A role for the gut microbiome in immune-mediated diseases

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Human diseases are broadly caused by interaction between the host genetics and environmental factors, of which the microbiome is one key grouping.

- There are 10x more bacteria than cells in our body.
  - 29% of all bacteria live in the gut.
  - 150 times more genes in our gut than our body otherwise.

Humans have evolved to become dependent on their gut microbiome and to develop systems of interacting with it.

- The gut microbiota regulate metabolism and immune development and function.
  - Therefore they are likely a priori to be involved in immune dysfunction and disease.

Why study the microbiome?
• Compared faecal flora between 15 AS cases and 15 controls by 16SrRNA/DGGE

• No difference in profiles noted.
Sequencing: A recent history

- 1977: 100bp, 1 day
- 1993: 1000bp, 1 day
- 1998: 96000bp, 1 day
- 2004: 30Gb, 7 days
- 2009: 200Gb, 10 days
- 2015: 1.6Tbases, 3 days
16S rRNA sequencing / meta-barcoding

Sample (e.g. water, soil, sediment, faeces, biopsy, ...)

DNA extraction

DNA

PCR

PCR-amplified rRNA genes

DNA sequencing

rRNA gene sequences

clustering (e.g. >99% identity)

BLAST-search rRNA sequence database with millions of taxonomically classified rRNA sequences (e.g. RDP, Silva)

OTU Table:

<table>
<thead>
<tr>
<th>OTU</th>
<th>Species</th>
<th>Sample1</th>
<th>Sample2</th>
<th>Sample3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E.coli</td>
<td>17</td>
<td>0</td>
<td>335</td>
</tr>
<tr>
<td>2</td>
<td>S.aureus</td>
<td>231</td>
<td>11800</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>unknown</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Counts of OTUs per sample

Relative Abundance
Shotgun metagenomics

1. DNA extraction
2. Fragmentation
3. DNA sequencing

Assembly

Metabolic reconstruction

Phylogenetic binning

Gene finding & annotation

Relative Abundance

>seq1
GCCGTAGTCC...
>seq2
TATGCCGGTA...
>seq3
...
...
Fig 1. Heat-plot representing the taxonomic composition of the samples at genus level.

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0148028
Extent of diversity is huge, and so far undercharacterised

- Shotgun sequencing of 11,850 human gut microbiomes
- Identified 1,952 novel bacteria
  - 26% did not belong to any known family
  - 40% did not belong to any known genus
  - This represented a 281% increase in the known genomic diversity.
- Suggests that strain specific diversity for example is far greater than currently curated.
• Noticed differences in Th17 numbers in intestinal walls of mice from JAX lab (low Th17) and Taconic Farms (high Th17)
• Found that cohousing of JAX mice with Taconic farm mice, or gavaging JAX mice with intestinal fluid from Taconic farm mice led to development of Th17 lymphocytes in JAX mice.
• Taconic farm mice had high levels of segmented filamentous bacteria (SFBs) adherent to the gut mucosa
  • JAX mice did not
• Transfer of SFBs into mice with low Th17 raised in germ free conditions led to the development of Th17 cells in those mice.
Ivanov, Cell, 2009
Effects of the gut microbiome on immune development

• Induce production of IgA by germinal centres
  Kamada, Gastroenterology, 2014

• Promote development of Peyer’s patches

• If absent leads to Th2 dominant gut mucosal adaptive immune system, deficient in Th1 and Th17 cells
  Mazmanian, Cell, 2005.

• Bacterial metabolites also shape the immune system
  • Short chain fatty acid (butyrate, acetate, propionate) are produced by gut bacteria such as Faecalibacterium prausnitzii
  • SCFA supplementation leads to increased T-regs, reduced Th17 numbers and activity.
    Zhang, BMC Gastroenterol, 2016 (and others)
Human Immune Diseases Associated with Gut Dysbiosis

• Gut dysbiosis has been associated with
  • Primarily intestinal diseases including IBD, IBS, celiac disease
  • Primarily extra-intestinal diseases including T1D, asthma, RA, multiple sclerosis, SLE, psoriasis, ankylosing spondylitis.

