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Replenishing our defensive microbes

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Abstract

Large-scale characterization of the human microbiota has largely focused on Western adults, yet these populations may be uncharacteristic because of their diets and lifestyles. In particular, the rise of “Western diseases” may in part stem from reduced exposure to, or even loss of, microbes with which humans have coevolved. Here we review beneficial microbes associated with pathogen resistance, highlighting the emerging role of complex microbial communities in protecting against disease. We discuss ways in which modern lifestyles and practices may deplete physiologically important microbiota, and explore prospects for reintroducing or encouraging the growth of beneficial microbes to promote the restoration of healthy microbial ecosystems.

Keywords

human microbiome; hygiene hypothesis; ecology; gut microbiota; pathogens; antibiotics

Introduction

The relatively recent transition of human populations from hunter-gatherer and agricultural societies to industrialized societies has been concurrent with a rise in previously absent “Western” diseases, including obesity, asthma, and inflammatory bowel disease. The ‘hygiene hypothesis’ is one of many hypotheses proposed to explain this increase. This hypothesis suggests that industrial societies reduce our exposure to microbes with which we have coevolved, leading to improper immune function and to establishment of microbial communities that differ substantially from those of our ancestors [1].

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Although the roles of specific pathogens have received intense scrutiny, we have only recently begun to understand the importance of microbes that can positively influence human health. The rapidly decreasing costs of DNA sequencing now allowing analyses of the microbes that live in and on the human body on a scale and with a resolution that has not previously been attainable. Large consortia, such as the NIH's Human Microbiome Project [2] and the EU's MetaHIT [3], together with many other human microbiome projects on different scales worldwide, have given us a first impression of the diversity of the human microbiome. These projects allow us understand the microbes that we harbor, how these microbes assemble into healthy communities, and the genes involved in specific microbial functions. In this review, we discuss the mechanisms by which our microbes train our immune system to recognize and overcome pathogens, how modern societal practices may derail our microbiomes from their ancestral tracks, and how replenishing our microbiota with beneficial microbes can improve human health.

Microbes provide health benefits and protection against pathogens

The modern infatuation with cleanliness stems in part from the misguided midcentury thinking that most microbes cause disease, and that the absence of microbes is therefore a key component of health. Over the last twenty years, the use of culture-independent methods that allow us to identify the members of human-associated microbial communities that are difficult to grow in the laboratory, together with epidemiological studies and studies of germ-free mice, has started to change this thinking. There is now compelling evidence that the opposite is true: rather than reducing microbial exposure, we should balance our symbiotic microbial communities to protect us from pathogens and disease states. The specific features of the microbial communities that provide protection varies considerably, and depends on what is being protected against. In some cases, such as [4], a single microbial species can provide protection; in others, such as [5,6], half a dozen specific members are required; and yet others, such as [7,8], require the action of a much larger community in aggregate. Although specific roles played by some important microbes have been identified, as outlined below, the full range of protective effects and their causative microbial agents remains unknown. In this review, we focus on bacteria, but it is important to recognize that viruses, eukaryotes, and even archaea are also important member of the human microbiota, and their effects on health are also important and increasingly studied [9,10]. Additional discoveries about the interplay between the host GI tract, immune system, and environmental microbial communities continue to accumulate at a rapid pace [11]. Here we focus on two of the best-established ways in which microbes contribute to resistance against pathogens and pathogenesis.

Microbes ‘educate’ the immune system through direct interaction, which is required for proper immune system response

The reaction of the human immune system to both constitutive and transient members of our microbiota requires a delicate balancing act. The immune system must produce enough pro-inflammatory signals to recruit, differentiate, and cause the proliferation of effector cells to control populations and localization, but must also avoid inflammatory responses that would damage the host [12]. Because both beneficial and harmful microbes exist, our immune

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system must be 'taught' to identify and respond to each microbe correctly. This education of the immune system requires direct contact with the microbiota (for example, by antigen-presenting cells and toll-like receptor signaling), and alters key components of both the adaptive and innate response. In the adaptive immune system, studies in germ free (GF) mice show that colonization of the gut is critical to the induction of regulatory T cell (T_{reg}) populations [13]. These induced regulatory T cells ($iT_{reg}s$) promote gut health by balancing the pro-inflammatory response with an anti-inflammatory one. Additionally, when microbes pierce the mucosal or epithelial barrier, they respond by releasing anti-inflammatory cytokines that reduce the intensity of the Th2 skewed response [14].In mouse colitis models, transfer of naive T cells and $iT_{reg}s$ can both ameliorate symptoms and prevent development of symptoms in mice that are genetically predisposed to develop colitis[15]. Different microbes induce different $iT_{reg}s$, and the community of $iT_{reg}s$ is therefore influenced by historical and ongoing microbial exposure [16]. For example, *Bacteroides fragilis* induces $T_{reg}s$ via secretion of polysaccharide A [17]. This induction can ameliorate colitis symptoms, but depends on induction of the correct $iT_{reg}s$. Similarly, certain species of *Clostridium* induce $T_{reg}s$ that prevent or reduce colitis in mouse models [18], although the specific mechanisms by which they trigger induction are not well understood. Prior exposure to *Clostridium* species can also be important for the induction of $T_{reg}s$ in the context of *Helicobacter pylori* infection, determining the severity of infection [19]. Because the iT_{reg} population is exposure-dependent, the iT_{reg} repertoire provides a mechanism by which past disruption of the gut ecosystem might cause later dysbiotic or pathogenic events. Shifts in the microbial population away from the iT_{reg} repertoire might decrease the anti-inflammatory capacity of the gut immune system, creating an aggressive response that can alter gut microbial community structure and/or cause host tissue damage.

Acute infection has long been known to transiently change the states of many components of the immune system, not just $iT_{regs,}$ but we are only now beginning to realize how nonpathogenic members of our gut microbiota alter the state of the immune system over longer timescales (for example, by affecting antibody and defensin production). GF mice without diverse microbial communities cannot produce normal levels of antibodies upon inoculation with pathogens [20]. Similarly, antibody responses to viral infection in the lung mucosa depend on specific commensal microbes [21]. Although the mechanisms by which these microbes alter the state of the immune system are not yet well understood, commensal microbes appear to prime the immune system outside the context of infectious disease. Thus the diversity of the microbiome, and the past microbiome of an individual, might be critical components of health.

Biodiversity of microbial communities plays an important role in preventing disease and infection

Ecological studies on larger scales suggest more diverse communities are in general more robust to invasion or disruptive events [7]. Biodiversity can also limit the emergence and spread of disease, in part through changes to the community structure that are not possible in less complex communities [22]. On the smaller scale of human-associated microbial communities, diversity may play a similar protective role. A diverse microbiome might provide protection by many different mechanisms. Some of the best-supported hypotheses

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about diversity-protection relationships are that diverse communities might: use resources that would otherwise be available to a pathogen [23], produce short chain fatty acids such as butyrate or other molecules that inhibit growth of pathogens [24], or directly modulate the immune system effector population and/or cytokine milieu [25,26]. However, support for all of these hypotheses is limited, and it is not yet even known in general whether the protective effect of biodiversity is a community-level effect, or whether high levels of biodiversity simply increase the probability that a particular species that is protective against the condition of interest is included in the community. Three specific cases are intriguing. First, in the locust gut, pathogen invasion was limited by overall community diversity, and not by the presence of any specific member [7]. Second, transferring the microbiota between strains of mice (NIH to C3H/HeJ) eliminated the susceptibility of C3H/HeJ mice to *Citrobacter rodentium* infection[5]. However, in contrast, *C. difficile* infection susceptibility could be altered by introducing only a small subset of a resistant host's community, suggesting that only a few specific members were involved in this case. These results are not necessarily contradictory -- some disease states or susceptibilities could stem from low biodiversity, others from the absence of a specific microbe. Further research could investigate whether antibiotics have deleterious effects proportional to the extent to which they chronically reduce bacterial diversity in the gut. Hypertrophic environments might also reduce the benefit of the endogenous gut microbiota by preventing them from scavenging the majority of available resources, thus allowing a ‘weedy’ or pathogenic species to establish and expand itself and reducing overall diversity (as is seen in other hypertrophic environments on other scales) [23].

Manipulation of the microbiota is a promising method for treating disease

Because the gut microbiota activate host immune defenses that are critical for protection against infection, microbiome manipulation is developing as an increasingly important treatment modality. For example, as noted above, NIH Swiss mice can resolve colonization with the murine pathogen *C. rodentium*, but the same infection in C3H/HeJ mice is lethal. However, transferring gut microbiota from NIH mice into C3H/HeJ mice delayed pathogen infection and mortality. These improvements were associated with increases in IL-22 in mice that received the NIH mouse microbiota transplant, suggesting that the microbiota is fine-tuning the host's innate immune system to prevent infection [5]. More work is needed to understand what role the microbiome plays in human diseases, and especially how many other effects attributed to host genetics may actually stem from shared vertically transmitted microbial species or communities.

