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GUEST COMMENTARY

Host as the Variable: Model Hosts Approach the Immunological Asymptote

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There is currently great interest in studying the interaction of nonmammalian hosts with microbes that are pathogenic to mammals. In recent years, numerous studies have reported the outcome of human pathogenic microbe interactions with such nonmammalian hosts as amoebae, slime molds, plants, worms, fish, and insects (18, 20, 24, 27, 32, 34). This trend is fueled by many forces, including the precedent established by the discovery of Toll receptors in flies (3), the realization that microbial virulence mechanisms are often shared and conserved among very different types of hosts (26), the suggestion that for certain microbes mammalian virulence may originate from interactions with nonmammalian hosts in the environment (7, 34), the attraction of simpler host-microbe systems with well-developed genetic tool boxes (32), fewer restrictions on the use of nonvertebrate hosts, and scientific curiosity about the extent to which host-microbe interactions are common. In this issue, we are presented with a timely Minireview on worms and flies as model hosts (20) and the description of yet another model host system for the study of fungal pathogenesis using moths (23). Clearly, the study of model hosts is a powerful tool in microbial pathogenesis; yet, their use poses questions that range from the proper lexicon to the justification for this type of scientific inquiry.

Host: alternate, alternative, either, or neither? When investigators attempt to glean new insights about the pathogenesis of a specific microbe from a new experimental system that utilizes a different type of host, the new system is sometimes referred to as an alternate or alternative host or model. Although this terminology is understandable in the context of differentiating the new experimental system from the vertebrate or mammalian host of interest, the terms "alternate host" or "alternative host" are already used in other fields, where they convey specific meanings. An alternate host has been defined as a secondary host, as either host of a heteroxenous rust (28), and as a facultative intermediate host (14). The alternate host also applies to the situation with some parasites, like *Plasmodium* spp., that alternate between different hosts as part of their life cycle. The phrase "alternative host" is sometimes used to refer to plant hosts that can be colonized by, but are different than, the main host and are not required for the completion of the life cycle of a specific pathogen (19). Since these experimental systems are not usually intended to allow completion of a life cycle, the term "alternative" is probably preferable to maintain some continuity of meaning with the scientific lexicon and the plant and mycology literature. However, a closer inspection of these terms suggests that neither term is really adequate to convey the intended meaning, since the use of a new host is not usually viewed as an alternative to the mammalian host but rather as an experimental system that will hopefully yield new insights by comparative analysis. Consequently, it may be more precise to simply use the phrase "model host" or "host model." Some recent papers have used this terminology (18, 32).

Model hosts: novelty or ancient history? The discovery of Toll receptors in *Drosophila melanogaster* has reinvigorated the study of other hosts in the disciplines of microbial pathogenesis and immunology, but it is worth remembering that Metchnikoff drew key immunological and developmental insights from observational studies of marine invertebrates (36). His observation of amoeboid cells congregating at a lesion in a starfish inspired his proposal of the phagocytic theory of immunity. Metchnikoff's work predated the identification of Toll receptors by 100 years, and both observations were possible because of the conservation of the innate immune system. In fact, a strong case can be made that model hosts are old experimental systems that date to the very beginnings of experimental biology and helped launch the science of immunology.

Microbial and immunological variables. Since microbial virulence is only one outcome of the interaction between a microbe and its host, investigators of microbial pathogenesis must consider at least two variables in any experimental system: the host and the microbe (9). However, since the interests of most investigators in the field of microbial pathogenesis are primarily microbecentric or hostcentric, most experimental systems are focused on either the microbe or the host. A microbe- or hostcentric focus is also encouraged by the current reductionist intellectual climate that encourages in-depth investigation of relatively narrow questions. Microbecentric views of virulence emphasize the existence of virulence factors and mechanisms by which microbes cause disease in susceptible hosts, while hostcentric views tend to focus on immunological variables that affect host susceptibility (9, 10). This dualism is reflected in the larger fields of microbiology and immunology, where the major experimental variables are usually microbe related and host

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related, respectively. Consequently, microbecentric investigators tend to modify microbial variables while keeping the host constant. For example, microbecentric investigators generate microbial mutants and evaluate the contributions of specific genes to the outcome of the host-microbe interaction. This approach is exemplified in Falkow's molecular postulates of virulence, where the object is to use the tools of molecular biology to rigorously identify those attributes that contribute to virulence (11). In contrast, hostcentric investigators tend to vary the host while keeping the microbe constant. Hence, immunologists evaluate the contributions of certain components of the host immune system by making directed gene deletions and comparing the outcomes of infection with given hosts. Another example where host variables are altered is found in vaccine studies where the efficacy of a potential vaccine antigen is evaluated by comparing the susceptibilities of immunized and naïve hosts to a microbe. The causes of this intellectual divide are probably multifactorial and reflect the need for reductionism in the study of complex systems, scientific tribalism, and the intellectual traditions that spawned the disciplines of immunology and microbial pathogenesis from the older fields of microbiology and medicine after the germ theory of disease was accepted in the late 19th century.

Viewed from the context of the microbe- and hostcentric divide, the use of nonmammalian hosts to explore questions of microbial virulence allows microbecentric investigators to approach the immunological asymptote, since changing the host makes the host an experimental variable. Conversely, for hostcentric investigators, the experiences with model hosts provide the basis for comparison that allows the identification of common themes in host defense. Hence, the introduction of model hosts into microbial virulence studies has the potential to narrow the intellectual divide between microbecentric and hostcentric investigators.

Limitations of other model systems. A major limitation of model host systems is that they are useful only for studying nonspecific pathogens (i.e., those capable of infecting and causing disease in more than one host). In this regard, the development of model systems for the human pathogenic fungus *Cryptococcus neoformans* involving amoebae (5, 31), slime mold (30), flies (1), worms (21), and now moths (23) is possible because of the remarkable host range of *C. neoformans*, which also extends to other mammals, birds, and reptiles (8, 13). Model host systems are usually not applicable to viral pathogens that have only one host or to parasites with highly specialized host systems. Another significant limitation of model host systems for mammalian pathogenesis is the need to work at lower temperatures. For example, *Dictyostelium discoideum* is not viable at temperatures above 27°C, and this precludes studies of microbial virulence expression at mammalian temperatures (32). The development of the wax moth (*Galleria mellonella*) as a model host for *C. neoformans* (23), *Aspergillus flavus* (33), and *Candida albicans* (4) is a significant advance because moths can tolerate mammalian temperatures. Interestingly, *C. neoformans* killed moths faster at 37°C than at 30°C (23), suggesting a temperature regulation of virulence factors or an impairment of moth immunity at the higher temperature range.

When model hosts are available as experimental systems, the advantages and disadvantages of the particular system being considered are also a function of the phylogenetic distance between the hosts compared and the experimental question being investigated. Hostcentric investigators interested in immunological questions must contend with the fact that the likelihood of finding commonalities between hosts decreases with their phylogenetic distance. On the other hand, the discovery of a shared immune or virulence strategy between phylogenetically distant hosts could provide major insights into host defense and microbial pathogenesis. Conservation of function across evolutionary distances suggests that the relevant function conferred a significant survival advantage across the evolutionary time scale. Alternatively, the identification of common host defense or virulence strategies could reflect convergent evolution to a particularly suitable solution to a microbial virulence problem. However, immune and virulence strategies specific to mammalian host-microbe interactions are less likely to be discovered by focusing on model hosts. Hence, the adaptive immune system would not have been discovered if immunological experimentation was limited to invertebrate or unicellular hosts. Similarly, a host defense mechanism discovered in a phylogenetically distant model host may have no relevance to host defense in mammals. For example, insects use melanin polymerization, a defense mechanism not found in vertebrates, to trap, contain, and kill microbial pathogens (29). However, such information may still be medically useful, since one could imagine genetically modifying the host of interest to express defense mechanisms of a phylogenetically distant host. Furthermore, microbes that infect phylogenetically distant hosts must have developed virulence strategies to cope with differences in immune mechanisms that in turn may rely on common attributes that can be discerned only by comparative studies. Returning to the melanin example, it is remarkable that this pigment is used both by insects for host defense (29) and by many microbes for virulence (25). Insights gained from model hosts could conceivably be exploited to enhance the resistance of other susceptible hosts. In this regard, the highly effective melanin-based defense strategies used by insects might be engineered into vertebrate or plant hosts, thus providing a new layer of innate immune defense.

