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What Is a Host? Incorporating the Microbiota into the Damage-Response Framework

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Since proof of the germ theory of disease in the late 19th century, a major focus of the fields of microbiology and infectious diseases has been to seek differences between pathogenic and nonpathogenic microbes and the role that the host plays in microbial pathogenesis. Remarkably, despite the increasing recognition that host immunity plays a role in microbial pathogenesis, there has been little discussion about what constitutes a host. Historically, hosts have been viewed in the context of their fitness or immunological status and characterized by adjectives such as immune, immunocompetent, immunosuppressed, immunocompromised, or immunologically impaired. However, in recent years it has become apparent that the microbiota has profound effects on host homeostasis and susceptibility to microbial diseases in addition to its effects on host immunity. This raises the question of how to incorporate the microbiota into defining a host. This definitional problem is further complicated because neither host nor microbial properties are adequate to predict the outcome of host-microbe interaction because this outcome exhibits emergent properties. In this essay, we revisit the damage-response framework (DRF) of microbial pathogenesis and demonstrate how it can incorporate the rapidly accumulating information being generated by the microbiome revolution. We use the tenets of the DRF to put forth the following definition of a host: a host is an entity that houses an associated microbiome/microbiota and interacts with microbes such that the outcome results in damage, benefit, or indifference, thus resulting in the states of symbiosis, colonization, commensalism, latency, and disease.

Microbial virulence is a measure of the outcome of an interaction between a given microbe and a susceptible host, whereby the host suffers damage and the responsible microbe is considered the causative agent and often characterized as a pathogen. Several essays have analyzed the question "what is a pathogen" (1–4), but we are not aware of any formal effort to define "what is a host." Why define a host? Virulence is a microbial property that occurs only in a susceptible host. Therefore, a precise definition of the term "host" is important to fully understand host-microbe interactions and their outcome and, equally as important, to tackle research questions on pathogenesis, host response, and approaches to therapeutic and vaccine design. The seemingly simple question of "what is a host" is actually very complex, particularly with respect to defining host boundaries.

We will begin this discussion by considering definitions for the word "host" that are currently in use. Browsing through online definitions, one sees that the word "host" is used in many contexts, most of which are associated with social functions involving the entertaining of guests, e.g., one who hosts a party or is a master of ceremonies. However, some dictionaries also include definitions relevant to biological questions. The Merriam-Webster Online Dictionary provides two definitions of the word "host" that fit in the context of microbial pathogenesis: (i) living animal or plant on or in which a parasite lives and (ii) the larger, stronger, or dominant member of a commensal or symbiotic pair (http://www.merriam-webster.com/dictionary/host). Another online dictionary (Dictionary.com) provides a definition for host as "a living animal or plant from which a parasite obtains nutrition" (http://dictionary.reference.com/browse/Host). In the 1950s, Garber developed a nutritional theory that viewed hosts suitable for microbial virulence as those capable of providing microbes with nutrition (5, 6). From these definitions, some themes emerge, including the suggestions that the host must be living and larger and provide nutrition. However, none of these definitions are sufficient to define a host in the context of dynamic interactions with a microbe or a resident microbiota.

The crisis of the late 20th century. Before considering the definition(s) of "host" further, we will briefly consider the evolution of thought about disease-causing microbes since the germ theory of disease was proven in the late 19th century. Acceptance of the germ theory established certain microbes as the cause of certain diseases. Such proof of causality for a number of microbes and diseases soon led to a generalized concept that disease-causing microbes were fundamentally different than microbes that did not cause disease. This concept led to rapid growth of the field of medical microbiology and the medical specialty of infectious diseases, as well as research efforts to identify microbial characteristics that resulted in disease. The concept and ensuing research were logical. Based on the then-available knowledge and scientific tools, the majority of microbes that could actually be associated with disease were bacteria that could cause disease in animals and be cultured in the laboratory. In addition, and perhaps most importantly, these bacteria also had attributes that appeared to be responsible for their virulence, namely, capsular polysaccharide and toxins. The rigor with which these attributes were linked to disease reinforced the concept that microbes that caused disease

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were different than those that did not. This concept evolved into a consensus, which led to development of the term "virulins" to describe the factors that conferred microbial virulence (reviewed in reference 7). This term would eventually be replaced by "virulence factor." The notion that virulence was a singular microbial trait held for much of the 20th century. However, by the 1990s it was clear that it was not possible to identify microbial factors that governed the ability of many, if not most, microbes to cause disease. Furthermore, the virulence factor concept could not explain the emergence of and rapid rise in infectious diseases caused by microbes that were long considered unable to cause disease. These microbes included members of the commensal flora, e.g., coagulase-negative *Staphylococcus* spp. and *Candida albicans*. The emergence of diseases due to these and other microbes, often in patients who had impaired barrier or other types of immunity, led to a crisis in thought as it became clear that available concepts could not explain how microbes thought to lack virulence factors or pathogenic potential could cause disease. For example, the fungus *Candida albicans* had only rarely been associated with disease until the 1950s, when widespread use of antibiotics led to an increasing frequency of oral candidiasis. Then, by the 1980s, *Candida* was a common bloodstream isolate in hospitalized patients. In response to the emergence of infectious diseases in patients with impaired immunity, the concept of microbial opportunism was developed to counter microbe-centric views of microbial pathogenicity with a more host-centric view (8–10). The concept of microbial opportunism viewed these new disease-causing microbes as having pathogenic potential that became manifest in the setting of a weakened host immune system. However, the concept of opportunism was flawed from the start because nonopportunistic microbes also caused disease in patients with impaired immunity, and opportunistic microbes were rarely isolated in apparently normal patients, making it impossible to separate "opportunistic" and "nonopportunistic" microbes on the basis of the host in which they caused disease. For example, patients with AIDS were more susceptible to pneumococcal pneumonia (11), in addition to AIDS-defining microbes, such as *Toxoplasma gondii*, *Pneumocystis* spp., and *Cryptococcus neoformans*. Consequently, *Streptococcus pneumoniae* was categorized as an "opportunist" in persons with HIV/AIDS, despite the fact that this bacterium causes disease in apparently immunologically normal adults and children. To add to the confusion, *S. pneumoniae* is also a transient, but recurrent (due to its many different serotypes), component of the normal respiratory microbial flora. Thus, neither microbe-centric nor host-centric views of microbial pathogenesis could account for how this single microbe with a known virulence factor could be characterized as a pathogen in one host, an opportunist in another host, and a commensal in yet another. Similarly, microbe-centric views could not explain the observation that some organisms, e.g., *Staphylococcus aureus*, could simultaneously exist as a pathogen causing bacteremia and a resident of the nasal flora in the same host (12).

The growing inability of the microbial pathogenesis and infectious disease fields to reconcile the state of a microbe in a host (e.g., colonizer, commensal, opportunist, or pathogen) with the immune status of the host and its very ability to cause disease at all stemmed from the microbe-centric view that virulence was solely a microbial property. Clearly, this view could not explain how the same microbe could cause disease in some, but not all, hosts or how it could be commensal and pathogen in the same host. Compounding this problem was the application of molecular biology to the study of microbial pathogenicity beginning in the 1970s. This line of research, which attempted to identify specific microbial genes that conferred virulence, reinforced microbe-centrism. Some knowledge gained from these studies reinforced the importance of known virulence factors in host damage. However, despite vigorous efforts, this line of investigation failed to explain why nonpathogens can also cause disease. What was missing was inclusion of the role of the host in microbial pathogenesis and virulence. The role of the host became obvious when diseases due to so-called nonpathogens began to emerge in patients with impaired immunity. This historical convergence challenged the reductionist approach to the study of microbial pathogenesis championed by both molecular techniques and the increasing specialization by scientists (13). Also, it became clear that the emergence of "nonpathogens" as the cause of disease could not be reconciled with available theories and intellectual constructs. We came face to face with this problem while designing and then teaching the inaugural course in microbial pathogenesis at the Albert Einstein College of Medicine from 1996 to 1999. As we confronted the conundrum of trying to explain what made some microbes pathogenic and others not, we sought to develop an integrated theory that accounted for the role of both the host and the microbe in microbial pathogenesis and infectious diseases. The result was the "damage-response framework" (DRF). It is noteworthy that the DRF traces its origin to class notes. At the time, we were frustrated by imprecise and incomplete definitions of the basic terminology of the field (14). Hence, the DRF began as an attempt to clarify the definitions of fundamental terms, such as virulence and pathogenicity, to provide students with a theoretical basis for solving problems in microbial pathogenesis that was independent of the phylogenetic characterization of microbes (15).