The skin microbiome also plays a critical role in defense against pathogens. The skin acts as the body's first line of defense against incoming pathogens, and production of effector T cells is linked to signals produced by host-associated microbiota. For example, GF mice monocolonized on the skin with *Staphylococcus epidermidis* produced significantly more proinflammatory IL-17A in the skin, but not in the gut, than did uncolonized mice [27]. Effector T cell production and function in the skin was unaffected by antibiotics that substantially changed the gut microbiota, suggesting that these two reservoirs of microbes modulate host immunity independently. *Staphylococcus epidermidis* also reduced dermal infection by the parasite *Leishmania major*, primarily by augmenting IL-1 cell signaling to

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activate local effect cell responses [27]. These studies demonstrate how the microbial communities at different body sites can be protective, and additional studies of the nares, vagina, mouth, and other body habitats are likely to extend these results to other body sites.

Modern behaviors reduce our exposure to possible beneficial microbes

Studies of the gut microbiome of modern humans living in remote, traditional communities, and of ancient humans from fossil or subfossil specimens, are beginning to provide a foundation for understanding how modern, “Westernized” humans have altered their gut microbiome from ancestral states [11,28-30]. Humans in rural, remote Malawian and Venezuelan communities have differ markedly in their gut microbiota and microbiomes from humans living in the highly westernized US. Some of these differences may have evolutionary roots. For example, 1400-year-old human fecal material from a high-altitude rock shelter in El Zape, Mexico preserved a gut microbiome that resembled the microbiome of humans currently living in Malawi and Venezuela, and differed from the microbiome of individuals living in the US [30] (Figure 1). For example, the spirochaete *Treponema berlinense* was found both in ancient El Zape fecal material and in rural, traditional populations of Malawi and Venezuela, but not in the United States population. As studies expand to include more human populations living traditional lifestyles and/or additional sources of ancient samples, general patterns and associations may allow us to characterize the pre-antibiotic, ancestral state of the human gut. These studies may even provide a pool of possibly beneficial ancestral microbes that have been lost due to recent lifestyle changes and that could be resupplied to improve health. We describe how several aspects of westernized societies -antibiotics, Cesarean sections, and lack of exposure to livestock - may be significant drivers of microbial change.

Antibiotics

Many studies have shown that antibiotic use in humans drastically decreases gut microbial diversity [31-33]. Although antibiotics are immensely valuable for clearing life-threatening infections, their overuse in patients may lead to unintended consequences. As noted above, a diverse gut microbiota can be protective against disease, and increasing evidence suggests that the depletion of this diversity by antibiotics may increase susceptibility to later infections. For instance, mice dosed with the antibiotic ampicillin were much less resistant to colonization when dosed with 10^8 CFUs of vancomycin-resistant *Enterococcus faecium* (VRE) than controls that did not receive antibiotics. The gut communities of the antibiotic-treated mice were completely dominated by VRE. Remarkably, the gut microbial communities of humans receiving the same antibiotics were dominated (>97%) by the genus *Enterococcus* just 7-18 days prior to VRE infection in the bloodstream, demonstrating that antibiotic use might reduce the community's ability to fight off invading microbes [34].

Repeated antibiotic use in humans may also increase the reservoir of antibiotic-resistance genes available to pathogens. For example, the microbiota of two healthy human adults harbored 115 unique inserts encoding transferable antibiotic-resistance genes, nearly half of which were 100% identical to resistance genes found in known pathogenic isolates [35]. In pigs, antibiotic treatment greatly increased the diversity of antibiotic-resistance genes over an already high background of resistance, even for classes of antibiotics that were not

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administered to these specific animals [36]. Similarly, when six human subjects were treated with clarithromycin and metronidazole (commonly used for treatment of *Helicobacter pylori* infections), the antibiotics greatly reduced gut bacterial diversity, and the communities remained perturbed four years after treatment in some individuals. Repeated and extensive antibiotic usage in humans thus likely selects an increasingly potent reservoir of antibiotic-resistance genes.

