THE ROLE OF INTESTINAL MICROBES IN THE IRRITABLE BOWEL SYNDROME; FROM BENCH TO BEDSIDE ... AND BACK

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Hospital Universitari Vall d’Hebron Nov 2010
OVERVIEW

1. Microbes as a trigger for the development of IBS: The story of Walkerton.

2. Commensal microbes and IBS

3. Commensal microbes and gut dysfunction

4. Psychiatric co-morbidity in IBS: Commensal microbes and the brain

5. A new conceptual model of IBS
Well established entity
Recent work has addressed the epidemiology and pathophysiology of PI-IBS
CANADA'S WORST-EVER E. COLI
CONTAMINATION
WALKERTON MAY 2000

Heavy rain & flooding
Friday May 26th 2000.

Pollution of town’s water supply

6 deaths

Local hospital overwhelmed

Town shut down
10 YEAR FOLLOW UP OF THE RESIDENTS OF WALKERTON

✓ THE INCIDENCE, PREVALENCE AND NATURAL HISTORY OF POST-INFECTIVE IBS

✓ RISK FACTORS FOR PI-IBS

✓ GENETIC PREDISPOSITION

✓ PATHOPHYSIOLOGY

? LONG TERM CONSEQUENCES
Local residents were invited to undergo structured assessments at research clinics established 2 years after the outbreak.

The cohort of 2069 subjects was divided into 701 controls without gastroenteritis, 904 subjects with clinically suspected gastroenteritis, and 464 subjects with self-reported gastroenteritis that could not be substantiated by another source.

Rome I criteria were met by:

- 10.1% of controls
- 27.5% of subjects with self-reported GE (P<.001)
- 36.2% of subjects with clinically suspected GE (P<.001)

POST-INFECTIOUS IRRITABLE BOWEL SYNDROME IS A CHRONIC CONDITION

EIGHT YEAR PROGNOSIS OF POSTINFECTIONOUS IRRITABLE BOWEL SYNDROME FOLLOWING WATERBORNE BACTERIAL DYSENTERY GUT 2010

- The prevalence of IBS among 742 eligible subjects who suffered acute gastroenteritis during the outbreak was:
  - 28.3% after 2-3 years
  - 15.4% after 8 years, but remained significantly increased compared with controls who did not have acute gastroenteritis (OR 3.12; 95% CI 1.99 to 5.04).

- Independent risk factors for PI-IBS at 8 years included
  - female gender,
  - younger age,
  - prior anxiety/depression,
  - fever or weight loss during the acute enteric illness.

- IBS symptoms were not stable over time.

GENETIC PREDISPOSITION TO PI-IBS

• 79 functional variants of genes with products involved in serotonergic pathways, intestinal epithelial barrier function, and innate immunity screened and fine mapping performed in regions of interest.

• Single nucleotide polymorphisms compared between Walkerton residents who developed gastroenteritis and reported PI-IBS (n = 228, cases) and those gastroenteritis but did NOT develop PI-IBS (n = 581, controls)

4 GENETIC VARIANTS ASSOCIATED WITH PI-IBS:

2 variants located in TLR-9 gene (rs352139, P545P; \( P = .0059 \) and rs5743836, -T1237C; \( P = .0250; r^2 < 0.14 \));

1 variant in cadherin-1 (CDH1) gene (rs16260, -C160A; \( P = .0352 \));

1 variant in interleukin-6 gene (IL6) (rs1800795, -G174C; \( P = .0420 \)).

The TLR9, IL6, and CDH1 variants all persisted as independent risk factors for PI-IBS when controlling for previously identified clinical risk factors.

• Lactulose-Mannitol urinary excretion measured in 132 Walkerton residents with PI-IBS and 86 controls.

• Lactulose-mannitol ratios increased among cases vs. controls (Mann-Whitney mean rank 118.8 vs. 95.3, \( P = 0.007 \)); cases were more likely to have a ratio >0.020 (\( P = 0.007 \)).

• Increased permeability associated with increased stool frequency.

• Acute gastroenteritis is the highest risk factor known for the development of IBS (RR= 11.9). García Rodríguez LA and Ruigómez, A. BMJ 1999).

• Inefficient down-regulation of the inflammatory response to acute bacterial infection implicated in development of PI-IBS. Gwee KA, Collins SM et al, GUT 2003.

