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The gastric microbial community, *Helicobacter pylori* colonization, and disease

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Long thought to be a sterile habitat, the stomach contains a diverse and unique community of bacteria. One particular inhabitant, *Helicobacter pylori*, colonizes half of the world's human population and establishes a decades-long infection that can be asymptomatic, pathogenic, or even beneficial for the host. Many host and bacterial factors are known to influence an individual's risk of gastric disease, but another potentially important determinant has recently come to light: the host microbiota. Although it is unclear to what extent *H. pylori* infection perturbs the established gastric microbial community, and *H. pylori* colonization seems generally resistant to disturbances in the host microbiota, it can modulate *H. pylori* pathogenicity. Interactions between *H. pylori* and bacteria at non-gastric sites are likely indirect—via programming of the pro-inflammatory vs. regulatory T lymphocytes—which may have a significant impact on human health.

Keywords: *Helicobacter pylori*, microbiota, microbial community, stomach, disease, rhesus monkey, human, mouse, 16S rDNA

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Introduction

The normal human stomach was long thought to be sterile, owing largely to the gastric acid barrier. But after the seminal discovery of Marshall and Warren in 1983,[1] it became clear that approximately half the world's human population is chronically colonized with *Helicobacter pylori*, which causes asymptomatic inflammation (gastritis) in virtually all infected individuals, and peptic ulcer or gastric adenocarcinoma in a few.[2] In a sense, *H. pylori* can be considered a commensal because of its near universal prevalence prior to the antibiotic era (and presently in most developing countries), and because of its extensive co-evolution with humans.[3] On the other hand, *H. pylori* sometimes causes serious disease,[2] so pathobiont may be a more appropriate designation.[4] Widespread treatment of *H. pylori* is generally considered impractical, and perhaps harmful, not only because of antibiotic toxicity and off target effects, but also because accumulating evidence suggests that some individuals may actually derive benefit from *H. pylori* infection. Therefore, it is critical to identify those who are at greatest risk of serious disease. To date the emphasis has been largely on bacterial virulence factors, host genetics, and environmental influences, particularly diet.[5-7]

But with the recent recognition that the stomach is also colonized by other bacteria, another potential determinant of the outcome of *H. pylori* infection is the composition or structure of bacterial communities in the stomach, either at the time of exposure or over the course of infection (**Fig. 1**). This might occur, for example, because the microbial context provides competition or alters the host physiology to render it more or less hospitable to *H. pylori*. Conversely, introduction of *H. pylori* to the stomach may disrupt a stable ecosystem and lead to disease indirectly by disrupting beneficial organisms and communities. The gastric microbiota may also affect the host immune response to *H. pylori*, shifting

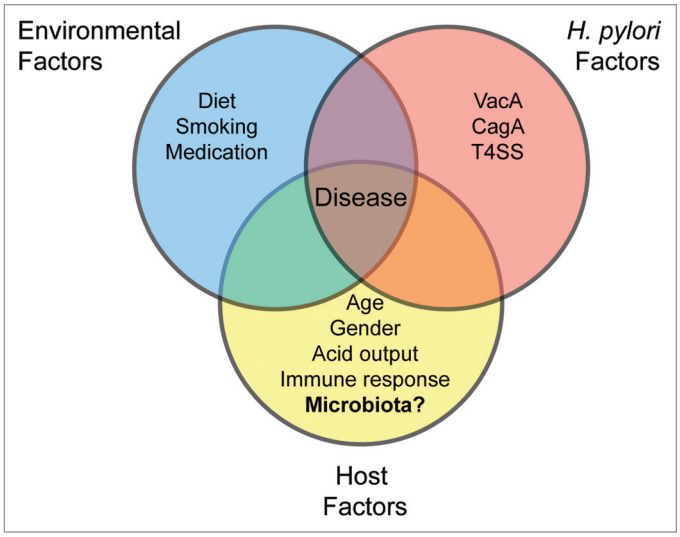

Figure 1. Risk factors for *H. pylori*-associated disease. Increased risk is associated with environmental factors such as a diet high in salt or low in iron, smoking, and the use of medications such as proton pump inhibitors and nonsteroidal anti-inflammatory drugs. *H. pylori* factors also play a role, with increased risk attributed to specific alleles of the *vacA* cytotoxin and the presence of the *cag* PAI, which encodes a type 4 secretion system (T4SS) and its effector, CagA. Host determinants of disease include the age of acquisition, gender, acid output following infection, an altered immune response due to genetic polymorphisms (*IL-1*β, *IL-10*, *TNF-*α, or *IL-1RA)*, and perhaps the composition or structure of the microbial community.