The damage-response framework of microbial pathogenesis. The DRF was formulated over a decade ago and put forth in a series of essays to provide an alternative to the aforementioned microbe-centric view that virulence is a microbe-dictated progress which had prevailed from the late 1890s to the late 1990s (7–10). The DRF is based on three tenets that are considered obvious and incontrovertible: (i) microbial pathogenesis requires a microbe and a host, (ii) the microbe and the host must interact, and (iii) the relevant outcome of host-microbe interaction is damage in the host, whereby damage results from microbial or host factors, or both. The main impact of the DRF was that it included a role for the host as well as the microbe in microbial pathogenesis. It also pioneered the concept that host damage could stem from either weak or strong responses to microbes. Although others had also considered the host to be a contributor to microbial pathogenesis, previous views were unable to define a role for the host that included hosts with normal as well as impaired immunity. As noted above, the development of tools and platforms to dissect microbial gene expression and biology perpetuated the longstanding focus on microbe-centric views of microbial pathogenesis. This led to increasing polarization because host- and microbe-centric views as exemplified by the concepts of opportunistic pathogens and the requirements of Falkow's Molecular Postulates (16, 17), respectively, were irreconcilable. In contrast, the DRF was able to incorporate the range of host responses to microbes with a parabolic curve in which host damage is plotted as a function of the host response. Initially, this led to a depiction of the outcome of host-microbe interactions by six different curves (or

classes) representing microbes known to cause disease (14). Importantly, the microbial classes were independent of the phylogenetic class (such as bacterium, virus, fungus, or parasite) or identifying characteristics (such as Gram negative or Gram positive) of the organism. The six classes were novel and useful when first proposed because they placed the focus on the outcome of host-microbe interaction, rather than microbial features. For example, classifying *Aspergillus* spp. as class 4 pathogens, which caused disease in states of either weak or strong immune responses, provided a clear explanation of how these organisms can cause invasive disease in neutropenic patients and hypersensitivity pneumonitis in otherwise healthy farmers. However, as more knowledge was acquired, it became clear that some of the classes needed to be reconsidered. In fact, the very ability to reclassify microbes and incorporate new information is a major strength of the DRF that distinguished it from other theories of microbial pathogenesis. Thus, the DRF was flexible and could evolve. As an example, *Cryptococcus neoformans* had initially been characterized as a microbe that caused disease only in the setting of a weak immune response (a class 2 pathogen), e.g., that which occurred in patients with AIDS. However, the advent of antiretroviral therapy and recognition of the immune reconstitution inflammatory syndrome (IRIS) revealed that cryptococcosis could also occur in the setting of a strong, albeit dysregulated, immune response. Similarly, the discovery that a significant inflammatory response is often found in seemingly normal patients with cryptococcosis indicated that *C. neoformans* can cause disease in the setting of a weak or a strong immune response. Hence, *C. neoformans* is better characterized by the parabolic curve of class 3 or 4 microbes. In fact, over time, close examination of the outcome of host-microbe interaction for many microbes suggests that most can be classified by a single parabolic curve in the majority of patients, with the important caveat that the curve can be tipped toward damage stemming from a weak or a strong response. Thus, the DRF incorporates the state of the host into the outcome of host-microbe interaction. Nonetheless, as the complexity of host-microbe interaction has been increasingly unraveled, the initial effort of the DRF to characterize microbes into individual classes has evolved into a focus on the outcome of host-microbe interaction as a function of host response and time.

By focusing only on hosts and microbes and the outcome of their interactions, the DRF introduced a set of concepts that provided a major departure from previous views of microbial pathogenesis. For example, the emphasis on host damage as the relevant outcome of host-microbe interaction allowed the DRF to connect the states of commensalism, colonization (carriage), latency, and disease as states that differed from one another only by the amount of host damage over time (14). This was a fundamentally different view than concepts that considered the states of commensalism or disease as properties of specific microbes. Importantly, the DRF did not imbue any microbe with a state-producing property, e.g., commensalism, opportunism, etc. Instead, the DRF shifted the focus away from microbial properties to the outcome of host-microbe interaction. According to this reasoning, to refer to a specific microbe as a pathogen is flawed, because this assumes that the microbe is solely able to cause damage when its ability to do so is (i) possible only in a susceptible host and (ii) a function of host or microbial factors or both (18). Similar reasoning rejects referring to a microbe as a commensal on the basis of traits that dictate the outcome of its interaction in a host. Commensalism is a state that cannot be defined or dictated independently of a host. According to the DRF, there are no pathogens, no commensals, and no opportunists; instead, there are only microbes and hosts that interact, with the resulting state being the outcome of their interaction. The DRF also dispensed with the need for host-centric views, such as those that regarded host deficits as the driver of microbial pathogenesis, as, for instance, the concept of microbial opportunism. Host-centric views led to the designation by the Centers for Disease Control and Prevention (CDC) of certain infectious diseases as AIDS-defining illnesses, such as pneumocystis pneumonia and cryptococcosis. AIDS-defining conditions were indeed useful to identify patients with severe immunodeficiency, but they were inadequate to explain the occurrence of common microbes in patients with HIV/AIDS, such as *S. pneumoniae*. Although the DRF was initially developed to bring order and precise definitions to the imprecise and exception-driven lexicon of microbial pathogenesis, it soon became clear that the mathematical approach, which viewed damage as a function of the host response, could easily accommodate all microbes, be they associated with disease pathogenesis or not, or even with symbiosis and commensalism. Regarding the latter, indifferent host-microbe interactions produce no known damage, while beneficial host-microbe interactions, which are often characterized as symbiotic, can be viewed as the opposite of damage. As such, they were incorporated into the DRF by drawing the host-response curve as negative damage (19), again underscoring the flexibility of the DRF and its ability to incorporate new information.

In summary, the DRF brought balance to views of microbial pathogenesis by emphasizing the role of the host in producing host damage and focusing on outcomes of host-microbe interaction as a function of the amount/degree/type of damage in the host (14). In hosts with weak responses, microbe-mediated damage can be dominant, while hosts with strong responses often exhibit primarily host-mediated damage. The DRF redefined commonly used, but imprecisely defined, terms such as pathogen, virulence, and pathogenicity as a function of host damage. Notably, despite its success in reemphasizing the role of the host in host-microbe interactions, the DRF never defined what was meant by a host. Nonetheless, the DRF implicitly linked the host with immune responses, thereby implicitly defining hosts as entities that respond to microbes.

Host-microbe interactions exhibit emergent properties. The fact that virulence is an emergent property (20) is critically important for the study of host-microbe interactions because it has an impact on how the subject is approached, conceptually and experimentally. The concept of emergence is ancient and can be traced to Aristotle, who commented that in certain situations the whole is greater than the sum of its parts. Emergent properties are generally considered to have two attributes: (i) the whole must be greater than the sum of the parts and (ii) there must be a form of novelty (21, 22). The application of these attributes to host-microbe interaction implies that once an interaction occurs in a given host, it will be very difficult to predict from first principles whether the outcome will be commensalism, colonization, mutualism, or disease. Like virulence, the microbial states of commensalism, colonization/carriage, mutualism, and disease are emergent properties. Each of these outcomes is novel and results from an interaction between two entities, host and microbe, that cannot be reduced to either entity alone. The phenomenon of emergence imposes serious epistemological limits on host- and microbe-cen-

TABLE 1 Host- and microbe-centric views of outcomes of host-microbe interaction, in comparison with DRF view

