Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome

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NATURE | VOL 519 | 5 MARCH 2015
Background

Incidence of IBD

• Fact 1: increasing in the past decades worldwide
• Fact 2: markedly higher in developed western countries
# Background

## Incidence of IBD

- Fact 1: increasing in the past decades worldwide
- Fact 2: markedly higher in developed western countries

![Graph showing incidence of Chron's disease](image)

**Chron's disease**

V. Loftus, Jr. Clinical Epidemiology of Inflammatory Bowel Disease: Incidence, Prevalence, and Environmental Influences, Edward. Gastroenterology 2004;126:1504–1517
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![Incidence of IBD](chart.png)
Background

Role of Microbiota

- Fact 3: gut microbiota provides important benefits: metabolism / immune development
- Fact 4: disturbance of the microbiota– host relationship: numerous chronic inflammatory diseases (IBD and metabolic syndrome)
- Fact 5: multi-layered mucus structures regulate this interaction
Hypothesis

• Agents that disrupt mucus–bacterial interactions might promote gut inflammation

• Fact 6: Emulsifiers can increase bacterial translocation across epithelia in vitro

Methods

1. wild-type C57Bl/6
2. two engineered strains (IL10 \(^{-/-}\) and Tlr5 \(^{-/-}\))
3. Germ free mice

- carboxymethylcellulose (CMC) or polysorbate-80 (P80) via drinking water (1.0% w/v or v/v, resp.) for 12 weeks.

*P80: FDA approved at up to 1.0%.
* CMC deemed ‘generally regarded as safe (GRAS)’ used at up to 2.0%
Endpoints and Results

Microbiota encroachment of the epithelium

- Wild Type, Water
  - closest bacteria 25 μm from epithelial cells
  - no bacteria within 10 μm

- Wild Type Emulsifier treated
  - average distance reduced > 50%
  - bacteria in contact with the epithelium

- IL10 \(^{-/-}\) and Tlr5 \(^{-/-}\)
  - basal microbiota encroachment
  - enhanced by both CMC and P80
Confocal microscopy + mucus-preserving Carnoy fixation

![Images of microscopy results for WT and IL10−/− mice under different conditions: Water, CMC, P80.](image)

- **Water**
- **CMC**
- **P80**

**Distance of bacteria from IEC (μm)**

- **Water**
- **CMC**
- **P80**
Endpoints and Results

Microbiota composition

• Both CMC and P80 dramatically altered composition of both faecal and intestinal-adherent microbiota in all groups.
• reduced levels of Bacteroidales / increased levels of bacteria with mucolytic properties
• marked reduction in microbial diversity
Endpoints and Results

Increase in colonic adherent bacteria
Endpoints and Results

Pro inflammatory activity in faeces

- Exposure to emulsifiers increased faecal levels of bioactive lipopolysaccharide LPS and flagellin in all groups

* lipopolysaccharide (LPS) and flagellin receptors TLR4 and TLR5 activate pro-inflammatory gene expression
Endpoints and Results

Gut permeability

- Emulsifier treatment increased gut permeability in wild-type and Il10-/- mice

*increased levels of serum antibodies to flagellin and LPS correlate with permeability

Conclusion 1

“exposure to dietary emulsifiers results in erosion of the protective function of the mucus, increased bacterial adherence and a more pro-inflammatory microbiota”
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Endpoints and Results

Features of colitis

– the presence of immune cell infiltrates
– changes in gross colon morphology
– elevated levels of the leukocyte myeloperoxidase
– Faecal lipocalin 2 (LCN2)

• Il10-/- and Tlr5-/-: promoted extent and incidence of colitis, 10fold increase in LCN2
• wild-type: subtle histopathologic changes, modest increase in LCN2
Conclusion 2

“emulsifiers may promote robust colitis in susceptible hosts and induce low-grade inflammation in wild-type hosts“
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Conclusion 2

Emulsifiers

Microbiota – Host Interaction
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Endpoints and Results

Microbiota required?

• Emulsifier treated germ free mice
  – No change in LCN2, Colon length, no splenomegaly
  – No reduction in mucus structure

• Transfer of microbiota from emulsifier-treated mice to germ-free mice
  – microbiota encroachment, low grade inflammation
  – elevated levels of faecal LPS and flagellin
  – altered microbiota composition
"emulsifier-induced changes in the microbiota have a role in driving the inflammation and metabolic changes promoted by these food additives"
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Discussion

• Steady increase in the consumption of food additives, many of which have not been carefully tested
• Testing has used animal models designed to detect acute toxicity and/or promotion of cancer
• Emulsifiers, can disturb the host–microbiota relationship and thus promote intestinal inflammation, which can manifest as colitis or metabolic syndrome.
• Dietary emulsifiers may have contributed to the post-mid-twentieth-century increase in incidence of inflammatory bowel disease and metabolic syndrome
Limitations

Animal model study
Continuous exposition to emulsifier
Withdrawal effect?

Overall

• Provides possible explanations for epidemiologic data
• Proves Hypothesis step by step
• Excludes interfering factors
Thank you for your attention