• In most cases no clear distinction can be made between causation and association.

• In all cases the microbial picture is complex
  • No common autoimmune disease has been shown to be caused by a single individual microbe.

• Replication of findings between studies has been very inconsistent.
Gut Microbiome and Cancer

- Recent studies have shown that clinical response to PD-1 and PD-L1 inhibitors are influenced by the gut microbiome.

- Non-responders were more likely to have been treated with antibiotics within 2 months before, or 1 month after, the first administration of PD-1/PD-L1 mAb and had a less diverse microbiome.

- Transfer of the non-responder vs responder microbiome to PDX mice led to concordant differences in survival.

Enteric Infections and Spondyloarthritis.

- Enteric infection is a common trigger of reactive arthritis
- Gut permeability is increased in AS patients and their first degree relatives
- Strong clinical association of AS and IBD
- Subclinical involvement of terminal ileum is common in AS
- In B27-transgenic rat model
  - Remains disease free in germ free environment
  - *Lactobacillus rhamnosus* colonisation protects against development of arthritis.
Subclinical Gut Disease in AS

- Mielants et al, J Rheumatol, 1995
  - Ileocolonoscopy on 123 patients with SpA, monitored for 2-9 years
  - 23% had acute ileal inflammatory lesions
  - 45% had chronic ileal inflammatory lesions
    - When found in cases with non-AS-SpA, risk of progression to AS was increased.
A Common Genetic Background for Inflammatory Bowel Disease and Ankylosing Spondylitis

A Genealogic Study in Iceland

Bjarni Thjodleifsson,1 Árni J. Geirsson,1 Sigurdur Björnsson,1 and Ingvar Bjarnason2

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
<th>First-degree relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td>205</td>
<td>94 (74–114)‡</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>261</td>
<td>3.7 (1.1–8.5)‡</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1,091</td>
<td>2.9 (1.6–5.0)‡</td>
</tr>
<tr>
<td>IBD</td>
<td>1,352</td>
<td>3.0 (1.8–4.6)‡</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>261</td>
<td>5.9 (1.8–11.7)‡</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1,091</td>
<td>2.1 (1.1–3.5)‡</td>
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<tr>
<td>Ulcerative colitis</td>
<td>1,091</td>
<td>5.4 (4.3–6.9)‡</td>
</tr>
<tr>
<td>IBD</td>
<td>1,352</td>
<td>4.8 (3.9–5.8)‡</td>
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</table>

ARTHRITIS & RHEUMATISM
Vol. 56, No. 8, August 2007, pp 2633–2639
Li et al, Genes and Immunity, 2017.
• Skg mouse model phenotype depends on animal research facility
• Skg SpA and ileitis are ameliorated by GF housing
• Disease can be triggered by altered Schaedler flora
• Cohousing Skg with BALB/c can transfer arthritis.
AS gut microbiome is different from HC

- Using 16S sequencing on terminal ileal biopsies, AS cases can be distinguished based on the microbial profile from healthy controls.

- AS cases have higher abundances of:
  - Lachnospiraceae (P=0.001),
  - Ruminococcaceae (P=0.012)
  - Rikenellaceae (P=0.004)
  - Porphyromonadaceae (P=0.001)
  - Bacteroidaceae (P=0.001)

- Decreases were noted in:
  - Veillonellaceae (P=0.01)
  - Prevotellaceae (P=0.004)

Costello et al., Arthritis Rheumatol, 2014
...it was me, right?
Does the Microbiome Differ by Genotype?

Brown et al, unpublished
**Effect of HLA-B27 on the gut microbiome**

**Aim:** To examine the effect of HLA-B27 on the composition of the intestinal microbiome using culture independent, 16S rRNA amplicon sequencing.

**Design:** Matched mucosal intestinal samples from HLA-B27 positive and negative profiled using 16S rRNA amplicon sequencing.

