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Short Communication

Cautionary tales from the microbiome: Finding what is real and reproducible

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ABSTRACT

Statement of the Problem: Commonly-used methods of analyzing microbiome or RNA-seq datasets can be misleading and all the available information in a consistent manner are not in use. These results in many analyses being dominated by either the most abundant, or the rarest features: In fact, it is often the case that the most abundant taxa dominate multivariate outputs, and the rarest taxa dominate univariate outputs in the same dataset. Furthermore, these datasets have extraordinary properties that make the use of correlation and network analysis problematic. Methodology and Theoretical Orientation: Data collected using high throughput sequencing (HTS) methods are sequence reads mapped to genomic intervals, and are commonly analyzed as either normalized count data or relative abundance data. One reason for these normalizations is to attempt to compensate for the problem that the sequencing instrument imposes an upper bound on the number of sequence reads. Positive data with an arbitrary bound are compositional data and are subject to the problem of spurious correlation. Thus, ordination, clustering and network analysis become unreliable. A second problem is that the data are sparse: i.e., contain many 0 values. A third problem is that the largest measurement error is at the low count margins in these datasets. Conclusion & Significance: We use microbiome datasets to show how Bayesian estimation combined with compositional data approaches that examine the ratios between taxa give robust insights into the structure and function of microbial communities. I will present example datasets drawn from the human and ecological domains and show that ordination, differential abundance and correlation can be interpreted in an internally consistent manner that provides reproducible insights.

INTRODUCTION

The concept of symbiotic microbiomes (yes, plural) influencing our health seems now, in hindsight, to be obvious, and the fact that the science has caught up to the folk medicine has all sorts of people buzzing. Some of the buzz is well informed (see below), some not, but all in all we are making progress understanding a few of the ways in which our vast mucosal environment interacts with the outside world. At the same time its fair to say that we know very little yet, and have a long way to go. Some recent findings drive this point home. We can think of the frontier mentioned in the title in two ways. One, maybe obvious, is to think about the frontier of science, as this is where we find ourselves as the technology to do the some of this work was not widely available until recently. More subtly, we can think of the mucosal environments – oral, pulmonary, digestive, excretory, reproductive – as frontier environments where self interacts with non-self in an exploratory manner, that is, not confrontational a priori. There is a lot at stake: pathogen recognition and defense, nutrient uptake, metabolic regulation, waste disposal, on and on. It makes sense that there are tightly controlled and very complex rules of engagement. The new findings I want to review touch on some of these rules and suggest layers of control and organization that we really don't understand yet. Secondarily, we can study these systems with an eye on drug discovery.

Back to back papers in the December 16/26 double issue of Nature identify a critical pathway for the development of regulatory T cells (Tregs) in the gut. Data from the Ohno lab in Japan and the Rudensky lab in NYC paint broadly similar stories of the role of the specific commensal bacteria in fostering Tregs (see references 1 and 2, below). Both papers show that the fatty acid butyrate stimulates the development of Tregs. This in itself is not a new finding. Butyrate is a major energy source in mammalian metabolism and not surprisingly it's production is driven by commensal bacteria, notably the abundant Clostridia class of bacteria (some species within Clostridia are pathogenic, but that's a different story). Again, it's not particularly surprising that one of the most abundant mammalian commensals gives off good vibes in the form of fatty acids that support a quiet immune system. The papers differ in some curious ways, in particular, the Ohno paper states that the induction of Tregs was limited to the gut, while the Rudensky papers highlight Treg production in the lymph nodes and spleen, but not the colon. Regardless, the reason these papers made it into Nature is that they identify the mechanism by which butyrate induces Treg differentiation, and this is by inhibiting a histone deacetylase (HDAC IIa) thereby allowing for the specific acetylation (and therefore activation) of DNA elements that support Treg differentiation, notably at the FoxP3 promoter

and enhancer.

But before we all run out and start swallowing a bunch of butyrate capsules and subject ourselves to butyrate enemas, lets be clear about what these papers are saying and what they are not saying. First, we are dealing here with inbred mouse strains on carefully defined diets. Translation of the results to outbred humans on diverse diets is not so straightforward. That said, the results support eating a high fiber diet, which will yield plenty of butyrate and related fatty acids. Second, the papers agree on one thing very specifically, which is that the generation of Tregs in the gut is a local phenomena, specific to the colon (large intestine, south of the caecum). This makes sense of course, as that is where the Clostridia are cranking out the fatty acids. The application of these findings to colonic disease, notably Ulcerative Colitis, is worth exploring. But broadening the scope to include general health, well-being and immune serenity is not warranted – despite the pile on by the Supplements and Wellness Industries.

A very different story just came out in PNAS, and this one concerns the response of different populations to a gut pathogen found in the gastric mucosa (lining of the stomach). The bacterium Helicobacter pylori is found in about half of the human population worldwide. H. pylori is a causative agent of gastric adenocarcinoma in a small percentage of the people who are infected, less than 1%, although hotspots are known. One such hotspot was studied by a team from Vanderbilt who found that the higher incidence of H. pylori induced precancerous inflammation correlated with the presence of a European strain of the bacterium infecting an Amerindian population in Columbia. In contrast, an African strain of H. pylori infecting the descendants of African slaves nearby did not cause inflammation and cancerous lesions. The investigators conclude that H. pylori is mainly pathogenic when it occurs in a population distinct from that with which it co-evolved. So, a fine line between commensal and pathogen is drawn.

Conclusion:

The gut microbiome has been implicated in the development of Th17 effector T cells, at least in mice. This is interesting in light of where we started, with the generation of Treg cells, since in some ways Tregs and Th17s are the result of different developmental pathways that T cells take. Note that the first two studies reviewed were focused on extrathymic nTreg generation. Mice that are raised with no pathogens in their environment, including their food, which is irradiated, don't develop very many Th17s as a percentage of the total T cell population. Since Th17 cells are associated with diseases it seems reasonable to ask whether a Th17 inducing microbiota is linked to any particular disease. Littman's lab at the Rockefeller in NY has done exactly that. Newly diagnosed RA patients were found to carry the intestinal bacterium Prevotella copri at much higher levels than PA patients or healthy control patients (21%). This association of a specific pathogen with an autoimmune/chronic inflammatory disease is very striking. When mice were infected with a rodent-compatible strain of P. copri they developed pronounced intestinal inflammation, but not arthritis. Still, the intestinal inflammation was associated with the induction of Th17 cells, and so the hypothesis that this may underlie more systemic inflammation is still reasonable.

There are some problems with the story. The clinical development of IL-17 targeting drugs has shown that these do very well in PA and psoriasis, perhaps in inflammatory bowel disease, but they have failed to show sufficient benefit so far in RA. So at the level of drug discovery the link of an intestinal pathogen to Th17 T cells producing IL-17 and then to the disease, RA, seems to falter.

Thinking more broadly, the application of microbiome studies to drug development is in its infancy, and I think there is some reason for optimism as these studies become more sophisticated. The H. pylori and P. copri studies mentioned make it clear that many factors influence the response of a given population or individual to their microbioma. One interesting approach, the use of fecal transplantation to treat severe diarrhea and also Crohn's disease, has made it into early clinical trials. Isolation of the critical components that reset the immune system in the local and systemic settings is going to take significant time and effort, so we'll have to stay tuned.