For microbecentric investigators, the identification of microbial virulence strategies that are used in both vertebrate and invertebrate hosts would imply highly conserved mechanisms of pathogenesis. Hence, the finding that *Legionella pneumophila* and *C. neoformans* employ generally similar strategies to subvert amoebae and macrophages indicates the development of a non-host-specific pathogenic strategy (31, 34). In this situation, identifying commonalities and differences between the host-microbe interactions could be extremely insightful for dissecting the relevant pathogenic strategy.

Justification. A hurdle faced by investigators wanting to use other hosts to study pathogenesis at a time of limited resources is the need for developing a compelling rationale for justifying their studies. Clearly, if support for biomedical research by society is largely intended for the goal of improving human health, then it is reasonable to ask about the potential benefits of committing scarce resources to the study of microbial interactions with other hosts. Fortunately, there are powerful rationales for using model hosts, some of which have also been proposed by others (15, 20, 32).

(i) Dissection of virulence mechanisms. Model hosts provide convenient systems for identifying potential attributes of virulence in pathogenic microbes. Model hosts are generally better suited than mammalian hosts for high-throughput screening techniques, including genomic and proteomic analyses. The finding that mechanisms of virulence are conserved across very different types of host-microbe interactions provides a compelling rationale for employing model hosts to screen for and identify virulence determinants. There are now numerous precedents whereby virulence determinants identified in model hosts have been shown to be important for mammalian virulence (16, 22, 32, 35). Insights made with model hosts can then be tested with mammal hosts to ascertain their relevance for mammalian microbial pathogenesis.

(ii) Comparative immunological studies. Model hosts can be used to screen for host genetic determinants of both susceptibility and resistance, which can then be tested with mammal host systems for relevance to host defense. The discovery of Toll receptors in flies and the subsequent identification of Toll-like receptors in vertebrates is the precedent that is often used to justify using model hosts to search for new host defense mechanisms. On the other hand, discovering host defense mechanisms in model hosts that have no counterpart in mammals can provide useful insights into other defensive strategies and may reveal microbial vulnerabilities that could be exploited by drug discovery or crop engineering to reduce microbial susceptibility.

(iii) Emergence and maintenance of virulence for certain microbes. For some pathogenic microbes acquired from the environment, the phenomenon of mammalian virulence may result from selection by other microbes, including predators (6, 7). Given that many emergent infectious diseases originate from the environment and that mammalian virulence is influenced by microbe-microbe interactions in environmental niches, one can justify studies of microbes and their likely natural hosts to understand, anticipate, and identify potential threats. Hence, the study of the interaction of microbes with other microbes and hosts could provide insights into the mechanisms responsible for the emergence of virulence.

(iv) Evolutionary studies. Host-microbe interactions are almost certainly ancient in the evolutionary timescale. In fact, eukaryotic cells may have originated from early host-microbe interactions between unicellular organisms which resulted in the emergence of organelles from ancient infection events that led to symbiotic microbial interactions (17).

(v) Drug screening. Invertebrates have been used for screening potentially useful medicinal compounds by taking advantage of conversed physiology between animals. For example, *Drosophila* has been used to screen for antiaging drugs (2), and *Caenorhabditis elegans* has been used to screen for antihelminthic microbial molecules (12). A paper by Mylonakis et al. demonstrates how moths infected with a fungal pathogen can be used to assess the efficacies of combinations of antifungal agents (23). It is noteworthy that the combination of amphotericin B and 5-flucytosine is most effective against *C. neoformans* in moths, a finding that parallels human experience and suggests the potential utility of model hosts to rapidly screen drug combinations for antimicrobial efficacy.

Model hosts are old experimental systems that have been employed for the study of virulence and host defense since the dawn of immunology. Model host systems are powerful adjunctive tools for studying virulence because they have the capacity to highlight similarities, contrast differences, and provide important insights. However, they are not substitute or alternative hosts, since each host-microbe system is unique and no one host can fully replicate another. Even within a species, the genetic diversity among individuals and microbes makes each host-microbe interaction unique. Model hosts are increasingly attractive systems that will undoubtedly continue to find new uses and applications.

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