PERSPECTIVE

Host-Pathogen Interactions: The Attributes of Virulence

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Virulence is one of a number of possible outcomes of host-microbe interaction. As such, microbial virulence is dependent on host factors, as exemplified by the pathogenicity of avirulent microbes in immunocompromised hosts and the lack of pathogenicity of virulent pathogens in immune hosts. Pathogen-centered views of virulence assert that pathogens are distinguished from nonpathogens by their expression of virulence factors. Although this concept appears to apply to certain microbes that cause disease in normal hosts, it does not apply to most microbes that cause disease primarily in immunocompromised hosts. The study of virulence is fraught with the paradox that virulence, despite being a microbial characteristic, can only be expressed in a susceptible host. Thus, the question "What is a pathogen?" begs the question, "What is the outcome of the host-microbe interaction?" We propose that host damage provides a common denominator that translates into the different outcomes of host-microbe interaction.

The words "virulence" and "virulent" derive from the Latin word "virulentus," meaning "full of poison" [1, 2]. "Virulentus" derives from the Latin words "virus" ("poison") and "lentus" ("fullness") [2], and, in turn, the term "virus" may be related to the Sanskrit word "visham," meaning "poison" [2]. The word "virulence" currently is used to characterize the relative capacity of a microbe to cause disease and has traditionally been used to describe a microbial characteristic [3]. This usage is consistent with the ancient meaning of virulence as a microbial characteristic that implies an ability to deliver poison and thereby to cause disease. Currently, this concept has been extended to imply that it is the characteristic of virulence that distinguishes pathogens, which are thought to be virulent, from nonpathogens, which are thought to be avirulent.

The Problem with Virulence

Virulence has been difficult to define since the early days of the microbial theory of disease [3]. Half a century ago, Watson and Brandly [4] concluded that "there is no uniformity of agreement among students of infectious disease on the meaning of the word virulence." This situation still exists today, because there is considerable confusion regarding what is meant by virulence [3, 5, 6]. The confusion stems from the fact that virulence is considered to be a microbial property, yet it is expressed only in a susceptible host. Therefore, rather than being an independent variable, virulence is a dependent variable that is contingent on the availability of a susceptible host and the context and nature of the host-microbe interaction.

This paradox also has been acknowledged by others, who noted that virulence is "a host-centered measure of a phenomenon that is neither host nor parasite but of the host parasite complex" [6]. The concept that virulence is an intrinsic microbial property that distinguishes pathogenic from nonpathogenic microbes is difficult to apply in the face of increasing evidence that host factors are critical determinants of the outcome of host-microbe interactions. For example, so-called virulent microbes are avirulent in hosts with specific immunity, and microbes that are usually avirulent cause disease in impaired hosts. Thus, virulence is not a separate microbial characteristic but, rather, a complex, dynamic, and changeable phenomenon that includes both host and microbial factors.

Furthermore, host-microbe interactions can range from the elimination of the microbe to the death of the host encompassing the states of latency, colonization, and commensalism, which can evolve to cause disease [7]. Thus, the view that virulence is a single characteristic is difficult to reconcile with the fact that the host-microbe interaction is continuous and subject to further change on the basis of host, microbial, and exogenous factors, such as medical intervention.

Classical Attributes of Virulence

The recognition that virulence is a multifaceted characteristic of certain microbes, but not of others, led to efforts to define

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its attributes. Early definitions of virulence were based on descriptions of microbial characteristics, including its degree of pathogenicity; its capacity to overcome host defenses; the severity of the disease that it caused; the percentage of death an infection with it induced; its invasive power; its infectivity or the damage it induced; and its capacity to grow and multiply in a host [3]. For example, Kolmer [8] suggested that virulence arose from 2 microbial factors, toxicity and aggressiveness (or invasive power), and Falk [9] defined virulence as the inverse of resistance. Nonetheless, the basis of the aforementioned definitions of virulence was the presence of certain microbial attributes that appear to have been consistently noted.