The impact of antibiotics, particularly during important developmental milestones, can be seen even when administered at subtherapuetic levels. In mice, subtherapeutic antibiotic treatment (STAT), commonly used to promote growth in domestic farm animals, led to increased adiposity and altered metabolic function [37,38]. The combination treatment of penicillin and vancomycin, as well as treatment with chlortetracycline alone, significantly decreased the Bacteroidetes/Firmicutes ratio. This ratio has been previously associated with obesity and increased weight gain in wild-type mice [39], and in mice genetically predisposed to obesity [40]. The caloric output of fecal samples collected from STAT-treated mice decreased, consistent with the hypothesis that the gut microbiota in STAT-treated mice extracts more energy from the diet than that in untreated mice [39]. The gene content of the microbiome was also affected: relative abundance of butyryl CoA transferase genes increased at 3 weeks, but recovered to baseline levels by 6 weeks. Relative abundance of formyltetrahydrofolate synthetase genes did not significantly differ at 3 weeks or 6 weeks, indicating that changes in gene levels are likely antibiotic-specific. STAT significantly upregulated genes involved in liver pathways associated with lipogenesis and triglyceride synthesis, perhaps leading to the observed increases in fat mass accumulation. This study is especially intriguing in the context of an epidemiological study of >11,000 children in the UK, which concluded that antibiotic use before 6 months of age was significantly associated with increased body mass between 10 and 38 months of age [41]. Thus, the developing microbiome of infants may be particularly susceptible to deleterious, long-lasting effects derived from antibiotic use.

Cesarean Sections

Maternal transmission has been shown to be a crucial factor in passing on protective microbes to offspring in many species. In *Drosophila neotestacea*, for example, the parasite *Howardula aoronymphium* causes near universal sterility in females and reduced mating success in males. In order to protect against this parasite, *D. neotestacea* transfer the bacterial endosymbiont *Spiroplasma* between mothers and eggs. In wild populations, females infected with *Spiroplasma* in addition to *H. aoronymphium* are more than ten times as fertile as *H. aoronymphium* infected females that do not also harbor *Spiroplasma* [4]. In humans, the earliest exposure to foreign microbes for newborns has historically been from the vaginal microbial community during birth. This natural route of inoculation is bypassed in Cesarean sections, which are performed with increasing frequency worldwide despite evidence of significant deleterious effects [42-44]. A study of 165 Finish newborns (141 delivered vaginally, 24 delivered by Caesarean section) showed that by 1 month of age the C-section delivered infants had significantly less *Bifidobacteria* than did their vaginally-delivered counterparts, and also had significantly reduced bacterial cell counts in their stool [44]. Similarly, Swedish children who developed allergies by age 5 were less colonized by

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several *Lactobacillus* species (*L. rhamnosus, L. casei, L. paracasei*) and *Bifidobacterium* at birth [45]. Thus, vaginal delivery may inoculate a newborn with *Lactobacillus* and *Bifidobacterium* species that confer protective benefits later in life. Studies of exogenous inoculation of newborns with these important microbes in cases where C-sections are medically indicated are therefore needed.

Exposure to animals and livestock

Another mechanism by which modern humans may have lost some of their ancestral microbes is the reduced exchange between individuals and their environment, particularly through reduced exposure to animals. Ancient and rural societies typically have larger extended families that live with one another in close proximity; they also tend to have more contact with farm animals including livestock, and with wild animals (e.g. those hunted as food), than do populations in more industrialized settings. In constrast, family units in many 'western' countries consist of only parents and their offspring living in one residence. These smaller households and decreased exposure to animals (other than domestic pets) likely reduce microbial transmission, including possibly beneficial microbes. For example, individuals living within a household share a greater proportion of their skin microbiota than non co-housed individuals [46]. Furthermore, the presence of a dog in the family facilitated the spread of rare, low-abundance microbes, including the family *Methylophilaceae* (class *Betaproteobacteria*) derived from canine oral communities, and families from within the *Actinobacteria* and *Acidobacteria*, likely derived from soil. The likely route of transfer was oral-skin transmission from the dog to household members. Exposure to animals, especially during the post-natal period, is especially important. In a study of 1,187 infants, Havstad et al. [8] found that IgE levels, typically elevated in diseases with an allergic component, were significantly lower in children who were exposed to pets early in childhood. These findings are consistent with the hygiene hypothesis, which states that exposure to certain microbes, including microbes obtained by pet-human transmission, trains the immune system to recognize foreign microbes and avoid harmless allergens.

Replenishing the host's beneficial microbiota

Manipulating the microbiome

Because deviations from a "normal" healthy microbiota are linked to many human diseases, it is increasingly important to discover how to "reset" and "replenish" our gut microbiota with beneficial microbes (Figure 2). Different nutrients from the host's diet probably help determine which niches are available for microbial utilization, and thus which microbes become established. Large-scale changes, including a steady increase in microbial diversity, are seen in an infants' gut microbiome over the first few years of life, in part as a result of changes in their diet [33,47]. The intrapersonal variation of the adult gut community is relatively stable over time compared to differences between individuals [48,49], and a core functional profile of the microbiome is present even though the species that contribute the functions to this profile vary among individuals [23]. In mice, large changes in the gut communities result from dietary changes over the course of 1-4 days, though the effects are easily reversible [50]. However, in humans the timescale appears to be much slower, and long-term diet as measured by food frequency questionnaires over the course of a year, but

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not short-term diet experimentally manipulated over 10 days in a laboratory setting, seems to have a major effect [51]. Dietary alterations may thus play a role in achieving stable, long-term microbiome manipulation, as has been discussed elsewhere in detail [52].

One of the best experimental systems for identifying members of the microbiota that are causally responsible for change is the method of personalized culture collections transferred into gnotobiotic mice [6,53]. An individual's stool sample can be serially diluted, cultured from single progenitor cells without interference from other, faster-growing microbes, the individual strains can be characterized, and communities mirroring the original community can be reassembled. By reintroducing specific sets of taxa back into germ-free mice, the effect of the gut microbiota on host physiology can be determined directly, including the possibility of adding or removing specific members thought to be important. This technique will allow researchers to discover which taxa, or consortia of taxa, are required for preclinical efficacy in mice, and will guide clinical trials.

Because antibiotics profoundly reduce gut microbial diversity, it seems reasonable that antibiotic pretreatment might assist establishment of a new microbial community. Counterintuitively, antibiotics may actually impede the establishment of new communities. For example, antibiotic pre-treatment impaired the establishment of many phylotypes in rats after cecal transplant. Only 12 phylotypes of the input community were readily established across all antibiotic-pretreated rats, whereas 22 phylotypes were reproducibly established in the transplantation-only recipient rats (without antibiotics) [54]. The finding that cecal transplantation increased the overall diversity of non-antibiotic treated rats, and that this diversity was maintained beyond three months, shows that the gut may be more amenable to manipulation of the microbiota than previously thought. Another example of microbiota remodeling comes from the observation that mice with reduced bacterial diversity after cefoperazone treatment recovered their full diversity when caged with normal mice, presumably assisted by coprophagy. Stool transplantations may thus help a gut community recover effectively even after antibiotic treatment [55]. These conflicting reports might be the result of individualized responses of community alterations following antibiotic treatment, as has been demonstrated in humans [32].

Probiotics – live microbes that, when ingested, have health-promoting effects – have also been used to treat individuals with gastrointestinal diseases (see Table 1 in [56]. However, public enthusiasm for probiotics has greatly outpaced the evidence of efficacy, and the hypothesis that probiotics affect the structure of the gut microbial community is not well supported by existing data. Consistent oral intake of the commonly-used probiotic strains *Lactobacillus delbrueckii* spp *bulgaricus*, *Lactococcus lactis* spp *cremoris*, *Bifidobacterium animalis* spp *lactis*, and *Streptococcus thermophilus* in humans did not significantly alter the gut microbiome in terms of community composition, structure, or gene content [57]. However, the probiotics did up-regulate bacterially encoded pathways involved in polysaccharide degradation in fecal and urinary samples [57]. Thus, probiotics might convey health benefits in some cases by modifying gene expression in the host and/or microbiota, rather than by changing the composition of the microbiota itself. Given the importance to infants of developing a healthy microbiota, it has been suggested that probiotics could place the infant's microbiota back on track developmentally when altered by antibiotics early in

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life (Figure 2). However, these first few years of life include crucially important developmental processes, as also demonstrated in mouse studies showing that early interaction with the microbiota can permanently affect brain development and behavior [58]. Therefore, there is substantial risk of unintended consequences and caution should be exercised [59]. Future research should seek to understand why and how our gut microbiome changes, understand the functional consequences of those changes, and develop new therapies to return our microbiome to a healthy state.