State	Microbe-centric	Host-centric	Damage-response framework
Disease	Microbes that cause disease have traits that allow them to cause disease. Such microbes are called pathogens. Pathogens are different from nonpathogens.	Host susceptibility allows some microbes to cause disease. The capacity of a microbe to be a pathogen depends on host immunity.	There are only microbes and hosts: disease is one state resulting from the host-microbe interaction where there is sufficient damage to affect host homeostasis.
Commensalism	Commensal microbes have traits that allow them to adapt to host niches.	Host defenses regulate and maintain microbial flora in host niches.	There are only microbes and hosts: commensalism is one state of the host-microbe interaction where there is no damage to the host but there could be a benefit. When mutual benefit results from the interaction, a state of mutualism results.
Colonization (or carriage)	Colonizing microbes are identified based on their propensity to reside in host tissues/niches. In most instances, the microbe is linked to a particular niche, e.g., *Staphylococcus aureus* in nares. In most/many instances, colonization is transient, rather than permanent/ongoing.	Colonizing microbes are characterized as such based on host immunity. Thus, some microbes could colonize host tissues, whereas others cannot. In transient colonization, the microbe persists until the immune system responds. In persistent colonization, the host is unable to eradicate the state.	There are only microbes and hosts: colonization is a state where the amount of damage incurred by the host is not sufficient to affect homeostasis. Host damage can trigger an immune response that eradicates the microbe. The interaction can also progress to disease. When damage is minimal, this state is indistinguishable from commensalism.

tric approaches to the study of host-microbe interactions. Microbe-centric approaches tend to vary the nature of the microbe, keeping the host constant. An example of such a microbe-centric approach is evaluating the contribution of a microbial component to pathogenicity by comparing the virulence of wild-type, mutant, and gene-complemented strains, a type of experiment that is common in microbiological and genetic research on pathogenesis (23). In contrast, host-centric approaches tend to vary the nature of the host, keeping the microbe constant. An example of a host-centric approach is evaluating the contribution of a host factor to susceptibility by comparing the virulence of the same microbe in a wild-type host and one with a deficiency in the factor of interest, a type of experiment that is common in immunology (23). Although these types of experiments are informative with respect to the specific conditions under which they are performed, neither approach can provide a complete understanding of the outcome of a host-microbe interaction because emergent outcomes are not reducible to the nature of either the host or the microbe under study. This problem is irrelevant in the DRF, since its focus is on outcomes that are a function of multiple factors that include the environment and time in addition to host and microbe. In fact, current experimental approaches are insufficient to simultaneously investigate the contributions of multiple complex entities (e.g., host, microbe, and environment) to host-microbe interaction. For example, we lack experimental technology that can measure host damage that does not impair homeostasis. Thus, most research continues to rely on extreme outcomes in an experimental animal, such as microbial burden, certain measures of inflammation, mortality, or weight loss, to assess the outcome of the host-microbe interaction. Table 1 summarizes host- and microbe-centric views of the outcomes of host-microbe interaction and compares them to the view of the DRF.

The microbiota in the context of the DRF. Over the past decade, the microbiome revolution has expanded our view of the host. There is overwhelming evidence that the microbiota has profound effects on all aspects of host physiology, immunity, and health (24). Although the words "microbiota" and "microbiome" are often used interchangeably, some have proposed precise definitional differences, with microbiota referring to the associated community of microorganisms and microbiome to their gene complement (25). We will use the term microbiota because it fits best with the notion of microbes and hosts. A large literature has now linked the microbiota to immunity as well as to resistance and susceptibility to certain diseases. There is strong evidence that alterations to the microbiota result in host damage (26). The introduction of broad-spectrum antimicrobial agents in the 1940s and 1950s provided the first evidence that the loss of constituents of the microbiota could result in host-microbe interactions that produce host damage and disease. An early example of this phenomenon was noted in the 1950s when the use of antimicrobial agents was linked to the increase in oral candidiasis in otherwise healthy people. Later, antibiotic-associated diarrhea was linked to *Clostridium difficile* colitis, and today, this condition is epidemic in hospitals and a major public health threat. Other examples of associations between disruption of the microbiota and damage in the host include a wide variety of diseases, including allergy, asthma, and obesity, and possibly developmental problems such as autism (27, 28).

