Identification of diet-determined microbial colonization profiles of infection-resistant mice.

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Introduction

Although microorganism are found ubiquitously throughout our planet, our understanding of them remains limited. This limitation stems from the fact that most of what we know comes from studying pure cultures of different microbes under \textit{in vitro} conditions. While these studies have been illuminating about basic biological processes, they have revealed little about how microbes function in their natural environment and how they interact with each other. Over the last few years there has been a growing interest in characterizing both the diversity of microbes in the environment and their importance to their respective ecological niches. There have been several studies which have surveyed the microbiota of different environments such as tropical deciduous forests (Noguez et al. 2005), the ocean (Venter et al. 2004), hypersaline lakes (Loy et al. 2002), and hot springs (Wilson et al. 2007). Other studies have focused on the interaction of macroorganism-colonizing microbes surveying systems like the human intestine (Eckburg et al. 2005), termite gut (Warnecke et al. 2007), and the cystic-fibrosis patient lung (Flanagan et al. 2007).

While all of these studies have given great the diversity and ubiquity of microorganisms, perhaps the most important finding has been the observation that each ecological niche contains
a distinct set of microbial inhabitants, and in a way this has been one of the greatest research impediments. Because of the enormous diversity of ecological niches, environments, and host genetic factors, teasing apart the precise role of different microbes in different environments has been difficult. However, recently published work from the laboratory of Jeffrey Gordon has really taken microbial community analysis to the next level, characterizing the role of host-gut microbiota in diet-induced obesity (Turnbaugh et al. 2006; Ley et al. 2006; Turnbaugh et al. 2008). Perhaps the biggest breakthrough was the development of a mouse model for obesity in which environmental and genetic variables could be eliminated as well as a system of establishing uniform colonization of multiple mice by transferring the microbiota from a single sacrificed mouse to multiple germ-free recipients.

In this study, I intend to use a similar mouse model to study the establishment and perturbation of the colonizing microbiota over the course of exposure to different potentially infectious pathogens. There will be three primary aims: (1) establish multiple diet-controlled microflora backgrounds that have different degrees of sensitivity to infection by different pathogenic organisms, (2) investigate the effect of exposure to sub-lethal doses of different pathogenic organisms, (3) investigate potential for probiotics in the prevention or treatment of infectious disease. Each of the aims are partially dependent on each other to narrow the scope and degree of microbial profiling, as only diets and pathogens with pronounced effects will be followed up on in later aims.

**Aim 1:**
We can begin with a set of genetically identical mice. After weaning, different diets will be fed to these mice. At minimum we will use diets known to promote distinct microflora profiles in mice previously published (Turnbaugh et al. 2008), high-fat/high-sugar, fat-restricted, and carbohydrate-restricted. In addition to these diets, we will also use different one-time inoculations of the mice by feeding them various natural products such as different fruits and vegetables as well as constant treatment with different anti-microbials. After 12 weeks when colonization has been completed, a single mouse from each dietary cohort will be sacrificed. The microbes will then be used to colonize a different cohorts of germ-free mice. These recipient mice will be kept under gnotobiotic conditions. After colonization has been stabilized, mice from the different cohorts will be sacrificed and used for analysis. The remainder of the mice in each cohort will be used to perform the infection experiments. An important note for using germ-free mice is that they will have impaired immune systems. We can actually take advantage of this in that the resistance or sensitivity to infection will be primarily a result of the colonizing microflora rather than variations in adaptive immunity.

Members from each cohort will be inoculated with varying doses of different pathogenic organisms known to affect the host gut, namely *Helicobacter* sp., *Vibrio* sp., *Salmonella* sp., *Listeria* sp. and *E. coli*. As a control, attenuated strains of each of these pathogens can be used. The mice will be monitored for changes in diet, excrement, body temperature, sleep patterns, or death. The microbiota of mice with particularly mild or severe symptoms will then be profiled. The tissue sampled to obtain microbial profiles will vary depending on the pathogenic organism and where it optimally colonizes.
The microflora profiles for each diet will be assessed in three ways. First, shallow sequencing of 16S rRNA will determine which species of organisms are most represented. Second, using microarrays spotted with 16S rRNA of species known to colonize mice, the presence of less abundant species can be detected without deep sequencing. Third, sequencing of mRNA will give a metabolomic readout of what biological processes are most actively used under each diet. The objective is not necessarily to understand precisely what metabolic processes are, but rather to be able to identify the key differences between each dietary cohorts.

**Aim 2:**

In this aim, we will take mice being fed a standard diets and investigate how they change over the course of an infection with a pathogenic organism. If we identify diets in Aim #1 that alter infection sensitivity, they will also be investigated as well. During an infection, the colonizing microbial profile will obviously change. The objective of this Aim is to identify what organisms drop out or bloom during infection. Organisms that drop out likely share compete with the pathogen for the same niche, while organisms that bloom are potentially organisms that may play a role in the infection. Understanding how the microbial profile changes during infection in addition to simply providing us with a better understanding of what is involved in maintaining and sustaining stable microflora, in it may give insight into possible pro-biotic therapeutics, the latter of which will be addressed directly in Aim #3.

Three different cohorts of mice will need to be analyzed for any given dietary conditions. The first will be a control, pre-infection groups. The mice profiled in Aim #1 can serve this
purpose. The second group will be sacrificed and analyzed shortly after infection. The final group will be profiled after the mice have recovered from the infection. Profiling this final group will inform us of whether or not infection results in long-lasting microbial colonization changes.

Profiling of organisms will be done similar to Aim #1. First, shallow sequencing of 16S rRNA will be done to look for drastic changes in species numbers. Second, custom microarrays can be made to specifically probe for the 16S rRNA of microbes in uninfected mice identified in Aim #1 and in the shallow sequencing. Finally, sequencing of mRNA should reveal significant changes in gene expression.

**Aim 3:**

This final aim will be an attempt to compile the different microbial profiling into a usable therapeutic. The first objective is to demonstrate that the microbial profiles can be altered once established. Initial results by Turnbaugh et al. suggest that this might be possible by simply changing altering the diet of the mice, but direct feeding of cultured microbes or isolated samples can also be tested. The 16S rRNA microarrays used in earlier aims can be reapplied here. The second objective is to demonstrate that mice with modified microbial profiles can actually be made resistant to infection by pathogenic organisms.

One final application of probiotics would be to test whether feeding cultured organisms that drop out of colonization during infection (identified in Aim #2) can speed the recovery of the mice after being infected. This can be assayed by monitoring the mice as described in Aim #1.
References: