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Development of the Human Gastrointestinal Microbiota and Insights from High-Throughput Sequencing

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Abstract

Little has been known about the development of the gastrointestinal tract microbiota, until recently, because of difficulties in obtaining sufficient sequence information from enough people or timepoints. Now, with decreased costs of DNA sequencing and improved bioinformatic tools, we can compare GI tract bacterial communities among individuals, of all ages from infancy to adulthood. Some key recent findings are that the initial bacterial community, even in the GI tract, depends strongly on delivery mode; that the process of early development of the microbiota is highly unstable and idiosyncratic; that the microbiota differs considerably among children from different countries; and that older adults have substantially different GI tract communities than younger adults, indicating that the GI tract microbiota can change throughout life. We relate these observations to different models of evolution including the evolution of senescence, and suggest that probiotics be selected based on patient age. Studies of the microbiota in older people might tell us which probiotics could increase longevity. Drug metabolism varies among individuals with different microbial communities, so age- and region-specific clinical trials are required to ensure safety and efficacy.

Introduction

Recent advances in sequencing technology[1] have revolutionized our view of the microbiota in many human body habitats[2], especially in the gastrointinal (GI) tract[3–5]. In particular, sequence-based approaches have increased our understanding of similarities and differences

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among individuals and populations[2–6], sources of the initial inoculum of bacteria[7], and developmental trajectories of the microbiome at the beginning[7–9] and end[10, 11] of life.

The healthy human fetus develops within an environment that is thought to be mostly sterile. However, although bacteria in the amniotic fluid are associated with preterm labor, babies exposed to bacteria in utero are often viable[12]. Human adults have highly differentiated bacterial communities in different body habitats[13–15], although, interestingly, in newborn infants, these communities appear to be largely undifferentiated[7].

We review the sources and effects of the initial inoculum and development of the infant microbiota over the first few years of life. We discuss evolutionary principles, proposing that bacteria with effects most apparent at different stages of development reflect different biological needs of the host at different ages. We also speculate on sources of variation for the adult microbiota.

Baby's First Microbes

How do babies first become colonized, and how does this first inoculum differentiate into the highly distinct microbiota found different adult body habitats? A first step in understanding developmental microbial ecology in humans is to describe the pioneer bacteria in the newborn. These founder species can define processes that lead to more complex and stable adult ecosystems, according to the ecological theory of succession originally developed by plant ecologists[16, 17].

It is often thought, incorrectly, that newborns are sterile, and are colonized after birth by environmental microbes. Placental mammals give birth through a birth canal that is heavily colonized by microbes. This is unlikely to be accidental; rather, the vagina has likely evolved to provide the primary inoculum for all mammals. The human vagina is an ecosystem dominated by relatively few bacterial species[18, 19]. Unique among body sites, vaginal communities are typically dominated by *Lactobacillus,* which constitute >50% of all bacteria present[9]. At the time of delivery, the vagina is invariably dominated by *Lactobacillus* and *Prevotella* spp[7]. Ravel et al.[9] recently reported that vaginal communities dominated were strict anaerobes, rather than lactobacilli, in non-pregnant, healthy black and Hispanic women. Nugent scores (which indicate the level of bacterial vaginosis) and pH each increase with the proportion of non-Lactobacillus sp.; environments of low pH are associated with community states dominated by *L. iners* and *L. crispatus*[9]. It is controversial whether poor representation of lactobacilli reflects normal inter-individual differences of the vaginal ecosystem or asymptomatic infections, but these findings indicate that the vaginal community changes during pregnancy, to provide newborns with beneficial microbes.

Little is known about the age-related, successional mechanisms involved in the development of the human microbiota from founder communities. A recent community-wide survey using multiplexed 16S rRNA pyrosequencing revealed that vaginally delivered babies acquire, at birth, their own mother's vaginal bacteria. These bacteria can be found in the skin and mouth, and are already present in the first meconium[7]. Therefore, neonates' different body sites are colonized with essentially the same microbiota that was inherited vertically from

their mothers, and only later develop the distinct microbial communities found at these sites in adults.

After the natural primary inoculation at birth, infants have multiple exposures to human microbes. Subsets of microbes colonize different body sites; in adults, these communities are highly differentiated[2]. According to studies of cultured microbes (as opposed to the culture-independent, 16S rRNA surveys and metagenomic studies primarily described in this review), facultative anaerobes establish in the intestine and help reduce the environment so that strict anaerobes can be established in sequence[20, 21].