Fecal Transplants

The evidence that out gut microbiota is important for educating our immune system is compelling, and modern behaviors may limit our exposure to specific and important microbial “teachers”. Can we replenish our microbiota to compensate for this loss? The best case study for beneficial manipulation of the microbiota can be seen in the increasingly popular use of fecal microbiome transplantations for the treatment of recurrent *Clostridium difficile* infection. *C. difficile* infections, inflammatory bowel disease, and irritable bowel syndrome have all been associated with dysbiosis of the host's gut microbiota, leading to recurrent inflammation, diarrhea and constipation, although the mechanism of pathogenesis remains unknown [60,61]. *C. difficile* is the most common cause of diarrhea associated with the use of antibiotics; the antibiotics permit particular *C. difficile* strains to dominate the community and release toxins A and B, which promote diarrhea [62,63]. Recurrences in diarrheal episodes are generally treated with antibiotics; however, up to 65% of patients receiving antibiotics suffer relapse [64].

In contrast to the general ineffectiveness of antibiotics for treatment of *C. difficile* infections, fecal transplantation is highly effective both in animal models and in humans. For example, Lawley et al. [6] infected mice with *C. difficile*, resulting in a chronic intestinal disease. When treated with vancomycin alone, the *C. difficile* returned within 5-7 days of antibiotic cessation. Conversely, when the infected mice received a fecal transplantation from a healthy donor, the *C. difficile* infection did not return even months after treatment in 23 of 25 mice. To test whether the whole community was required or whether a lower-diversity subset would be sufficient for recovery, the authors cultured a healthy microbiota fecal sample through several generations (or passages), to reduce the community to only its culturable members. *C. difficile* infected mice were successfully treated using communities that underwent Passage 1 and 2, which already were reduced in phylogenetic diversity, but not Passage 3, where the community was very low diversity and dominated by *Enterococcus* spp. and *Enterobacteriaceae* spp. These experiments showed that the full community diversity of a gut microbiota is not required for clearing persistent *C. difficile* infections, but that replenishing the gut with specific members of the microbiota drive the transition from a diseased to healthy state. Ultimately, the authors identified a minimal mixture of 6 phylogenetically diverse taxa consisting of three novel species of *Bacteroidetes* sp. nov., *Enterorhabdus* sp nov., *Anaerostipes* sp. nov., and the previously identified *Lactobacillus reuteri*, *Enterococcus hirae*, and *Staphylococcus warneri*, that could resolve *C. difficile* infections. This study underscores the current interest in the intersection between personalized medicine and microbial ecology for identifying communities that can modulate health status: simple communities with culturable members provide the advantage that they

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can be more easily packaged, characterized and dispensed, but the full diversity of the community may be required for some disorders.

Conclusion

The field of human microbial ecology is evolving, and has recently transitioned from demonstrating that specific microbial consortia are associated with disease states towards learning how to directly manipulate the human microbiome for therapeutic purposes. The use of whole fecal transplants and highly defined microbiota transplants for *C. difficile* infections has demonstrated that microbiome manipulations can achieve high efficacy in at least one case where traditional pharmaceuticals fail. Furthermore, they suggest that mouse models represent a highly tractable system for investigating microbiome manipulation that can then guide clinical applications in humans. In the future, antibiotics might be used to treat the most severe infections, but their long-term effects on the microbiota may be mitigated by reintroducing species from the same person in a state of health, from other people (and perhaps from populations living more traditional lifestyles), or from engineered microbial consortia. Replenishing our defensive microbes will allow us to direct our microbiota back onto its evolutionary tracks, which may be especially important given that modern behaviors and practices likely create microbial detours not previously encountered in our evolutionary history.