Analysis using normalised OTU counts and sparse Partial Least Square Discriminant Analysis in MixOmics (le Cao et al, PIOS One, 2016)

**Samples:**
568 samples from 6 body sites from 107 individuals (9 of those B27 Pos)
- 90 ileum
- 101 left colon
- 97 right colon
- 104 Cecum
- 92 rectum
- 84 stool

http://mixomics.org/mixmc/
BMI and the microbiome

Asquith et al, in Biorxiv, 2019
Gender and the microbiome

Asquith et al, in Biorxiv, 2019
Sample size and the microbiome

Asquith et al, in Biorxiv, 2019
HLA-B27 positive samples cluster distinctly from HLA-B27 negatives

Permanova = 0.04

Asquith et al, in Biorxiv, 2019
How does the microbiome induce immune-mediated disease?

• Role of host genetics remains unclear.

• Host genetics may:
  • Favour a more pro-inflammatory gut microbiome
    • Directly alter the immune response to ‘normal’ microbiota, higher proportion of inflammatory microbiota
    • Direct interaction effect of bacteria on immune system, or via metabolic factors eg. SCFA?
  • Increase invasiveness of the gut mucosa
    • Disruption of the epithelial barrier of the gut, allowing for pathogens to permeate the intestinal barrier and elicit immune responses
  • Increase of certain bacteria may elicit aberrant immunological response
    • Molecular mimicry. Bacterial peptides mimicking HLA-B27-restricted epitopes my stimulate activation of autoreactive T or B cells.

• More than one of these mechanisms may operate!
A pro-inflammatory microbiome?

- Shotgun metagenomics. 250 case-control cohort of Han-Chinese individuals.
- ~50% of AS patients treated with TNFi
  - Common treatment for inflammatory arthritis
- Replicated in two datasets disturbance of gut microbiome in AS cases vs controls
- Demonstrated depletion of *Faecalibacterium prausnitzii*, corrected by TNFi treatment
  - Notable ‘peace keeping’ microbe
  - Depletion has been associated many diseases. (a range of inflammatory bowel disease such as Crohn’s disease, obesity, coeliac disease, colorectal cancer, pediatric arthritis...etc)
  - Produces short chain fatty acids (acetate, butyrate, propionate) which are anti-inflammatory.
  - Do TNFi work in part through effects on the microbiome?
Potentially cross-reactive arthritogenic peptides

• Calculated the overall abundance and diversity of bacterial peptides which mimic HLA-B27-presented peptides in AS case and control stool microbiomes.

• AS cases had greater abundance AND diversity of bacterial peptides which are known to be presented by HLA-B27, than did healthy controls.

• Does HLA-B27 fail to control the microbiome in AS patients, leading to immune stimulation and disease?
• Bacteria are seen invading the gut mucosa in AS patients (A-C) but not healthy controls (D)
• The extent of bacterial invasion correlates with the number of inflammatory cells in the gut mucosa
• This is associated with higher serum LPS levels in AS patients.
Vedolizumab – a two-edged sword

- Vedolizumab is an anti-α4β7 integrin antibody that prevents migration of lymphocytes into the gut.
- It is highly effective for IBD presumably by preventing proinflammatory cells entering the gut mucosal wall.
- It causes paradoxical axial spondyloarthritis
  - Main hypothesis is that this is due to increased bacterial migration into gut wall driving proinflammatory pathways, notably IL-23 production.
- Suggests microbiome drivers of IBD and axial spondyloarthritis operate through different mechanisms.
Microbiome for diagnosis?

• Gut microbial profiling distinguishes cases from controls in many diseases
  • One test could be used to assist in diagnosis of many conditions.
• Likely to have additive discriminatory capacity to genetic tests like polygenic risk scores.
• Utility prior to and at different stages of diseases, and in relation to ethnicity, diet and environment needs to be assessed.
Conclusions

• The gut microbiome has major effects on the human immune system development and later life function.
  • This is important in development of immune-mediated diseases and in resistance to cancer.

• Data suggests that the effects of the microbiome are driven by multiple interacting, rather than individual, bacterial species.

• The mechanisms by which the microbiome influence the immune system are not clear but may be as simple as metabolic properties rather than more complex immunological effects.
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