The microbial habitats of the host select for a group of well-adapted communities from the microbiota available for colonization, and host genetics influence the composition of the microbiota by influencing the environmental conditions of the habitats. Physiochemical and immunological properties and diet shape the diverse niches of the stomach and small and large intestines. Among mammals, gastrointestinal tract physiology is a powerful predictor of bacterial community composition of feces[22]. In humans, genetic differences that affect niche space also affect the composition of the microbiota. Variation in the mouse genome has recently been linked to variation in the GI tract microbiome; several genes that are believed to help establish the microbiome have also been associated with innate immunity[23]. Although quantitative genetics techniques have not been integrated into studies of the human microbiome, these approaches will likely reveal genes that influence variation in the GI microbiome (perhaps those involved in immunity and metabolism).

Several twin studies of the GI tract microbiome have sought to determine whether host genotype can influence the composition of the GI tract microbiota. The classical approach for assessing the heritability of the microbiota is simple and effective—if the microbiota of monozygotic twins are on average more similar to those of dizygotic twins, host genotype must affect how the GI tract is colonized. Host genotype appears to influence the composition of human GI tract microbiota. In one study, fingerprinting-based comparisons (temporal or denaturing gradient gel electrophoresis of 16S rRNA PCR products) between the fecal microbiota of monozygotic and dizygotic twins found greater similarity among the microbiotas of monozygotic twins[24]. However, evidence for the influence of genotype on the microbiota remains equivocal. For example, in a study of 31 monozygotic and 23 dizygotic twin pairs, Turnbaugh et al. reported that the microbiota of monozygotic twins overall were not significantly more similar than that of dizygotic twins[25]. No study has implemented an adequately powered analysis of the heritability of the human GI tract microbiome. Another possible reason for conflicting results regarding the heritability of the human microbiota is that twin studies have compared whole communities, and heritable subsets of the microbiota might have been missed.

Vaginal Exposure and the Infant's Microbiome

Many babies are not exposed to vaginal microbes at birth. In the United States, more than 30% of all live births in 2007 were C-section deliveries (http://www.cdc.gov/nchs/births.htm), and many women have C-sections by choice, in part because effects on the baby are assumed to be minimal. However, birth by C-section impacts the profile of initial bacteria acquired by the baby. C-section babies, in contrast to vaginally-delivered babies,

harbor bacterial communities that resemble those of the skin, comprising *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp[7, 26]. These communities in the baby are not closer to that of the mother's skin than to the skin of other women[7], indicating that the *Staphylococcus*-rich initial microbiota in C-section babies is provided by other people with whom the babies are in contact. Human-associated bacteria are common in hospital environments[27], and incidental exposures to skin bacteria in the hospital environment could contribute to the microbiota of C-section babies. C-section babies maintain differences in the intestinal microbiota for months after birth[16, 26, 28, 29], perhaps longer.

The lack of the natural first inoculum in C-section babies affects the development of the bacterial community in their GI tract;[16, 30] it might also account for their increased susceptibility to certain pathogens, compared with vaginally delivered infants. For example, 64%–82% of cases of skin infection with methicillin-resistant *Staphylococcus aureus* (MRSA) in newborns occur in C-section babies[31]. The altered composition of the intestinal microbiota could also contribute to risk for atopic diseases[32], allergies and asthma, which are higher in C-section than in vaginally-delivered babies[33, 34]. Direct transmission of vaginal microbes to the baby might have a defensive role, occupying niches and reducing colonization by pathogens such as MRSA. It was shown that *Lactobacillus* probiotics reduce the incidence of allergies at age 5 in C-section, but not in vaginally delivered, infants[35].

Mutualistic relationships with intestinal bacteria influence energy balance[36–39], the metabolism of xenobiotics[40, 41], resistance to pathogen colonization[42, 43] and the maturation of the intestine and the immune system[44, 45]. Differences in the initial microbiota can alter developmental pathways of the infant's microbiome, which could have important implications for infant development and health.

Development of the Microbiome

The strains of GI bacterial that are first detected in infants, acquired from the mothers' vagina and skin, are replaced by other strains of less-certain origin[46–48]. The newborn's GI community has relatively few species and lineages, but diversity increases rapidly (albeit with a considerable degree of instability) over the first few years of life[8, 49]. However, the reason for this increase in diversity is unknown—it is possible that new bacteria are incorporated at a constant rate as they are experienced in the environment, or that growing a larger gastrointestinal tract provide more distinct niches for bacteria, or a larger habitat for them to live in (biogeography studies have shown that larger islands support more species)[50]. Another alternative is that increasing functional complexity produces taxonomic complexity, until states of equilibrium are reached[100]. In a case study of one individual, the introduction of very simple solid food (rice cereal) did not alter the composition of the fecal microbial community or diversity of the species; the functional genes necessary to degrade simple plant-derived substrates were already present in the metagenome.[9] These functional genes likely did not belong to the Bacteroidetes, however, which are the principal degraders of complex plant polysaccharides in the adult GI tract. Once peas and other more complex plant-derived foods were introduced, the relative proportion of bacterial phyla in the GI tract changed from a community dominated by Actinobacteria and Proteobacteria to one dominated by Firmicutes and Bacteroidetes, leading to the establishment of an adult-like

microbiota characterized by a full suite of functions[49] and greater stability. Even though comparisons of individual babies show large differences in colonization dynamics[8], within a single baby, the consortia of bacterial taxa is not random, at any given time point, indicating that the microbes depend on each other within the consortium. Infancy is therefore a period of rapid colonization by groups of microbes that can change in response to events such as illness or changes in diet[49]. This microbial plasticity provides an efficient means for adaptation to the changing circumstances of development.

It is not clear how changes in lifestyle, illness, puberty, etc. affect the microbiota, and the stability of the microbiota over an individual's lifetime. Interestingly, human family members tend to have more similar microbiotas, compared to unrelated individuals[5], and the same bacterial strains can be shared among family members[48, 51, 52]. The process of bacterial succession in infancy varies among individuals[8], as does the process of recolonization of adults after antibiotic therapy[6, 53]. Intriguingly, children from different countries (e.g. Burkina Faso and Italy) have marked differences in GI tract microbiota[54], although the extent to which these differences are due to diet, genetics, or environmental exposures is not clear. Similarly, the elderly have different GI tract microbiota than younger adults[10, 11, 55]—this observation could result from differences among cohorts or differences directly associated with aging itself. Regrettably, the extensive longitudinal study required study these effects would exceed the typical 5-year period of funding from a grant from the US National Institutes of Health, making these types of studies a challenge to coordinate and fund. Evidence that differences in the GI microbiota can have radical effects on drug metabolism[56] indicate that drug trials should be performed in age- and location-matched populations, to avoid the effects of differences in the metabolic capabilities of the human GI tract microbiota.

Diet and the Microbiome

Diet is one of the most important determinants of microbial diversity in the GI tract; its effect on microbial community composition is reviewed elsewhere[28, 29]. Diet might further enhance differences in the initially inherited microbial populations that co-diversified with human populations. The selected bacterial populations might, in turn, affect the physiological performance of the human host. For example, breastfeeding has been shown to enrich vaginally acquired, lactic-acid producing bacteria in the baby's intestine,[57, 58] although long and detailed longitudinal studies of the dynamics of this species or of the community generally have not yet been performed.

A recent study of rural children in Burkina Faso and in Italy showed that the Bacteroidetes were far more abundant in microbiomes of African children [54]. Additionally, the types of Bacteroidetes present in the African microbiomes differed from those that are typically found in Western or Asian microbiomes. These types of Bacteroidetes might be suited to liberate energy from the plant-rich diet of the African children. These observations raise the possibility that microbiomes vary geographically with their hosts, in part because they are adapted to local diets. Populations of *Bacteroides plebeius* were recently shown to adapt, among groups of people, to the local food. Japanese strains of *B plebeius* harbor a gene acquired from marine bacteria that is necessary to degrade porphyran in edible

seaweed (Nori); North American microbiomes do not carry this gene [59]. Members of the GI microbiota can therefore adapt, via genomic changes, to changes in diet. What was particularly interesting about this study was the finding that the adaptation could be achieved by incorporating genes from bacteria in the environment.

