors

biopsy

HIO
Mesenchyme+Epithelium
Simple growth conditions (ENR)

Enteroid
Epithelium only
Complex growth conditions (ENR, p38i, TGF®i, etc)

Sato et al., Gastroenterology, 2011
HIOs contain major cell types of the small intestine
Organoids as an alternative?
Do HIOs have a functional epithelial barrier?

Nuclei/DPP4/ZO1/Ecad
Fluorescent 4kDa dextran (FD4)

Barrier permeability assay

Leslie et al. (2015) Infect Immun
2015 Jan;83(1):138-45
Pruitt & Lacy (2012)
Front Cell Infect Microbiol 2:28
Luminal TcdA and TcdB disrupt barrier function

Fluorescent 4kDa dextran (FD4) + purified *C. difficile* TcdA or TcdB
Control

TcdA

TcdB
Toxigenic *C. difficile* strains disrupt the HIO epithelial barrier!

Fluorescent 4kDa dextran (FD4) + vegetative *C. difficile*
C. difficile bacilli and spores within the lumen of infected organoids
But wait a minute: Didn’t we know that *C. difficile* is an anaerobe?

- Interesting observation: in one experiment, there actually appeared to be growth (multiplication) of *C. difficile* within organoids
- Upon further investigation, appears that the microinjection system was contaminated with *E. coli*
- Could *E. coli* alter the environment in a way to permit *C. difficile* growth?
E. coli and modulation of luminal oxygen tension

Hill et al. (unpublished data)
Conclusions

- Microbe-microbe and microbe-host interactions in *C. difficile* infection are complex and range from antagonistic to facilitative.

- Understanding the myriad interaction between *C. difficile*, the gut microbiota and the host could lead to new modalities for prevention and therapy.
Acknowledgments

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