The role of mucus associated bacteria in Inflammatory Bowel Disease

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The role of microorganisms in IBD

HoeVeR

the microorganisms involved in IBD
remain in dispute

Evidence for a range of microorganisms

- Paramyxoviridae
- Mycobacterium paratuberculosis
- Bacteroides vulgatus
- Yersinia species
- Fusobacterium varium
- Escherichia coli subtypes

nO COStISStEN'T DATa

ARE WE LOOKING AT THE WRONG BACTERIA?

If bacteria are involved in the initiation of IBD, the most likely candidates are those closest to the mucosal surface!

- Early studies in rodents
  Intestinal mucus & crypts colonised by
    - Spiral/helical bacteria
      - *Borrelia* spp
      - *Treponema* spp
      - *Spirillum* spp
      - “Others”
  “Others” now known to include:
  - Members of *Helicobacteraceae*
    - *Helicobacter* and *Wolinella* genera
  - *Campylobacter* species

Lee A Advances in Microbial Ecology, Vol.8.
Aim and study population

- **Aim**
  To investigate the role of members of the *Helicobacteraceae* and *Campylobacter* species with CD

- **Study population**
  Children
  Newly diagnosed
  Free from many confounding factors
Detection of members of the *Helicobacteraceae* and *Campylobacter* species in biopsy samples

**COHORT 1**

- **Children**
  - 54 CD (Newly diagnosed)
    - 2-16yrs; mean 9.7 years
  - 57 symptomatic Non-IBD controls (No pathology)
    - 2-15yrs; mean 9.4 years
  - 22 symptomatic Non-IBD controls (Pathology)
    - 2-15 yrs; mean 8.6 years

- **Diagnoses**
  - Allergic diseases, Colonic eosinophilia
  - Coeliac disease, Yersinia infection
  - Reflux oesophagitis, Rectal prolapse,
  - Active proctitis, Peutz Jeghers syndrome
Materials and Methods
Detection and identification of members of the *Helicobacteraceae* and *Campylobacter* genus in biopsies and fecal samples

- **DNA Extraction**
  Qiagen Mini stool kit

- ***Helicobacteraceae* specific PCR and Sequencing**
  Amplify 16S rRNA gene with *Helicobacteraceae* specific primers
  - C412F/C1288R (~400 bp) (Riley *et al.* 1996)
  - GC658F/1067R (~400 bp) (Grehan *et al.* 2002)

- ***Campylobacter* genus-specific PCR and Sequencing**
  Amplify 16S rRNA gene with *Campylobacter*-specific primers
  - C412F/C1288R (~800 bp) (Linton *et al.* 1996)
Detection of members of the *Helicobacteraceae* in biopsy specimens using PCR

Summary of sequencing results
Prevalence of non-*pylori Helicobacteraceae* species significantly higher in CD children (32%) when compared with controls (12%) (p<0.05)
A significantly higher prevalence of *Campylobacter* species detected by PCR in biopsy samples from children with CD

Summary of sequencing results
Prevalence of *Campylobacter concisus* significantly higher in CD children (39%) when compared with controls (2%) (p<0.0005)

Detection of members of the *Helicobacteraceae* and *Campylobacter* genus in fecal samples of CD children and controls

**COHORT 2**

- **Children**
  - 29 CD
    - Mean age 12.6 years
  - 11 Healthy controls
    - Mean age 7.2 years
  - 26 Non-IBD controls
    - Mean age 10.2 years

- **Diagnoses**
  - Functional bowel disorders, Reflux esophagitis
  - Eosinophilic gastrointestinal disease
  - Perianal fistula, Anaemia, Systemic inflammatory process,
  - Duodenal ulcer, *Helicobacter* gastritis, Constipation,
  - Mild focal cryptitis
A significantly higher prevalence of *Helicobacteraceae species* detected by PCR in fecal samples from children with CD

Summary of sequencing results

Prevalence of non-*pylori* *Helicobacteraceae* species significantly higher in CD children (38%) when compared with controls (9%) (p<0.001)
A significantly higher prevalence of *Campylobacter* species detected by PCR in fecal samples from children with CD

