RESEARCH ARTICLE

MICROBIOME

Identifying Gut Microbe–Host Phenotype Relationships Using Combinatorial Communities in Gnotobiotic Mice

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Introduction

We know that a dysbosis in the microbiota can influence host well-being:

Mice deficient in the T-bet transcription factor develop spontaneous colitis by 8 weeks of age. Although the T-bet deficient background and microbiota are required for the onset of colitis, once developed aseptic transfer of the microbiota induces colitis in new hosts that lack genetic predisposition or immunodeficiency known to induce colitis.


In mice lack TLR5 signaling, the composition of the microbiota becomes skewed and induces the onset of metabolic disease in the host.

Vijay-Kumar, M. et al. 2010. Science 328, 228–231

Transplantation of fecal microbiota from adult female twin pairs discordant for obesity into germ-free mice resulted in increased total body and fat mass. This effect could be rescued by colonising the obese mice with Bacteriodes (dominant microbe in lean).


Aim: to develop a measurable, unbiased approach for identifying human gut bacterial strains that modulate phenotypic variation in recipient mice
Large-scale bacterial isolation

Arrayed donor culture collections
Each well contains a unique bacterial strain with a draft genome sequence

Fractionate community into different subset sizes with randomly selected membership

Gavage each gnotobiotic mouse with a unique, random subset of the arrayed donor culture collection

Gnotobiotic mice
Colonized with subsets of the arrayed donor culture collection

Phenotype screen
(immune profiling, metabolomics, adiposity)

Phenotype response

% FoxP3+ among CD4+ T cells
Serine (arbitrary units)
Fat pad weight / body weight

Phenotypic modulator identification
(feature selection, monoclonizations)

\[ \text{phenotype}_i = f(\text{species}_{1_i}, ..., \text{species}_{n_i}) \]
Experimental design:

Human fecal flora from 5 healthy US lean-female donors → one donor per germ-free C57BL/6 male mouse (8-10 wks) → 2 wks → measured Treg induction and adiposity

Induction of colonic Treg

Induction specific to colon

Induction depends on flora

Colonisation increases epididymal fat pad weight
Generated a clonally arrayed collection of sequenced anaerobic bacterial isolates from donor F60T2

17-member strain collection generated:

1) capable of recapitulating the Treg and adiposity phenotypes transmitted by the intact uncultured microbiota

2) large enough to develop experimental framework and computational tools to identify effector strains

3) these strains covered Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria

Generated a synthetic community in silico—here they assume bacteria respond in an additive manner in order to induce metabolic, phenotypic, and immunologic phenotypes
Max-saturation rate: the addition of more effector strains would not increase the effect size beyond a certain point.

Importance of model:
“Identifying the point at which the mean effect size has saturated can provide insights into the proportion of community members capable of perturbing that phenotype, and can inform follow-up experiments to identify the specific bacterial strains that mediate the phenotype.”

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**fig. S1 – Simulating phenotype effect size and saturation as a function of microbial community size.** If all members of a microbiota are capable of saturating the effect size of a given phenotype, then the mean effect size as a function of community membership size for randomly drawn communities will be a step function (upper left panel). As communities are simulated with fewer effector strains, the function steepness and point of saturation are reduced until the number of effector strains equals one. In the latter case, the mean effect size is seen to increase linearly with community size; i.e., the only community where the single effector strain is certain to be present is when all community members are used. Color code; blue dots, individual data points; red circle and lines, mean ± SD.
Non-saturating systems or systems that only saturate at large populations

One effector strain

>1 effector strain

Bimodal

**fig. S2 – Simulating phenotype effect size as a function of microbial community size.**

(A) When phenotype effect size is simulated with an additive model as a function of community subset size, in the case of no saturation the mean effect size will linearly increase as community size is increased. The underlying raw data points (blue dots) will be bi-modally distributed for phenotypes with a single effector strain. (B) As the number of effector strains increases, effect size becomes multimodal with N+1 modes, where N is the number of effector strains. Color key: raw data, blue circles; red circle and lines, mean ± SD.
Robotically fractionated the culture collection into random subsets from 1 to 17 strains on average each strain was tested in 46 diff. subsets (7.6 ± 3strains/mouse) total of 124 mice

1) adiposity
2) SCFA concentration of caecal contents (non-targeted GC-MS & LC-MS)
3) cLP Treg analysis
Identifying strains that influence gut metabolism

Based on their in silico analysis concentrations of more than 25% caecal metabolites = saturation (<3 strains per community)

-since a low number of bacteria in a community is required, then this means that a large proportion of the bacteria in the original human feces are capable of influencing caecal metabolites

Model data

Experimental data
Identifying strains that modulate host adiposity

Germ-free mice are leaner than their colonized counterparts;
- this reflects a complex set of interrelationships, both known and yet to be characterized, between the gut microbiota, biotransformation of dietary components, and regulation of host metabolism

Saturates with few members of the community
- suggests that many strains are capable of influencing effector
- they did monocolonizations with 5 bacterodies strains and all colonisations resulted in an increase in fat tissue