the balance of Th1, Th17, and regulatory T cell activity that is thought to largely determine the outcome of infection.[8]

To begin to probe the interactions between *H. pylori* and the microbial community of the stomach, we recently characterized the gastric microbiota of specific pathogen (*H. pylori*) free rhesus monkeys (*Macaca mulatta*), which serve as a robust and physiological model that closely resembles human *H. pylori* infection, and compared the results to the microbial communities in the mouth and the gut.[9] Deep sequencing of the bacterial 16S rDNA gene identified a community profile of 221 phylotypes that was largely distinct from that of the distal gut and mouth, although there were taxa in common. The gastric community also included a second *Helicobacter*, the commensal *H. suis*, which is occasionally seen as a zoonotic infection in humans.[10] We then performed longitudinal analyses of the microbiota following inoculation of *H. pylori*. Although we expected that the community membership and structure would shift in response to *H. pylori* colonization, we found instead that the gastric microbiota was dominated by *H. pylori*, leaving the underlying community largely unchanged. The one exception was *H. suis*, which was in apparent competition with *H. pylori*, because dynamic increases in levels of the one were accompanied by decreases in the other. Here we put these results in a broader context, and we consider four questions regarding the relationship between *H. pylori* and the gastric microbial community.

Is There a Truly Distinct, Autochthonous Gastric Microbiota?

There is little doubt that prior to the decline of *H. pylori* prevalence in the modern era, the native microbiota of the stomach was universally dominated by *H. pylori* since humans evolved out of Africa more than 60 000 years ago.[3] But the question here is whether in the absence of *H. pylori*, or even in its presence, albeit in lower numbers, there is a distinct, resident gastric microbial community. Answering this question is not easy. DNA sequencing methods are not specific for autochthonous members of the gastric microbiota, but will also identify, for example, naked DNA, environmental bacteria in food or water, oral bacteria, and organisms passing through stomach to take up residence in the gut. These problems are not necessarily unique to analysis of the gastric microbiota. But identification of species that comprise the "normal" microbiota of the stomach is more difficult than in the gut because human gastric contents are not as readily available as feces, especially from asymptomatic hosts. Additionally, the overwhelming numerical dominance of *H. pylori* in the stomach[11,12] means that extremely deep sequencing is required to reliably sample minority species. Additional information could be obtained by sorting bacterial cells on the basis of their activity level in the stomach,[13] using, for example, metatranscriptomic analysis to identify genes expressed in the gastric environment, or even culture-based methods, which are increasingly sensitive. Comparison between *H. pylori* positive and negative individuals may also be difficult because *H. pylori* is often present in low abundance when detected by DNA methods, even when clinical diagnostic assays suggest that it is absent.[11,14]

These caveats aside, cultivation-independent methods suggest that the gastric community is in fact distinct, even when *H. pylori* is not present.[11,14-16] This conclusion is supported by both sequence abundance weighted and unweighted phylogeny-based community analyses, irrespective of *H. pylori* infection status or gastric anatomical site. While communities sampled from oral (dental plaque, tongue, pharynx) and gastric habitats (antrum, corpus) frequently segregate by site, there is some degree of overlap among samples, which is perhaps to be expected given the continual flow of food and saliva from the mouth to the stomach. However, even bacteria that are not autochthonous to the gastric habitat—but are routinely present due to flow from upstream sites—may contribute to the microbial ecology of the stomach and thus deserve consideration as members of this unique assemblage of bacteria.

Does *H. pylori* Infection Impact the Gastric Microbiota?

In a sense, it is hard to imagine that the profound changes in gastric physiology

induced by *H. pylori* infection would not alter the structure and membership of the gastric microbial community. Infiltration of PMNs, Th1, Th17, and Treg lymphocytes, induction of antimicrobial peptides, chemokines and cytokines, and changes in pH and mucus composition are just a few of the changes that might well be expected to alter the gastric microbiota.

Based on 16S rDNA sequence signatures, the human gastric microbiota is comprised of several hundred operational taxonomic units (OTUs),[11,12,15,17] which span 44 bacterial phyla.[14] Despite this impressive diversity, the species distribution is highly uneven, with four phyla dominating the human stomach.[14] PhyloChip analysis found no difference in phyla richness between *H. pylori* positive and *H. pylori* negative stomachs, although there were shifts in the relative abundance of particular taxa.[14] In contrast, a clone library-based community analysis estimated higher phylotype diversity and evenness in *H. pylori* positive samples, once *H. pylori* sequences were excluded, but did not observe significant changes in community composition.[11] These studies were performed on different anatomical sites within the stomach (corpus, largely antrum, respectively), subject number and profiles (10 Amerindians, 2 immigrants, 20 New York citizens), and sensitivity (high, low), which may explain the discrepant conclusions.

The rhesus monkey gastric microbiota was also seemingly indifferent to *H. pylori* colonization, with the exception of *H. suis*, a related gastric Helicobacter species that frequently colonizes rhesus macaques and other animals.[10] Using six age- and gender-matched rhesus monkeys, we observed no correlation between *H. pylori* infection status and phylogenetic diversity or community membership at the genus level, in either the antrum or corpus.[9] However, there was a striking effect on *H. suis* levels. While *Helicobacter* was the most abundant bacterium in post-*H. pylori* inoculation biopsies, individual biopsies were dominated by only one Helicobacter species, suggesting that *H. pylori* and *H. suis* are competitively exclusive (**Fig. 2**). *H. pylori* inoculation also knocked down the *H. suis* 16S rDNA levels in animals that were *H. suis* PCR-positive prior to inoculation. Both *H. pylori* and *H. suis* are ubiquitous among rhesus macaques housed at primate research colonies,[10] and although each species predominates in different gastric anatomical sites (antrum corpus, respectively), their distributions clearly overlap. However, they have distinct lifestyles in the rhesus monkey stomach. *H. pylori* localizes primarily to the mucus layer just above the epithelium and induces inflammation, while *H. suis* associates with parietal cells and does not provoke histologic inflammation. Consequently, the mechanism of their mutual antagonism is not clear.

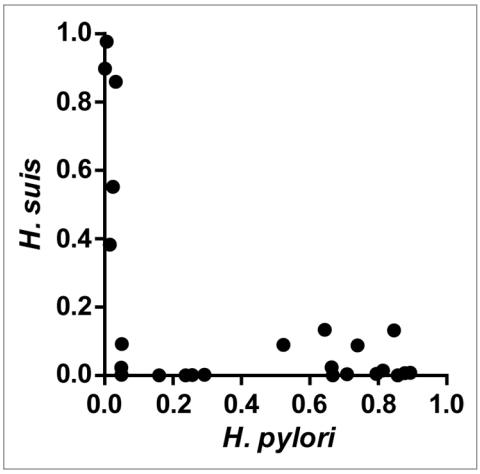

Figure 2. *H. pylori* and *H. suis* are competitively exclusive in the gastric mucosa. Biopsies were collected from SPF rhesus monkeys at five different time points following inoculation with *H. pylori*. DNA extracted from individual biopsies was subjected to pyrosequencing of variable regions 1–3 of the 16S rDNA gene. Shown is the proportion of total sequence reads that mapped to *H. pylori* or *H. suis* in individual biopsies. Adapted from Martin et al.[9]

Rodent models have also been used to assess the impact of *H. pylori* colonization on the resident gastric microbiota. In wild type mice, culture-independent studies found that neither long nor short-term *H. pylori* infection had a substantial impact on the gastric microbiota.[18,19] Studies in hypergastrinemic INS-GAS mice demonstrated that *H. pylori* infection caused a reduction in Bacteroidetes and an increase in Firmicutes.[20] When these mice were gnotobiotically colonized with members of the altered Schaedler's flora (ASF), *H. pylori* infection was accompanied by decreased *Bacteroides*, and both a decrease (*Clostridium*) and increase (*Lactobacillus*) in Firmicutes.[21] The Mongolian gerbil is probably the most relevant rodent model for *H. pylori*-microbiota interactions in the stomach, with a human-like gastric pH of <2,[22,23] and comparable pathology induced by gerbil-adapted *H. pylori*.[24] Although one study suggests that there may be a dynamic interaction between *H. pylori* and the gastric microbiota in the gerbil model,[25] the results are preliminary and based on small numbers of animals.

On balance, current evidence suggests that *H. pylori* does not have a consistent impact on the underlying structure or composition of the underlying gastric microbiota, though deeper sequencing will be required to confirm that relatively rare taxa are not impacted by *H. pylori* infection.

Does *H. pylori* Infection Alter Microbial Communities or Disease at Other Mucosal Sites?

Accumulating data suggest that *H. pylori* infection can sometimes modulate diseases at mucosal sites outside the stomach. For example, epidemiological studies suggest that while *H. pylori* infection promotes gastric cancer, it actually protects against esophageal cancer, which is increasing in epidemic proportions in developing countries where *H. pylori* prevalence is declining.[26] *H. pylori* can also enhance humoral immunity to an oral vaccine of the enteric

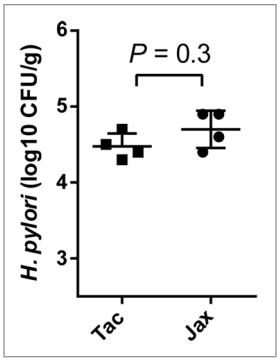

Figure 3. *H. pylori* colonization is similar in mice from Taconic Farms and from the Jackson Laboratory. C57BL/6 mice from Taconic Farms (Tac) and the Jackson Laboratory (Jax) were inoculated with *H. pylori* strain J166 and assayed for *H. pylori* CFU/g gastric tissue five days later. There was no difference in *H. pylori* colonization level in mice from the two vendors. The Mann-Whitney *U* test was performed using GraphPad Prism ($P > 0.05$ is nonsignificant).

pathogen *Salmonella enterica* serovar Typhi in humans,[27] and repress *S.* Typhimurium-induced colitis in mice, through a Treg dependent mechanism.[28] The mucosal effects of *H. pylori* infection may even extend beyond the gastrointestinal tract. For example, *H. pylori* infection is associated with a decreased risk of asthma,[29] particularly in young children.[30] These observations have been supported by mechanistic studies demonstrating that *H. pylori* mitigates allergic airway disease in a murine model of asthma due to the induction of anti-inflammatory regulatory T-cells.[31] Observational studies also suggest that *H. pylori* may protect against active tuberculosis in humans and cynomolgous macaques, which is associated with an increased production of IFNγ and an enhanced Th1 immune response to *M. tuberculosis*.[32] Thus, there is immune "crosstalk" between *H. pylori* in the stomach and organisms at distant mucosal sites. However, the protection afforded by *H. pylori* against other mucosal pathogens is not uniform, even among those that also provoke a strong Th1 immune response. For example, *H. pylori*-infected mice that were subsequently inoculated with influenza A virus had viral titers equivalent to *H. pylori*-uninfected controls.[33]

How might *H. pylori* modulate disease at mucosal surfaces outside the stomach? One possibility is that *H. pylori* may in fact be resident in these other sites, though it is usually thought to colonize only the stomach and proximal duodenum.[2] Perhaps more likely, *H. pylori* could affect mucosal diseases at distant sites via its effects on immune cells that traffic between the mesenteric lymph nodes and other mucosal sites, a mechanism that has been proposed to explain protection against asthma.[31,34] In either case, whether by direct colonization or by immune mediated effects, *H. pylori* might alter the microbiota at mucosal surfaces outside the stomach. In the rhesus monkey model, the inoculation of *H. pylori* did not affect the community membership or structure of the oral microbiota.[9] However, there are few if any other studies that address this question, and it remains a topic for future investigations.