Summary of sequencing results
Prevalence of *Campylobacter concisus* higher (31%), but not significantly, in CD children when compared with controls (13%) (p<0.05)
Culture of mucus associated bacteria

- Culture of mucus associated bacteria problematic
  - Pre-induced diarrhea prior to colonoscopy flushes out intestinal contents
    - Reduces bacterial load in biopsy?
  - Fastidious nature of *Helicobacteraceae* and *Campylobacter* genus makes culture difficult - slow growers

- Current success with culturing
  - **Crohn’s Disease**
    - *H. pylori* cultured from 1 child but NOT in pure culture (identified by sequencing)
    - *C. concisus* cultured & mixed culture of *H. pylori* (identified by sequencing) from 1 child
    - *C. hominis* cultured from 1 child
    - *C. showae* cultured from 1 child
    - *B. ureolyticus* cultured from 1 child
  - **Non-IBD controls Pathology**
    - *C. jejuni* cultured from 1 child
    - *B. ureolyticus* cultured from 3 children
  - **Non-IBD controls NO Pathology**
    - *H. bilis /H. canis* in mixed culture (identified by sequencing)
    - *B. ureolyticus* and *C. jejuni* cultured from 1 child
    - *B. ureolyticus* cultured from 1 child
    - *C. hominis* cultured from 1 child
Can non-jejuni *Campylobacter species* invade intestinal cells?

- **Campylobacter invasion assay**

  Human intestinal epithelial cell line (CaCO_2_

  Gentamicin Protection assay

  Multiplicity of infection (MOI) = 20

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>% invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em> LT2</td>
<td>0.21</td>
</tr>
<tr>
<td><em>C. concisus</em></td>
<td>0.01</td>
</tr>
<tr>
<td><em>C. showae</em></td>
<td>0.00</td>
</tr>
<tr>
<td><em>C. hominis</em></td>
<td>0.00</td>
</tr>
<tr>
<td><em>B. ureolyticus</em></td>
<td>0.00</td>
</tr>
</tbody>
</table>

Seed 5 x 10^5 cells/well

24 hrs

Wash monolayer 4 times

Infect monolayer with Bacteria

6 hrs

Wash monolayer 4 times

Kill extracellular bacteria using 200 ul/ml gentamicin

1 hr

Wash monolayer 4 times

Lyse mammalian cells using 1% Triton X-100

Viable plate count
Can *Campylobacter concisus* invade intestinal cells?

Does increasing the MOI improve the ability of *C. concisus* to invade Caco 2 cells?

Does the presence of inflammation increases the invasive ability of *C. concisus*?

<table>
<thead>
<tr>
<th>MOI</th>
<th>CFU Invaded (SEM)</th>
<th>CFU Inoculated (SEM)</th>
<th>% Invasion (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (nx3)</td>
<td>4.6×10⁴ ± 9×10³</td>
<td>3.9×10⁷ ± 5.0×10⁶</td>
<td>0.11 ±0.02</td>
</tr>
<tr>
<td>200 (nx3)</td>
<td>9.0×10⁴ ± 3.4×10⁴</td>
<td>7.4×10⁷ ± 6.0×10⁶</td>
<td>0.13 ±0.05</td>
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</tbody>
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Pre-existing inflammation may increase the ability of *C. concisus* to invade intestinal epithelial cells.
Preliminary studies of IgG antibody response to non-jejuni *Campylobacter* species and *Campylobacter concisus*

Significantly higher mean IgG response to non-jejuni *Campylobacter* species and *C. concisus* in children PCR positive for non-jejuni *Campylobacter* species and *C. concisus*
Conclusions

- Increasing evidence that a range of mucus associated bacteria may play a role in the initiation of CD
- Further studies are required to investigate
  * Pathogenic mechanisms of enteroheptic *Helicobacter* and *Campylobacter* species in CD
  - Mechanism of *C. concisus* attachment to and invasion of intestinal cells
  * The immune response to mucus associated bacteria
  * The role of mucus associated bacteria in relapsing CD
  * Improved culture techniques for enteroheptic *Helicobacter*, *Wolinella* and *Campylobacter* species
  * Sequencing of pure cultures of *H. pylori* to investigate genetic differences from gastric *H. pylori*
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