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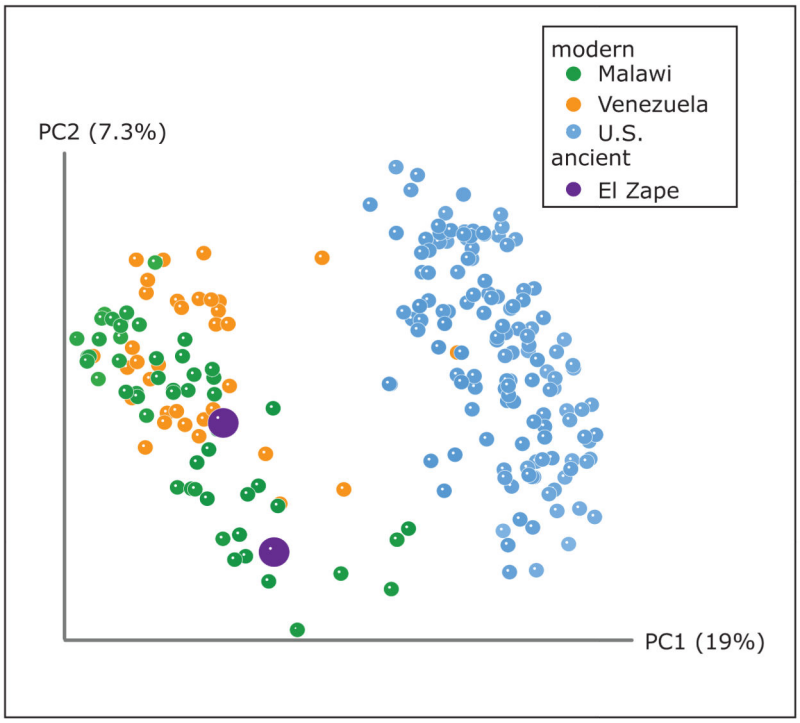

Figure 1. 16S rRNA gene sequencing survey reveals major differences in community composition of ancient vs. present-day humans

Fecal samples were collected from four different sources; adults in present-day Malawi, adults in present-day Venezuela, adults in present-day United States, and a ∼1400 year old deposit in an ancient rock shelter in El Zape, Mexico. Briefly, the 16S rRNA gene was sequenced, taxonomy was assigned against a reference database, and the communities were compared using the unweighted UniFrac phylogenetic distance metric. Microbial communities that are more dissimilar are located further apart in principal coordinate space, while similar communities are found clustered together [28,30].

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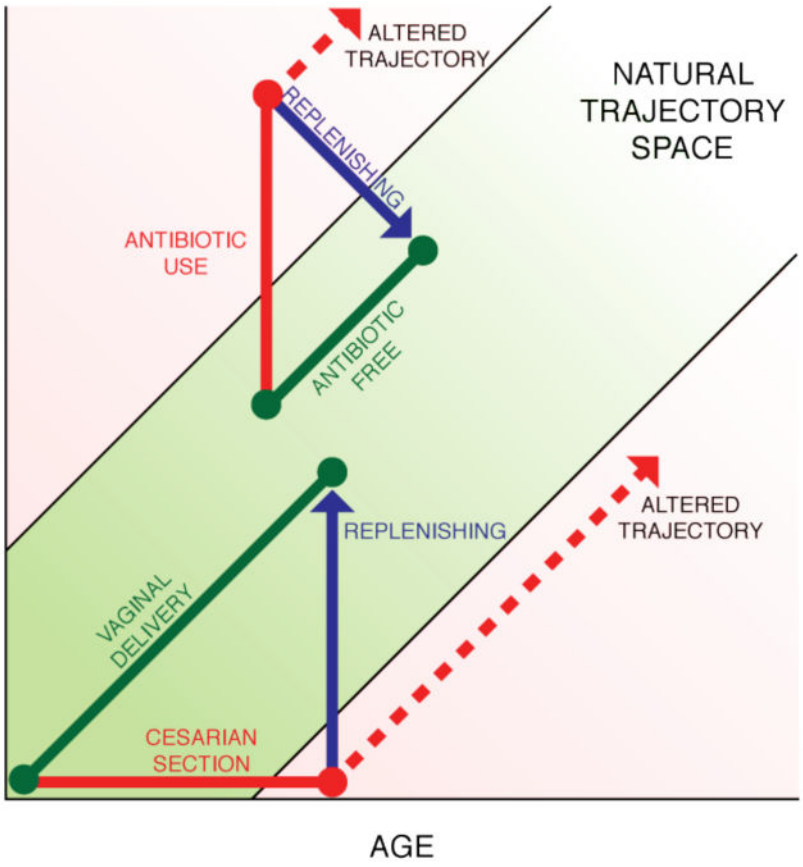

Figure 2. Diverting our microbial communities back towards ancestral states

Modern behaviors such as Cesarean sections and antibiotics may have the ability to push our microbial communities away from their natural, ancestral trajectories. However, microbiome manipulation may allow us to push our microbial communities back on track by replenishing the microbes that were affected by the disturbances. In this way, the impact on our microbial communities through events such as antibiotic use can be repaired such that our microbial communities maintain their protective benefits.