*this result does not exclude the effect of diet, bacteria-bacteria interactions, host effects
Identifying strains that modulate the host immune system

During our phenotypic screens, we observed that as few as two different bacterial strains were sufficient to induce a level of colonic lamina propria Treg accumulation equivalent to that achieved with the complete uncultured microbiota stained with FoxP3, Nrp1, CD103, CD4

Other strains from these families purchased from ATCC also induced Tregs - different bacterial donors (than gut-adapted strains) can drive Treg induction - also observed in *B. caccae* monocolonised NMRI mice
Expression of neuropilin-1 (Nrp1) in FoxP3+ Tregs [tTregs are Nrp1\text{hi}, whereas pTregs are Nrp1\text{lo}/-]

Greater proportions of Nrp1\text{lo/-} cells among Tregs were documented under each colonization condition

Tregs expressing the \(\alpha_E\) integrin CD103, suggest a more activated phenotype
Conclusions

I like the concept of using both in silico and real data to further explore how the microbiota influences the host through microbe-microbe interactions.

Nice paper and concept, although details in the methods are lacking

They are trying to modernize Koch’s postulates where groups of microbes that modulate phenotypic responses are identified along with the envrio. factors (like diet) that contribute to the overall host response (change fat pat)

Methodology is lacking, and their web application to stimulate phenotypic responses with a probabilistic additive model (http://faithlab.mssm.edu/model_viz/) does not work (yet?)
Genetically dictated change in host mucus carbohydrate landscape exerts a diet-dependent effect on the gut microbiota

Introduction

- Fucose residues are common in mucin glycans (terminally added), therefore the fucose sugar is at the interface of microbiota-mucus interactions

- **FUT2** gene encodes a galactoside 2-α-L-fucosyltransferase 2 responsible for adding a L-fucose residue in α1–2 linkage to the terminal β-D-galactose residue of gut mucus glycans

- **FUT2** gene expression is influenced by the microbiota and their ability to use fucose as a carbon source (*B. thetaiotaomicron*)

- Approx. 20% of humans lack functional copies of **FUT2**

- Susceptibility to diseases such as Norwalk, respiratory diseases, *V. cholerae*, *Helicobacter pylori* infection are influenced by the hosts’ secretor status of this gene

**Question**: how do host genotype and diet interact to shape gut microbial communities?
Effect of gut mucus fucosylation on the diversity and composition of a human microbiota transplanted into gnotobiotic mice fed a plant polysaccharide-rich standard diet.

Fut2-/- and Fut2+/+ (WT) mice had no difference in microbiota - consistent with both expressing fucose on mucus.

-interchangeably use HT and WT mice for controls

-in PCA Fut2-/- mice cluster slightly away from littermate controls (1b)

The ability of fucosylated glycans affects the overall diversity and composition of the microbiota.
Diet overrides the effect of gut mucus fucosylation on microbial composition

hypothesis: that a diet lacking complex polysaccharides would increase the microbiota dependence on the host mucus as an alternative source of glycans

This suggests that the elimination of complex polysaccharides has a greater impact on microbial composition than host genotype
Was there functional differences in the microbiota of mice on the SD vs the PD diet?

Genes involved in carbohydrate metabolism were enriched in the microbiota of PD fed mice compared to SD fed mice
-specifically glycoside hydrolases (involved in hydrolysing terminal host mucin glycans)
-could this be the host effect they are looking for? To answer this they did a case study

A Case Study of a Prominent Member of the Human Gut Microbiota Known to Adaptively Forage on Host Fucosylated Glycans → *B. theta*

Fut2+ (n = 4) and Fut2− (n = 4) adult GF male mice that were fed a PD diet were colonized with *B. thetaiotaomicron* and killed after 10 d.

In all mice, cecal contents including the mucus layer were removed, and RNA was extracted for transcriptional profiling of *B. thetaiotaomicron* by using custom GeneChips
Fucosylation determines transcriptional responses of B. thetaiotaomicron in the intestines of monocolonized Fut2− and Fut2+ mice fed a PD diet.

Upregulated genes involved in fucose catabolism that encode (Fut2+):
- L-fucose isomerase,
- L-fuculose-1-phosphate aldolase,
- L-fuculose kinase,
- fucose mutarotase,
- L-fucose permease

Where 11 polysaccharide utilisation loci were upregulated in Fut2−/−-mice

We concluded that in the absence of pressure to use host glycans (SD diet), host fucosylation status has minimal impact on B. thetaiotaomicron.

However, dietary polysaccharide deficiency interacts with host fucosylation status to significantly impact B. thetaiotaomicron’s glycan foraging in vivo.
Conclusions

Host mucus structure can influence the microbial populations - but that diet can also have a dominant effect over the microbiota.

Our data demonstrate that differences in host genotype that affect the carbohydrate landscape of the distal gut interact with diet to alter the composition and function of resident microbes in a diet-dependent manner.

I think it is a nice paper, and sheds light on to the importance of intestinal mucus in shaping the microbiota.