• Increased intestinal permeability associated with increased EE cells and lymphocytes in gut wall (Spiller R et al GUT 2000) and inflammatory cells (Gwee KA et al GUT 1999).

• Antibiotic use during acute gastroenteritis higher in those who subsequently developed PI-IBS (Gwee KA et al Lancet 1996)
CONCEPTUAL MODEL OF PI-IBS

- Microbial Pathogenicity
- Bacterial Recognition
- Intestinal Permeability
- Inflammatory Response
- Genetic Susceptibility
- Stress

Altered Gut Function & Symptom Generation → CHRONICITY
2. COMMENSAL MICROBES AND IBS

• INFECTION OR ANTIBIOTICS AS TRIGGERS FOR IBS

• EVIDENCE OF ALTERATIONS IN THE COMPOSITION & ACTIVITY OF COMMENSAL INTESTINAL BACTERIA IN IBS PATIENTS
The Aetiological Role of Antibiotic Prophylaxis with Hysterectomy in Irritable Bowel Syndrome

J Obstet Gynaecol 1984, Vol. 5, No. s1, Pages S22-S23
Virginia Alun-Jones, A. J. Wilson, J. O. Hunter and R. E. Robinson
Departments of Gastroenterology and Gynaecology, Addenbrooke's Hospital, Cambridge, UK

300 patients undergoing hysterectomy were randomized to metronidazole or placebo.

Significantly more IBS in antibiotic treated group than controls.
ANTIBIOTIC USE ASSOCIATED WITH DEVELOPMENT OF IBS


ANTIBIOTICS TRANSIENTLY PERTURB THE MICROBIOTA, BUT HOW DOES THIS LEAD TO CHRONIC GUT DYSFUNCTION
3. COMMENSAL MICROBES AND GUT FUNCTION & DYSFUNCTION

• COMPARISONS OF GUT FUNCTION IN AXENIC & COLONIZED MICE.

• EFFECTS OF PERTURBATION OF COMMENSAL BACTERIA ON GUT FUNCTION
GUT MICROBIOTA INFLUENCE VISCERAL PERCEPTION – EFFECT OF PROBIOTIC BACTERIUM

Anti-nociceptive properties of *Lactobacillus acidophilus*

**In Vitro**
- Cannabinoid CB2-R
- Opioid-R µ1
- NF-κB
- Epithelial Cell Line

**In Vivo**
- Analgesic effect of administered L.acidophilus to control of hyperalgesic rats = 1mg morphine sc

Rousseaux et al Nature Medicine 2007
GUT MICROBIOTA DETERMINE NORMAL ABNORMAL GI PHYSIOLOGY – STUDIES IN AXENIC MICE

**IMMUNE SYSTEM**

- IgA small intestine
- IgA Peyer’s patch
- CD4 small intestine

**INTESTINAL MOTILITY**

- Germ-free vs. post colonisation

**Graph:**
- Germ Free
- MMC periodicity
- Colonized
- Husseby et al
ABNORMAL INTESTINAL MOTILITY ALTERS GUT FLORA

Drug-Induced Suppression of MMCs

Normal motility

The Migrating Motor Complex (MMC)

Titers of microorganisms in duodenal segments for each experimental group. Each bar represents the mean ± SE.

Scott et al Gastroenterology

1982
THE INTER-RELATIONSHIP OF GUT MICROBIOTA AND GUT PHYSIOLOGY

THE MICROBIAL COMPOSITION OF THE GUT

• Number of bacteria
• Types of bacteria
• Bacterial metabolism

THE PHYSIOLOGY OF THE GUT

• Changes in motor activity/bacterial clearance
• Changes in epithelial activity/mucus secretion and biofilm
DOES PERTURBATION OF GUT FLORA INDUCE LOW GRADE INFLAMMATION & ALTER GUT PHYSIOLOGY?
NIH Swiss mice received by gavage a combination of non-absorbable antibiotics or drinking water (placebo) for 10 days.

Responses to CRD were measured before, and at 10 and 30 days after starting the antibiotics.

For the first 5 days, mice received bacitracin 4 mg/ml, neomycin 4 mg/ml, and primaricin 0.2 g/ml of drinking water during the first five days. The doses were halved for the second 5 days.