The microbiota is an active participant in the outcome of certain host-microbe interactions. The finding that the composition of an insect's gut microbiota was essential for the insecticidal activity of *Bacillus thuringiensis* (29) implicated the very nature of the entity considered the host in its susceptibility to a microbe. Even more compelling, the elimination of gut microbiota with antimicrobial drugs rendered the insects resistant to *B. thuringiensis* (29). Hence, the outcome of disease required at least three contributors: the bacterium, the insect, and the gut microbiota. Similarly, the mosquito gut microbiota influences susceptibility to Chikungunya virus (30) and malaria (31). This concept has been extended to mammals, whereby poliovirus replication in mice was influenced by the gut microbiota such that the presence of certain intestinal bacteria increased poliovirus virulence (32). On the other hand, *Clostridium* produces molecules that attenuate the virulence of *Salmonella* spp. (33). The microbiota is not only a

factor in host susceptibility to disease. It can also be altered by acquisition of certain microbes. In mice, *Citrobacter rodentium* infection produced dynamic alterations to the intestinal microbiota (34). In humans, a bout of *Salmonella* infection was temporally associated with a disruption in the gut microbiota (35). Recently, gut nematodes were shown to predispose the host to viral infection by modulating host cytokine networks (36). In humans, ingestion of probiotic bacteria was associated with an increased likelihood of colonization by vancomycin-resistant enterococcus (37). The microbiota is also sensitive to the health status of the host, since chronically ill patients had significantly reduced microbial diversity in their guts (38). Hence, the host microbiota can facilitate, protect, and respond to intercurrent host-microbe interactions with other, nonmicrobiota microbes.

Hosts and holobionts in the context of the DRF. The original formulation of the DRF did not incorporate a role for the microbiota, in part because it did not define the term "host." Given that the DRF is flexible and has been able to incorporate other advances in knowledge in the fields of microbial pathogenesis and infectious diseases, we now revisit the DRF to incorporate the microbiota. According to the DRF, the host experiences damage or benefits or is indifferent to host-microbe interaction. As such, the host is the readout of host-microbe interaction. For animals, the host is separable from its microbiota, which for mammals is acquired during birth. Animals can also exist in a germfree/axenic/gnotobiotic state, albeit with some physiological and immunological abnormalities. It can be argued that the germfree state is artificial, because it can exist only under nonphysiological laboratory conditions. Nonetheless, the existence of germfree/axenic/gnotobiotic animals makes it possible to consider two entities, animal and microbiota, as separable. In nature, all hosts associate with microbial communities, and these communities, the microbiota, establish a lifelong association with and exert profound effects on the entity in which they live, namely, the host. Together, the host and its microbiota are arguably a new entity. As such, the term "holobiont" has been coined to refer to the combination of microbial and host genes (39, 40). This term, which originated from an evolutionary theory proposing that the unit of evolution was the associated host-microbe community, provides a useful concept for considering the microbiota in the context of the DRF.

In its original formulation, the "response" part of the DRF referred to host immunological responses. The microbiota is known to be critical for proper immunological function. Thus, arguably, the microbiota is already a part of the DRF via its effects on host immunity. Nonetheless, we also need to consider immunity-independent effects of the microbiota on host-microbe interactions. In so doing, it may be helpful to view the microbiota as a nonimmunological system, or a factor that can affect the outcome of host-microbe interactions. If we reconsider the DRF using the term holobiont instead of host, it is apparent that the DRF can accommodate new information emerging from microbiota studies. Thus, it is the holobiont that responds to new or existing interactions with microbes, with the ensuing response being damage, benefit, or indifference. Consider the case of antibiotic-associated diarrhea: in this scenario, broad-spectrum antimicrobial drugs damage the holobiont by depleting the microbiota, rendering the holobiont susceptible to infection, damage, and disease with *Clostridium difficile*.

What is a host? Returning to the original question posed by this essay, we use the information above to define a host as the entity that houses its associated microbiome/microbiota, interacts with microbes, and responds to them in a way that results in damage, benefit, or indifference, thus producing the states of symbiosis, colonization, commensalism, latency, and disease. For a germfree/axenic/gnotobiotic animal, the host definition can be amended to an entity that can potentially house a microbiome/microbiota, and when this occurs, the previous definition applies. We note that a key word in the definition is that a host "responds" to the interaction and thus is different from inanimate microbial niches that also house microbes, such as a rock. This definition incorporates the dictionary definitions noted above, as the host must be living and larger and associated with the acquisition of nutrition, since microbes can feast on hosts and hosts can acquire food for microbes.

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