Stage-Specific Bacterial Interactions

Organisms that have definite lifespans, such as animals, have conflicts between young and old. In resource-limited settings, nature must eliminate older individuals after their peak reproductive age, to provide resources for the next generation[60]. Populations that increase the proportion of aged individuals are unstable and therefore transient[61]. Nature has solved this problem through creation of biological clocks that keep track of the position of each individual in the aging matrix; these clocks are phylogenetically deep and well-conserved[62–64].

One type of clock includes bifunctional genes (antagonistic pleiotropy). These genes are beneficial to young individuals but become costly with aging[65]. In one model, senescence is beyond the power of selection, because it occurs after reproductive age[66]. An alternative model is that selection operates on individuals at all ages, through its effects on resource allocation, and that population structures predict survival of social groups[67].

Subsets of endogenous microbiota might also have bifunctional genes. The congruence between host and bacterial phylogenies[13, 68] provide evidence for selection of microbes that co-evolve with their host. If microbial genes provide adaptive functions during reproductive life, but shorten host lifespan during the post-reproductive period, they could reduce resource competition between young and old, and promote resilience of the population structure[69, 70].

The dominant, human gastric bacterium *Helicobacter pylori* could have such a role. *H pylori* infection appears to benefit the host during early stages of life, providing resistance to infections with other bacteria that cause disease (e.g. diarrheal illnesses and tuberculosis), controlling energy homeostasis, and protecting against asthma[71, 72]. During post-reproductive life, *H. pylori* is associated with a log-linear increase in mortality from gastric cancer[72]. This bifunctional relationship with humans might account for its ubiquity in all populations studied before the widespread use of antibiotics[73]. It will be interesting to investigate whether other species in the human GI tract have similar, dual effects. Particular species that are beneficial to the young (e.g. *Lactobacillus* and *Bifidobacterium*) might actually be harmful, if transplanted into the elderly. These are important topics for study, especially for the development of probiotic therapies. Perhaps the healthy old, rather than the young, are the best donors of probiotics for other older individuals (there is evidence that the elderly have distinct GI microbiomes[10, 11]). Similarly, benefits that evolved in the young, over evolutionary time, might disappear with use of antibiotics, excessive hygiene, and other factors.

Probiotics offer a way to restore the original ecology to a disrupted microbiome. Before interventions can be effective, however, we need a better understanding of the ecology of the organ at the particular stage of development of the host. Probiotic research began

before research into the human microbiome. It has focused on a handful of bacterial species (including some that do not belong to the human microbiome) and has been guided by a trial-and-error approach based on minimal knowledge of the biological role of the probiotics. Many studies involving probiotics had serious limitations in their design and scientific rigor, lacked controls, or did not completely define the probiotic tested; these studies therefore have not clearly associated observed effects with specific microorganisms [74, 75].

Scientific advances in the field of probiotics have mainly focused on avoiding infections in immunocompromised subjects, mostly in the elderly. Ageing is associated with reduced immune function, increased disease and use of medications, and changes in nutrition—all modify the composition of the microbial community of the GI tract[76]. Reported positive effects of probiotics in the elderly include: increased natural killer cell activity following administration of *Lactobacillus bulgaricus* OLL1073R-1[77], increased cytokine responses[74], increased titers of specific antibodies after vaccination[78, 79], improved stool output following administration of lactic acid bacteria to constipated subjects[80]; reduced duration of respiratory infections following consumption of fermented milk[75]; reduced blood glucose concentrations, and increased white blood cell counts [81].

Some research has also been done in babies, showing a potential for probiotics in reducing necrotizing enterocolitis risks in preterm infants[82, 83]. However, more research is needed to understand how is the microbiome is modified by age and genetic environmental factors, and how to restore a dysfunctional microbiome at the right time, with the right species.

Conclusions

The human microbiota develops from an initial inoculum that is determined by mode of delivery. Through a dynamic process that is unique to every individual and unstable, the microbial community towards, but only approximating, its adult state during the first 1–3 years of life. Although the GI microbial communities of adults are often believed to be stable, there is evidence that it changes through life—at lower rates than in childhood, with unknown effects on health. Antibiotics have a radical effect on the GI microbial community at all stages of life, and responses vary among individuals. Global use of antibiotics and disappearing indigenous lifestyles could be eliminating a key source of information about the microbes with which we have evolved, and perhaps important insights into the normal developmental process. Preserving samples of these endangered GI tract microbes, at all stages of human development, might be an important goal of expanded human microbiome projects, worldwide.

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