The probiotic *Lactobacillus paracaseii* (NCC246, Nestle Research Institute) was administered in a dose of 100 µl of $10^{10}$ concomitantly with the same combination of antibiotics *paracasei* 1/ml in spent culture medium, or placebo, by oral gavage. for 10 days

Verdu et al GUT 2006
Total lactobacilli populations from colonic content and tissue on days 10 and 30 in antibiotic treated mice (ATB, n=5 per group) and in controls.
PERTURBATION OF INTESTINAL MICROBIOTA
INCREASED MPO ACTIVITY IN GUT AND
TRANSIENTLY INCREASED RESPONSES TO
COLORECTAL DISTENSION

(A, B) Percentage of colorectal distension (CRD) responses to 30 mm Hg versus day 0 in placebo and antibiotic treated mice.

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(A, B) Percentage of colorectal distension (CRD) responses to 30 and 60 mm Hg versus day 0 in placebo, antibiotic (ATB), and antibiotic + Lactobacillus paracasei resuspended in spent culture medium (ATB+L pa-SCM) treated mice.
PERTURBATION OF GUT MICROBIOTA INCREASED IMMUNOREACTIVE SUBSTANCE P IN THE GUT WALL; REVERSED BY LACTOBACILLUS PARACASEII

Substance P (SP) immunostaining:

(A) Day 0.
(B) Day 10 after placebo therapy.
(C) Day 10 after antibiotics alone.
(D) Day 10 after antibiotics + Lactobacillus paracasei.
(E) High power view of (C). Showing Thick arrow indicates SP staining in the myenteric and submucus plexi.
INTERIM SUMMARY

CHANGE IN SENSORY NERVE FUNCTION

INCREASE IN SUBSTANCE P.

SUBCLINICAL INFLAMMATORY RESPONSE

PERTURBATION OF PREVIOUSLY STABLE MICROBIOTA
A PROPOSED RELATIONSHIP OF THE MICROBIOTA AND GUT DYSFUNCTION IN IBS

UNSTABLE GI ENVIRONMENT

IBS RISK FACTORS:
ACUTE GASTROENTERITIS
ANTIBIOTICS
STRESS

INSTABILITY OF MICROBIOTA

ABNORMAL GUT PHYSIOLOGY

LOW GRADE SUB CLINICAL INFLAMMATION
CHANGES IN THE INTESTINAL MICROBIOTA IN IBS PATIENTS

IS IT “ABNORMAL”?

IS IT UNSTABLE?
RISK FACTORS FOR IBS ARE KNOWN TO DISRUPT GUT FLORA

1. Acute gastroenteritis
2. Antibiotic usage
3. Stress
4. Surgery
ABNORMAL BACTERIAL FERMENTATION IN IBS

• INCREASED COLONIC FERMENTATION OBSERVED IN IBS PATIENTS COMPARED TO CONTROLS (King et al 1998; Treem et al 1996)

• BACTERIAL OVERGROWTH ASSOCIATED WITH GI SYMPTOMS IN SOME IBS PATIENTS (Pimentel et al 2006)
# The Intestinal Microbiota in IBS

<table>
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<tr>
<th>Author</th>
<th>Methods</th>
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<th>Description</th>
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<td>Balsari 1982</td>
<td>Culture</td>
<td>20</td>
<td>Lactobacilli &amp; bifidobacteria</td>
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<tr>
<td>Bradley 1987</td>
<td>Culture</td>
<td>1</td>
<td>Considerable variation 18m</td>
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<td>Matto 2004</td>
<td>PCR-DGGE</td>
<td>21</td>
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<td>Malinen 2005</td>
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<td>Kassinen 2007</td>
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<td>Lyra 2009</td>
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<td>Most differences seen in IBS-D</td>
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<td>Tana</td>
<td>qPCR+Culture</td>
<td>26</td>
<td>Increased lactobacilli and Veillonella</td>
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<td>Codling 2010</td>
<td>PCR-DGGE</td>
<td>47</td>
<td>Changes in IBS seen in fecal &amp; mucosal niches</td>
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• Superficial mucus layer significantly thicker in patients with diarrhea than in healthy controls (B vs A).

• Mucus inclusions in the feces leading to mucus septa prominent in patients with diarrhea (D) v controls (C).

• Homogeneous web structure of the bacterial assembly in controls (E) disrupted by multiple striae in loose, diarrheal stools (F).

FISH: orange fluorescence at original magnification 400×.

Swidswink A et al Gastroenterology 2008
POOR MUCUS PENETRATION BY *FECALIBACTERIUM PRAUSNITZII* IN FUNCTIONAL DIARRHEA

- Multicolor FISH in a patient with functional diarrhea.

- The mucotrop *Enterobacteriaceae* (Cy3, *orange fluorescence*) is located on the border between feces and mucus and readily enters mucus in significant numbers.

- In contrast, *F prausnitzii* (Cy5, *red fluorescence*) is mainly located in feces and enters mucus in low concentrations.

- *F prausnitzii* has been shown to induce anti-inflammatory responses in gut epithelium. (Sokol H PNAS 2008)

Swidswinki A et al Gastroenterology 2008
• Majority of studies demonstrate altered microbiota in IBS; reduced diversity of bacteria.

• Reduction in *Lactobacillus* or *Bifidobacterium* seen in >1 study but finding has not been consistent.

• Greater temporal instability of microbiota over time seems to be a reproducible finding in IBS patients compared to controls (Bradley 1987; Matto et al 2004; Maukonen 2006).

• Disruption of biofilm may change access of selected bacteria to host tissue (Swidswinski 2008)
4. PSYCHIATRIC CO-MORBIDITY IN IBS:

COMMENSAL MICROBES AND THE BRAIN
PSYCHIATRIC CO-MORBIDITY IN IBS: THE TRADITIONAL MODEL

>60% of IBS patients have psychiatric co-morbidity

? Role of gut

Symptom reporting & health care seeking behavior

Symptom generation
THE BIO-PSYCHO-SOCIAL MODEL

Behavioural Factors

BI-DIRECTIONAL GUT-BRAIN AXIS

CHRONIC GUT DYSFUNCTION

LOW GRADE INFLAMMATION

SYMPTOM GENERATION

SYMPTOM REPORTING & HEALTH CARE SEEKING BEHAVIOR

Environmental triggers e.g. Infection
A MICROBIAL-CENTERED HYPOTHESIS

Environmental triggers e.g. Infection, antibiotics, diet…

- ALTERED BEHAVIOR

- CHRONIC GUT DYSFUNCTION
  - LOW GRADE INFLAMMATION

- SYMPTOM GENERATION

- SYMPTOM REPORTING & HEALTH CARE SEEKING BEHAVIOR

UNSTABLE MICROBIAL CONTENT OF THE GUT
CAN THE INTESTINAL MICROBIOTA INFLUENCE THE BRAIN & BEHAVIOR?
EXPERIMENTAL EVIDENCE SUPPORTING A LINK BETWEEN GUT MICROBIOTA AND THE BRAIN

1. OBSERVATIONS IN AXENIC MICE.

2. EFFECT OF PERTURBATION OF THE INTESTINAL MICROBIOTA ON BRAIN & BEHAVIOR.

3. TRANSFER OF BEHAVIORAL PHENOTYPE VIA THE MICROBIOTA.
Ia. Germ-free Mice Exhibit Impaired Learning & Memory

- Behavioural Testing
- BRAIN BDNF

Section of hippocampus from germ-free mouse - Reduced mRNA expression of BDNF in CA1 region

*Courtesy of McVey-Neufeld KA BBI, SJH & McMaster University*
Ib COLONIC BACTERIA ARE CRITICAL FOR THE DEVELOPMENT OF THE HPA STRESS RESPONSE

- Mild restraint stress
- Enhanced increase in ACTH
- Normalized by Bifidobacterium infantis
- Reduced following colonization by SPF feces

Increased plasma ACTH in GF mice following stress

Sudo N et al J Physiol 2004
5 week old male CF1 mice randomly assigned to receive standard rodent chow (PP diet) or chow containing 50% lean ground beef (BD diet) for 3 months.

Stool analysed by pyrosequencing (bTEFAP) showed greater bacterial diversity in the beef supplemented diet.

Improved working (P=0.0008) and reference memory (P<0.0001), slower speed (P<0.0001) in seeking food as well as reduced anxiety level in the first day of testing (P=0.0004).