Does the Gastric Microbiota Change *H. pylori* Colonization or the Host Response to *H. pylori* Infection?

The gastric microbiota is another potential factor—in addition to bacterial genetics, host genetics, and environment—that may alter the colonization dynamics of *H. pylori* and the host response to infection. The critical role of the microbiota in protecting the host from enteric pathogens is increasingly clear; *Clostridium difficile* colitis in the setting of antibiotics is perhaps the best example, but there are others as well.[35-37] Changes in the microbiota can even affect the host response to influenza virus.[38] More subtle differences in microbiota have also been linked to host susceptibility to enteric *Helicobacter* spp. For example, Yang et al. found that *H. hepaticus* could induce colitis in *IL10*$^{-/-}$ mice bred at only one of two breeding facilities, and this trait was associated with a distinct intestinal microbiota in uninfected mice.[39] A complex gut microbiota has also been associated with enhanced clearance of *H. felis*, another gastric helicobacter.[40]

However, studies performed by us and others that have looked for similar vendor or antibiotic effects on *H. pylori* colonization have met with negative results. A single strain of mouse purchased from different laboratories can have profoundly different microbiota and immune profiles in the lower GI tract,[41] yet carry similar levels of *H. pylori* in the stomach.[19] We have made similar observations (**Fig. 3**). Nevertheless, the composition of the gut microbiota does appear to play a role in the host response to *H. pylori* infection and severity of disease in mice. For example, antibiotic treatment prior to *H. pylori* infection led to a reduction in overall gastric inflammation and CD4+ T-cell recruitment compared with untreated mice.[19] This phenotype coincided with altered membership in the gastric community, although it is not clear if the differences in pathology were due to changes in the gastric or non-gastric microbial communities. Modulation of *H. pylori* pathogenesis has also been observed in mice co-infected with non-*pylori Helicobacter* spp. or with the parasitic roundworm *Heligmosomoides polygyrus*. These species are all common inhabitants of the murine gut that appear to redirect the T-cell response away from Th1. Co-infection with the *H. polygyrus* was associated with reduced pathology, possibly through the synergistic increase in immunosuppressive Treg cells,[42] and mice co-infected with *H. muridarum* developed less gastritis and had lower levels of both Th1 and Th17 cytokines.[43] Conversely, *H. hepaticus* co-infection increased gastritis and was associated with a decrease in Th1 and increase in Th17 cytokines.[43] These studies add to the mounting evidence that the presence of other species at distant mucosal sites can influence the host response to *H. pylori* by shifting the balance among Th1, Th17, and Treg lymphocytes.

Strong support for the effects of host microbiota on the pathology associated with *H. pylori* infection comes from recent studies of the INS-GAS mouse, a transgenic model of gastric cancer in which gastrin is overexpressed from the insulin promoter.[44] Specific pathogen free (SPF) INS-GAS mice, which harbor a

complex gut microbiota, progressed more rapidly to gastritis and neoplastic lesions following *H. pylori* infection than germ free mice colonized with *H. pylori* alone.[20] Strikingly, even colonization with just three members of the altered Schaedler flora (ASF) was sufficient to recapitulate the more aggressive pathology found in conventional INS-GAS mice.[21] Since three members of the ASF recapitulate the neoplastic effects of conventional microbiota, it seems unlikely that there is a single or even a few unique species that, together with *H. pylori*, promote neoplasia. Nevertheless, if the fingerprint of a cancer promoting microbial community could be identified, this might provide another biomarker that could be used to identify *H. pylori*-infected patients who are at greatest risk of gastric cancer.[45]

Conclusion

The stomach is not sterile, nor is *H. pylori* alone. Although there may not be a consistent dynamic between *H. pylori* colonization and the distinct gastric microbiota, it appears that the constituency of bacterial communities can sometimes promote and other times mitigate *H. pylori* disease, depending on its composition. If so, this may provide an opportunity for translational application as a biomarker for the risk of serious *H. pylori* disease, and perhaps even identification of organisms for therapeutic eradication.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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