Allies and Adversaries: Roles of the Microbiome in Infectious Disease

Studying how nonnative pathogens and host-associated microbial communities interact help us better understand infectious diseases

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Recent studies of host-associated microbial communities highlight how little we know about the roles played by microbial residents during infections. Historically, research on pathogenesis neglected the roles of resident communities within which most pathogens function. Indeed, many researchers eliminate the native microbial communities of their model hosts to simplify analysis of pathogens that infect them. Because interest in the involvement of those communities in host health centers largely on how they modulate host defenses, more effort is needed to address the purely microbial interactions that influence how pathogens affect those microbial communities as they also colonize their hosts.

Differences among pathogens, commensals, and mutualists are blurred and context dependent. A bacterium that is beneficial in one tissue can prove pathogenic in another. Benign members of the resident microbial community can become aggressive pathogens when that community is disrupted. Therefore, forcing host-associated microorganisms into rigid functional groups is counterproductive. Rather, we prefer using the term, "microbiont," which imputes neither beneficial nor harmful characteristics, capturing only a microorganism's association with its host.

Here we explore two roles of resident microbes during pathogenesis. First, we survey evidence for host-associated microbial communities as barriers to infection and review current efforts to identify mediators of this protective function. Second, we examine how disrupting the microbiota can transform commensal residents into pathogens.

SUMMARY

- **Studies of bacterial pathogens should consider more fully how the host microbiome contributes to pathogenesis.**
- **Microbial residents of a host can act as a barrier to invasive pathogens.**
- **The idea that disrupting host-associated microbial communities spurs a commensal-to-pathogen switch needs further study.**
- **A comprehensive understanding of how resident communities promote or prevent commensal-to-pathogen switching could lead to new treatment strategies.**

Microbial Communities Block Some Infections

More than a century ago, the Russian microbiologist and Nobel Laureate Ilya Metchnikoff uncovered evidence of colonization resistance, the ability of microbial communities to resist pathogens. When he consumed lactobacilli in sour milk, he learned that they did not survive for very long within his gut community and needed to be replenished frequently to exert salubrious effects on his health. American scientists Christian Herter (1865–1910) and Arthur Kendall (1877–1959) reinforced Metchnikoff's work, reporting that lactobacilli do not survive after being introduced into the monkey gut.

Many subsequent studies of probiotics substantiate the challenge of establishing new strains of bacteria in the mammalian gut. In general, levels of newly introduced bacteria drop to undetectable levels within days. Thus, microbial communities apparently withstand challenges from nonnative microorganisms, suggesting that host tissues that harbor a resident microbiota resist further colonization.

Handelsman: from a Focus on Microbial Communities to White House Science Policy

Jo Handelsman loves research so deeply that she nearly turned down an offer to work at the White House Office of Science and Technology Policy (OSTP), advising President Obama on scientific issues. "I couldn't imagine being away from bacteria, experiments, and students for two years," she says. Nonetheless, she accepted the offer to become associate director for science at OSTP in June 2014. "I absolutely love what I'm doing," she says. "People are inspiring and driven by the hope of making a difference in people's lives. I am thrilled to have the chance to contribute to that progress, particularly in the areas of microbiology, agriculture, public health, forensic science, and education."

Handelsman, 56, is on leave from Yale University, where she is the Howard Hughes Medical Institute Professor and Frederick Phineas Rose Professor in the department of molecular, cellular, and developmental biology. "Despite missing my lab and my students terribly, this is a chance of a lifetime to make a difference, and I intend to work as hard as I can to do just that, and then return to my research, no doubt with a new perspective," she continues. "My lab members have been absolutely wonderful about this period."

At Yale, Handelsman focuses her research on microbial communities, specifically how microorganisms interact with each other, and with hosts and inert surfaces. "We use both genetics and genomics, utilizing mutant analyses to identify genes that enhance or detract from success in a community," she says. "We also are interested in antibiotics, both in their native microbial community environment to understand their role in nature, as well as in antibiotic discovery and resistance for improving management of human infectious disease."

Handelsman, who "fell in love with microbiology in 7th grade looking at paramecia under a microscope," grew up in and around New York, N.Y. She received a B.S. from Cornell University in 1979 and a Ph.D. in molecular biology from the University of Wisconsin-Madison in 1984. Before joining Yale, she was professor of plant pathology at the University of Wisconsin-Madison from 1985 to 2009, and professor and chair of its department of bacteriology from 2007 to 2009. She served as president of ASM in 2013.

Handelsman, who has a keen interest in the status of women and minorities in science, rues "the talent we are missing due to explicit and implicit biases," she says. Several years ago, she and her colleagues sent a fictitious student resume to professors in biology, chemistry, and physics departments at six top universities, asking them to evaluate an imaginary candidate—randomly assigned the name John or Jennifer. "The results were stark and highly significant, both statistically and socially," she says. "The faculty would be more likely to hire John, pay him 15% more, believed he was more competent, and would be more likely to mentor him than Jennifer, although they liked Jennifer better."

Handelsman is married to Casey Nagy, a lawyer and anthropologist who is chief of staff for the president of the new Yale-National University of Singapore College. "He has been in Singapore full time since 2013, so . . . it will be nice to have him back in the States and even better when we are living in the same house again," she says. "I'm not sure I remember what a hobby is . . . . But I used to garden, and still read and lift weights to maintain sanity."

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.

While Metchnikoff, Herter, and Kendall illustrated host resistance to probiotic bacteria, other researchers in the latter half of the 20th century determined that this resistance also can apply to pathogens. In 1956, Rolf Freter successfully established a streptomycin-resistant strain of *Vibrio cholerae* in antibiotic-treated mice and guinea pigs. Shortly thereafter, C. Phillip Miller, Marjorie Bonhoff, and David Rifkind postulated that indigenous microbes prevent infections, following experiments in which they used streptomycin to change the composition of the mouse microbiome. In 1965, Rose Mushin and Rene Dubos noted their inability to infect adult mice with enteropathogenic *Escherichia coli*, positing that mice develop "a microbiota which is antagonistic to *E. coli*."

The term "colonization resistance" was coined in 1971, though usage of the synonymous phrase

Proposed Terminology

Microbiont	A host-associated microorganism
Pathogen	A microbiont under conditions in which it causes disease
Mutualist	A microbiont under conditions in which it and its host provide mutual benefit
Commensal	A microbiont under conditions in which it neither provides benefit nor causes harm to its host while receiving some benefit from the association. The term has also been used to describe bacteria that are benign in some situations but detrimental to their hosts in others

"microbial interference" dates back to the late 1950s. Recent observations that germ-free mice are more susceptible to infection than normally colonized animals support the fundamental principle of colonization resistance, which can reflect the interactions of the indigenous microbiota with either the host immune system or the invading pathogens.

Several recent reviews explore the relationship between the microbiome and host function; here, we focus on the interplay between residents and invaders. Colonization resistance can result from either exploitative or interference competition between microbes. For instance, *E. coli* strain Nissle 1917 is a normal gut resident that limits *Salmonella* Typhimurium colonization in mice by competing for and sequestering iron, an example of exploitative competition.

V. cholerae colonization of the intestine illustrates interference competition, or direct antagonism between competitors. Several of its genes confer immunity to the type VI secretion system (T6SS) of other bacteria, enabling *V. cholerae* to defend against attack by members of the indigenous microbiota and thus to establish infection. Moreover, specific bacterial taxa inhibit *V. cholerae* colonization in humans, and recovery from *V. cholerae* infection involves recolonization by indigenous bacterial residents, suggesting that the roles of other community members in response to infection warrant further investigation.

Resident Microbes Gone Rogue: Commensal-to-Pathogen Switching

Although indigenous microbial communities can protect their hosts from invading pathogens, under certain conditions some of these community members can be detrimental to their hosts. The transition from the neutral to the detrimental role is referred to as the commensal-to-pathogen switch, and these community members are known as opportunistic pathogens or pathobionts. Unlike pathobionts, "true" pathogens can produce intracellular infections, stimulate the host immune system, express virulence determinants, and infect healthy hosts.

The commensal-to-pathogen switch can follow an active change in behavior, but is more often associated with either passive translocation of a microorganism to a different host tissue or to host-created changes in its environment. For example, as a gut resident, *Bacteroides thetaiotaomicron* benefits the host by stimulating immune development and fermenting carbohydrates. Yet, when it is translocated from the gut, *B. thetaiotaomicron* infects tissues in the brain, liver, pelvis, and lungs. Similarly, *B. fragilis* strains are benign in the human intestine, but cause very serious infections in the bloodstream and are recovered from up to 30% of abdominal infections. Other gut residents such as *Clostridium sordellii* and extraintestinal pathogenic *E. coli* (ExPEC) escape the gut to colonize distal sites and disrupt host homeostasis.

The ability to cause disease following translocation to a new site in the host is not limited to intestinal microbes. *Streptococcus pneumoniae* is an innocuous resident of the upper respiratory tract, where it outcompetes other members of the microbiota, including *Haemophilus influenzae*, by producing bactericidal hydrogen peroxide. However, at other sites, it expresses a pore-forming toxin that damages host tissues in the middle ear, lung, and bloodstream, causing disease. Similarly, *Staphylococcus aureus*, an ordinary member of the skin consortia in up to 25% of the population, is frequently pathogenic when it enters the blood, causing pneumonia, abscesses, and systemic sepsis such as toxic shock syndrome.

Changes in human physiology that alter microbial habitats also can potentiate the commensal-to-pathogen switch. For example, when the skin is exposed to elevated temperatures and humidity, endogenous corynebacteria can cause several skin diseases, including pitted keratolysis, trichobacteriosis, and erythrasma. Similarly, suppression of the host immune response encourages *Streptococcus mitis* to shift from being a harmless member of the oral microbiota to a virulent pathogen that causes endocarditis, bacteremia, and septicemia.

Meanwhile, chronic stress in animals induces them to produce catecholamines, which bind iron, removing it from the host iron-binding proteins, transferrin and lactoferrin, and thus making it available for *E. coli* to use in causing infections. Since mammalian hosts limit iron to suppress pathogens, their producing catecholamine hormones proves self-destructive for those hosts when it is exploited by pathogens. This dynamic interplay between commensal microorganisms and their hosts regulates the spatial dis-

Additional Terms Used in This Article

Microbe-microbe interactions	Effects, measured at the individual, species, or community level, that populations of microbes exert on one another
Invasion	Perturbation to a microbial community in which a microorganism that does not exist within the community ("the invader") colonizes and establishes in the community
Colonization Resistance	Ability of a host-associated microbial community to resist invasion by pathogens
Mutualism	Relationship in which both partners benefit
Parasitism	Relationship in which one partner benefits at the expense of the other
Opportunistic Pathogen	Microorganism that causes disease when host defenses are disrupted
Pathobiont	Resident microorganism that can be benign or harmful
Dysbiosis	Changes to the structure of a microbial community that are detrimental to its host

tribution of microbes and influences whether they cause pathologies within the host.

Dysbiosis, Colonization Resistance, and the Commensal-to-Pathogen Switch

Although microorganisms in host-associated ecosystems adapt readily to minor environmental changes, extreme changes may challenge the entire community and lead to substantial consequences for the host. Perturbing the microbiota can cause dysbiosis—that is, changes that so disrupt the structure of a microbial community that they impair critical functions, including its ability to resist invading microorganisms. Dysbiosis can manifest itself in changes of community structure, metagenome structure, or gene expression.

Enterococcus infection provides a clinically important system for understanding the relationship between dysbiosis and commensal-to-pathogen switching. *Enterococcus* spp. can induce pelvic, neonatal, and urinary tract infections. In immunocompromised stem cell transplant patients, overgrowth of vancomycin-resistant enterococci (VRE) in the intestine precedes bloodstream infection. In these disrupted gut communities, populations of lactobacilli and related taxa are replaced by clostridia, enterococci, and members of the Enterobacteriaceae family. These changes in the microbiota can withstand antibiotic treatment, leading to persistent infections. However, fecal transplants containing a *Barnesiella* species cure these infections in some patients, suggesting that changes in the gut community accompany VRE invasion, domination, and infection.

Although the well-documented association between antibiotic treatment and *Enterococcus* infection suggests that the gut community plays a role in *Enterococcus* infection or its prevention, the community's role in commensal-to-pathogen switching during *Enterococcus* infection is unknown. In the insect *Manduca sexta,* the loss of gut integrity enables *E. faecalis* to translocate to the hemolymph, where it induces hemocyte aggregation and host death due to sepsis. Similarly, in mice treated with antibiotics and irradiated to deplete macrophages, *E. faecalis* migrates to the mesenteric lymph nodes from which it induces sepsis, consistent with a commensal-to-pathogen switch.

These findings support the idea that disrupting the gut community reduces its resistance to being colonized and enables commensal bacteria to become pathogens. The results also suggest that the microbiota has a dual protective role—modulating host immunity and outcompeting *Enterococcus* populations. Studying *Enterococcus* in both insects and mammals provides complementary insights. In insects, the role of the innate immune system can be studied in the absence of adaptive immunity and the gut community is simple, whereas studies in mice probe a more complex immunological response involving a more highly diverse gut microbial community. The well-studied case of *Enterococcus* commensal-to-pathogen switching provides a foundation for the study of other pathogens.

Conclusions

Following the application of Koch's postulates to identify genetic determinants of virulence, an immense research effort has helped to elucidate how host and microbial factors contribute to infections. However, many of those studies focused primarily on binary interactions between hosts and exogenous pathogens while ignoring host-associated microbial communities. This narrow

focus simplified the problem, making it approachable with 20th-century analytics.

Recent advances, however, make it possible to address infectious disease as a more complex system involving many additional factors. To determine the role of communities in disease, many familiar models of pathogenesis need to be amended to accommodate the microbiome. Future inquiry should include comprehensive assessment of the structure and function of microbial communities at all stages of infection, while also defining community and metagenome changes that antibiotic treatments induce. Likewise, the impact of the microbial community on host, pathogen, and disease outcomes should be considered an essential aspect of infectious disease.

Disease management based on ecology of the human microbiome is still in its infancy. Deliberately manipulating microbial communities will require an understanding of genetic and molecular factors that modulate community stability and vulnerability. Such research will depend on us learning which members of the microbial community play clear roles during infections, as well as how their absence and the loss of their metabolic capacities affect those infections. Integrating genomic and metabolomics data with classical genetic analysis will also provide critical insights into the role of the resident community as a barrier to and source of infectious agents in the human ecosystem.

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