However, behavioral changes could be due to higher tauramine and methionine. These have been shown to influence memory and learning. Rattiner LM, Biol Psychiatry 2000; Cao XJ, Eur J Pharmacol 2008. Li W, et al: Physiol Behav. 2009
III STRATEGY: PERTURBATION OF THE MICROBIOTA BY ANTI-MICROBIALS

10-14 days of:
- neomycin (4 mg/ml)
- bacitracin (4 mg/ml)
- primaricin (0.2 mg/ml)

Verdu et al GUT 2006
THE STEP DOWN TEST
Latency to step down

EXPLORATORY BEHAVIOUR

The Light Box – Dark Box Test

Time spent in light box
Number of white zone entries
RESULTS

ANTIBIOTICS ADMINISTERED ORALLY PRODUCED A TRANSIENT ALTERATION OF THE INTESTINAL MICROBIOTA.

ORAL BUT NOT SYSTEMIC ANTIBIOTICS PRODUCED A TRANSIENT CHANGE IN BEHAVIOR, RESULTING IN ANXIOLYTIC BEHAVIOR.

BEHAVIORAL CHANGES INDEPENDENT OF VAGAL INTEGRITY OR IMMUNE ACTIVATION

ORAL ANTIBIOTICS DID NOT INFLUENCE BEHAVIOR OF GERM FREE MICE
4. THE ADOPTIVE TRANSFER OF BEHAVIOURAL TRAITS TO GERM FREE MICE VIA THE MICROBIOTA
EXPLOITATION OF ESTABLISHED DIFFERENCES IN BEHAVIOR AND MICROBIOTA AMONG COMMONLY USED MOUSE STRAINS

Balb/c                                 NMRI OR AKR                        NIH SWISS
BEHAVIORAL DIFFERENCES BETWEEN: NIH SWISS & BALB/C MICE

Latency to Step-down:
1. To what extent does the flora of recipient NMRI mice resemble that of the donor Balb/c mouse?

2. To what extent does the behavior of recipient NMRI mice resemble that of the donor Balb/c mouse?
RESULTS

PERTURBATION OF THE MICROBIOTA ALTERS BEHAVIOUR BY A MECHANISM THAT IS INDEPENDENT OF IMMUNE ACTIVATION OR VAGAL INTEGRITY.

TRANSFER OF INTESTINAL MICROBIOTA BETWEEN MURINE STRAINS RESULTS IN BEHAVIORAL CHANGE IN RECIPIENT MICE

BEHAVIORAL CHANGE IN RECIPIENT MOUSE ACCOMPANIED BY ALTERATIONS IN HIPPOCAMPAL BRAIN DERIVED NEUROTROPIC FACTOR

CHANGES MAY OCCUR IN EITHER DIRECTION, BASED ON THE BEHAVIORAL PHENOTYPES OF DONOR AND RECIPIENT MICE)
Antibiotic induced perturbation of microbiota results in transient alteration in behavior & brain chemistry.

• Cannot be attributed to a malaise effect

• Independent of vagal integrity

• Not accompanied by inflammatory cytokine response
CANDIDATE MEDIATORS

• Factors that alter tryptophan metabolism. Desbonnet L et al 2008
• Butyrate has anti-depressant effects. Schroeder F at al 2007
• Proprionic acid induces autism-like behaviour Schultz SR 2009
• Bacterial production of neuro-active substances: Forsythe P et al 2009
  e.g. GABA, benzodiazepine receptor ligands
A NOVEL UNIFYING CONCEPT

Infection, Antibiotics or other factors (e.g. stress)

Perturbation of the Microbiota

Low Grade Inflammation

CHRONIC GUT DYSFUNCTION

GI SYMPTOM GENERATION

PSYCHIATRIC CO-MORBIDITY

ALTERED BEHAVIOR
CLINICAL IMPLICATIONS & FUTURE DIRECTIONS

• PROBIOTICS AS THERAPY FOR IBS
  – INCLUDING PYCHIATRIC CO-MORBIDITY

• MINING THE INTESTINAL MICROBIOTA FOR NOVEL THERAPEUTIC TARGETS & THERAPIES…

McMaster Centre for Microbial Chemical Biology

Analysis of naturally occurring molecules