Toxicity. The view that virulence depended on the elaboration of poisonous substances from microbes was common in the early 20th century [10–12]. Kolmer [8] proposed that the term "toxicity" applied to the type and amount of poison or toxin produced; he further proposed that soluble poisons could be released from a microbe or were endogenous within the body of the microorganism itself [8]. The classification of bacterial toxins involved a consideration of their immunogenicity and ability to produce antitoxins, although it was recognized that toxin-producing microbes could kill their hosts quickly, presumably before the development of an immune response. Karsner and Ecker [10] described 4 types of bacterial toxins: ptomains, produced by decomposition; exotoxins or true toxins; endotoxins that developed after the death of bacteria; and poisonous bacterial proteins. Ptomains consisted of small molecules, such as methylamines, putrescine, and cadavarin, which did not elicit humoral immunity [10]. Endotoxins elicited antibody responses that could agglutinate the microbe of origin, but such responses were seldom protective [10]. In contrast, true toxins were substances produced by the "life activity of bacteria" that elicited protective antitoxin responses [10]. This terminology reflects the fact that early investigators discerned that exotoxins represented a special virulence attribute distinguishable from media-related toxins or the toxicity that ensues after inoculation of dead bacteria. Stewart [13] provided a broader definition for the attribute of toxicity by defining it as the capacity to damage host tissue. According to Stewart [13], toxicity included not only damage from microbial toxins but also damage from toxic metabolic end products, production of substances that elicited allergic reactions, and interference with nutrition of host cells.

Aggressiveness. The ability of a microorganism to invade, survive, and multiply in the tissues of a host was considered to be the attribute of aggressiveness [8]. Although aggressiveness was considered to be different than toxicity, the 2 terms were often difficult to separate, possibly because some toxins contributed to the ability of the microbe to invade, survive, and multiply in the host. For instance, it was noted that *Staphylococcus aureus* and *Streptococcus pyogenes* made toxins called leukocidins that killed host leukocytes, but they were not toxic in the sense of diphtheria toxin, which was seen to directly promote tissue invasion [13, 14]. However, other organisms provided clear examples that aggressiveness and toxicity were different attributes. *Streptococcus pneumoniae* was considered to be highly aggressive and moderately toxic, whereas the toxin-producing bacteria *Clostridium tetani* and *Corynebacterium diphtheriae* were considered to be highly toxic but only slightly aggressive [13]. A microbial capsule was thought to contribute to the aggressiveness of a microorganism, because it allowed for survival in the host by promoting resistance to phagocytosis [10]. In contrast, Stewart [13] defined aggressiveness as the capacity of bacteria to multiply in tissues, but interestingly, he noted that replication alone was not sufficient to cause disease unless damage resulted. Presumably, any factor that contributed to the ability of a microbe to grow in tissue also would contribute to aggressiveness.

Bails's "aggressin" theory was based on the observation that bacterial exudates contained toxic substances (for a review of Bails's experiments in English, see [11]). "Aggressins" was a term used for substances produced by microorganisms that had the power to inhibit or destroy the ability of the host to defend itself against microbes [10]. Although Bails's "aggressins" were subsequently shown to be endotoxins [15], his "aggressin" theory can be regarded as the intellectual ancestor of today's concept that pathogenic microbes have virulence factors that mediate their pathogenicity. The attribute of invasiveness has not been well defined [16] and appears to be a newer formulation of the term "aggressiveness," which was used in the earlier literature. In one definition, the term refers to the capacity of a microbe to replicate in host tissues, which, in turn, is dependent on the capacity of the microbe to resist host defense mechanisms [16]. In another definition, the term meant the ability to disseminate from a portal of entry [17]. By the 1920s, it was clear that not all pathogenic microbes produced toxins, and further attempts were made to separate virulence from toxicity. By 1949, toxigenicity, infectivity, communicability, and invasiveness were each considered to be attributes of virulence [4].

Replication and transmission. Contagiousness has been considered to be as important as replication for certain microbes to persist in their hosts [18]. This concept led to the incorporation of infectiousness and the ability of microbes to reproduce and adapt in their hosts into the definition of virulence [15]. Although it has been stated that virulence reflects selection for characteristics that have maximized microbial fitness via transmission [19], there is some controversy as to whether in vivo microbial growth per se is a sufficient condition for virulence. The relationship between microbial virulence and transmissibility is complex and currently unresolved for most pathogens (reviewed in [20, 21]). Some authorities have considered the role of the microorganism in contagiousness to be a passive one, suggesting that communicability should not be considered to be an attribute of virulence [16]. On the other hand, there are proponents of the view that the mode of microbial transmission itself—for example, symptoms of diseases that

transmit microbes from one host to another—are attributes of virulence [4, 22]. However, this view cannot apply to all pathogens. For example, the fungus *Cryptococcus neoformans* can cause life-threatening meningoencephalitis, yet it is not contagious. Contagiousness is presumably a function of the route of transmission, location of the infectious process, ability of the microorganism to disseminate from one host to another, and immune status of the host.

The ability of certain organisms to replicate and sustain themselves in protozoan, invertebrate, or nonhuman mammalian vectors is consistent with the concept that virulence is linked to the mode of transmission and may be greater for microbes that do not require host fitness for transmission [23]. In this category, one could also consider the phenomenon of microbe-mediated alterations in host behavior, fitness, or both that facilitate transmission. For example, rats infected by *Toxoplasma gondii* lose their fear of cats, an event that can lead to increased predation and a higher likelihood of the parasite entering its definitive host to complete its reproductive cycle [24]. Along the same lines, a *Yersinia pestis* gene locus increases the feeding of its flea vector, which, in turn, can promote transmission of the microbe [25]. The latter phenomenon also is found among other microbes that replicate in insect vectors [26].

Adherence and attachment. For many microbes, adherence to host tissue is believed to be essential for virulence, and the microbial characteristics that promote adherence to mucosal surfaces are considered to be attributes of virulence [27]. This attribute may be particularly important for the virulence of certain bacterial strains, such as enterotoxigenic strains of *Escherichia coli* [27]. For some microorganisms, virulence has been defined in terms of the capacity to regulate the expression of adherence factors [23]. However, the fact that many commensal microbes also adhere to host cells but are not virulent underscores that some form of host damage is required for microbial virulence [7].

Antigenic variation. Microbes can adapt to evade selective pressures in the host was illustrated by trypanosomal phase variation and the emergence of bacterial strains resistant to serum during infection [15]. Other examples include variation in the expression of surface proteins by *Neisseria* spp. (reviewed in [28]) and the mutability of viral agents, such as retroviruses and hepatitis C virus [29]. Antigenic variation can increase microbial fitness by enhancing the ability of the microbe to evade host defenses and to survive in a host [23].

Immunologic reactions as manifestations of virulence. The ability of certain microbes to elicit deleterious immune responses has been considered to be an attribute of virulence. Hoeprich [17] characterized pathogenic host-microbe interactions, depending on their relative invasiveness, intoxication, and hypersensitivity and considered hypersensitivity to be as important for virulence as toxicity and invasiveness. Consistent with this concept, hypersensitivity reactions, such as the occurrence of Arthuslike phenomena, the development of vasculitis in people convalescing from streptococcal infections, and the intense cellular reaction and tissue destruction in tuberculosis, are all proposed to reflect the virulence of these microorganisms [14].

Virulence Factors as Determinants of Pathogenicity

The concept of virulence factors. Historically, virulence has been defined with a focus on microbe-induced effects on host fitness [6]. Proponents of this view have attributed the ability of a microbe to cause disease to the expression of particular microbial characteristics. Such characteristics, or virulence factors, have been defined classically as components of a pathogen that impair virulence when deleted, but not viability [3]. Microbial attributes, such as the capsule of *S. pneumoniae,* the toxins of *C. diphtheriae* and *Vibrio cholera,* and the M protein of group A *Streptococcus,* are consistent with this definition. Virulence factors can have a myriad of functional roles, including the capacity to facilitate microbial attachment, invasion, or both, as well as the promotion of the growth of a microbe in a host through avoidance of host detection, inhibition of phagocytosis, and regulation of the capacity for intracellular survival. Virulence factors may or may not directly enhance microbial growth in a host. For example, the virulence of some microbes with polysaccharide capsules is related to their capacity to evade host defense mechanisms and to replicate in tissue, which, in turn, induces damage and causes disease, largely as a by-product of the host inflammatory response to microbial growth. Conversely, for microbes that secrete preformed toxins, virulence may not be related to facilitating growth or replication but, instead, to the capacity for invasion or interference with host defense because the secretion action of the toxins does not require microbial growth.

Virulence factors can function in an all or none (requisite) or relative (contributory) fashion. Some requisite virulence factors, such as the toxins or polysaccharide capsules expressed by microbes such as *S. pneumoniae* and *V. cholerae,* confer pathogenicity and the ability to cause disease and therefore serve to discriminate pathogens from nonpathogens. In contrast, contributory virulence factors, such as the proteases and phospholipases of *Candida albicans,* modify the magnitude and extent of disease. However, they are not singular determinants of virulence, because their influence on pathogenicity is a matter of degree as mutant strains retain the capacity to cause disease [30]. Cutler [31] proposed that the virulence phenotype of *C. albicans* requires the expression of multiple genes (reviewed in [32]) that in aggregate confer the virulence phenotype, although singly, the genes are insufficient to determine virulence. Although the distinction between requisite and contributory virulence factors holds true for certain microbial characteristics, it is less clear-cut in hosts with immune defects, because microbes that lack requisite virulence factors may be virulent in the setting of immune impairment. For example, strains of the fungus *C. neoformans* that lack

its requisite virulence factor, the polysaccharide capsule [33], and are avirulent for normal mice can cause meningoencephalitis similar to that caused by encapsulated strains in mice with severe immune deficiency [34]. Thus, the immune status of the host modifies the expression of virulence factors and their ability to confer pathogenicity or to cause damage in the context of a given host-microbe interaction.

Identification of virulence factors. The concept that virulence factors are microbial characteristics that determine the capacity for virulence has pointed the investigation of microbial pathogenesis toward the identification of microbial traits that mediate virulence. The discovery of pathogenicity islands in some pathogenic bacteria [35] and the finding that the acquisition of certain lysogenic bacteriophages confers virulence to the host bacterium [36] are incontrovertible examples of factors that are required for virulence, or virulence factors. The identification of genes that control certain microbial traits and validate the molecular version of Koch postulate [37] has been facilitated by the development of molecular tools to search for genes that may be essential for virulence [38]. The quest for the molecular genetic basis of virulence has spawned the development of technology to look for multiple genes that may ultimately unravel the regulation of virulence at the genetic level [39–42].

Microbial virulence factors can be the target of effective immune responses, such as the antibody response [43], underscoring the fact that host immunity influences virulence. For example, the capsule of *S. pneumoniae,* the toxins of *C. diphtheriae* and *V. cholera,* and the M protein of group A *Streptococcus* are virulence factors that elicit antibodies that prevent these microbes from causing disease. Although not all vaccines or protective immune responses target virulence factors, knowing the target of protective responses can identify microbial characteristics associated with virulence. The successful use of virulence factors as vaccine antigens is consistent with the principle that effective immune responses can modify and reduce, if not negate, the virulence of certain microbes [44].

Limitations of the virulence factor concept. The concept that virulence is conferred by virulence factors applies best to pathogens that are free-living and able to cause disease in hosts with intact immunity. However, the view that pathogenicity is conferred by virulence factors is difficult to apply to many microbes whose pathogenicity is limited mostly to immunocompromised hosts, such as *C. albicans* [32] and *Aspergillus fumigatus* [45]. *Mycobacterium tuberculosis* [46] and *C. albicans* [31] serve as a reminder that classical virulence factors have not been identified for many pathogens. To account for the fact that these microbes are obviously virulent in some hosts but not in others, it has been suggested that factors essential for microbial replication and survival in a host be included as virulence factors [32, 43], although this is inconsistent with the classical definition of virulence factors that exclude constituents that are essential for microbial growth [3]. Along these lines, fungal heat-shock proteins needed for survival at mammalian temperatures have been considered virulence factors for human infection [32]. Furthermore, inclusion of characteristics that permit growth in a host as virulence factors allows for extension of this concept to many viruses for which virulence factors are difficult to define by classical terminology, because the process of replication in host cells is intrinsically associated with pathogenicity.

Constitutively expressed components of microorganisms, such as endotoxin and molecules referred to as modulins (e.g., cell wall polysaccharides and lipids), can contribute to the pathogenic process [47]. Although extension of the definition of a virulence factor to such microbial components is inconsistent with the concept that virulence factors distinguish pathogenic from nonpathogenic microbes (because they are present in all microbes), these molecules have been considered to be virulence factors by some authorities [27, 47, 48]. Clearly, some structural components of microorganisms produce tissue damage [49], possibly by inducing cytokine responses that damage the host [47]. Hence the virulence of some organisms is intrinsically linked to the ability of constitutively produced elements to damage host tissues, often by inducing a host inflammatory response (reviewed in [47]). For example, it has been suggested that *A. fumigatus* lacks virulence factors but possesses instead "physiological factors" such as melanin pigments that contribute to virulence [45]. Despite the problems inherent in the use of conventional definitions of virulence factors, most would agree that, regardless of whether they are needed for growth or are physiologic, a microbial component that can lead to host damage confers virulence. One solution to this conundrum is to change the definition of a virulence factor. Our recent proposal to define virulence factors as microbial attributes that mediate host damage [3] omits the problematic qualifier of whether the microbial trait is needed for survival in the host, includes traits that are necessary for survival and replication in vivo, and encompasses the interplay of host and microbial factors in the emergence of the virulence phenotype.

Virulence Is Influenced by Host Factors

By the 1930s, it was apparent that interference with host defense mechanisms could increase the virulence of certain microbes. Clinical isolates of *Neisseria meningitidis* rapidly lost virulence for normal mice in laboratory conditions, but their residual virulence could still be demonstrated in mice with peritoneal cavities damaged by infusion of gastric mucin [50]. The caveat that immune defects can predispose to diseases caused by certain microbes is exemplified by the fact that avirulent microbes (e.g., *Pneumocystis carinii, Cryptosporidium* spp., and atypical *Mycobacteria*) are virulent in the setting of impaired cell mediated immunity such as that due to human immunodeficiency virus (HIV) type 1 infection. Although the nature of the immune defects that promote the virulence of certain microbes is unknown, the defects that have been identified have accounted for a major inconsistency of Koch's postulates—

namely, that microbial virulence could be manifest in one host but not in another.

The microbial characteristics that promote disease in the absence of a necessary host defense mechanism represent possible virulence factors that would not otherwise be recognized, because the microbe is avirulent in normal hosts. Along these lines, *Legionella pneumophila* infection, which occurs predominantly in patients with defects of cell-mediated immunity, led to the description of a novel mechanism of intracellular parasitism and pathogenesis [51]. Similarly, *Haemophilus influenzae* type b infection and vaccine failure have been linked to a defective immunoglobulin light chain allele that is required to produce opsonic antibodies to *H. influenzae* type b capsular polysaccharide [52]. In people with HIV infection, susceptibility to encapsulated pathogens may be a function of HIV-associated depletion of B cells expressing the VH3 gene family immunoglobulin elements [53, 54] that are used in the response to polysaccharide antigens [55–57]. Thus, an insufficient antibody response can promote virulence, whereas an appropriate antibody response is associated with reduced or no virulence. Virulence also can be modified by intrinsic host factors. For example, HIV strains are not virulent in people who lack certain chemokine receptors, and toxigenic strains of *C. diphtheriae* are avirulent in hosts immunized with diphtheria toxoid. However, the emergence of antigenic variants can restore virulence to microbes, even among immunized populations, and such emergence serves as a reminder that virulence encompasses the attributes of both host and pathogen.

The Measurement of Virulence

The ability of a microorganism to cause disease in an animal model, which is central to Koch's postulates, has been the cornerstone of the measurement of virulence. This method was based on the observation that the inoculum required to kill an animal after experimental infection varied, depending on the microbe. For example, it was noted that infection with only a few pneumococci killed a mouse, whereas much larger inocula were required for other bacterial species [12]. As a result of these observations, virulence was defined as inversely proportional to the number of microorganisms required to cause an infection [58]; by the 1930s, the standard means of determining microbial virulence was to measure the smallest inoculum necessary to kill a susceptible animal [58]. Nonetheless, the limitations of the use of an inoculum size as a measure for virulence were apparent to early investigators. Park and Williams [12] noted that "virulence in test animals does not usually correspond with the severity of the case from which the organism was derived." In addition, the route of infection also was recognized to be an important variable in the experimental measurement of virulence [10], albeit a less important variable for determining the outcome of infection for organisms with great aggressiveness [8].

The use of the inoculum required to kill 50% of experimental animals (LD_{50}) to determine virulence is limited by the availability of a susceptible experimental animal model and the reliance on death as the measurable end point. Certainly, LD_{50} determinations are not applicable to infections that do not disseminate or that are not lethal. Furthermore, death is an all-or-none outcome that does not reflect accurately the continuous nature of the host damage or disease that results from host-microbe interactions. Alternative approaches to characterize virulence have significant limitations—for instance, they may not correlate with lethality. For example, mice infected with wild-type and chitin-deficient strains of *C. albicans* have a comparable organ fungal burden, but only the animals infected with the wild-type strain die [59]. Hence, the microbial burden and animal survival may be discordant. Similarly, antibody administration to mice with *C. neoformans* [60] and *M. tuberculosis* [61] infection prolongs survival, despite having little or no effect on microbial organ burden. These observations underscore the contribution of host factors to survival from microbial challenge. Although recent studies with immunodeficient mouse strains have identified some of the host factors that alter the virulence of certain microbes [62], the influence of host immunity on virulence remains difficult to assess by use of available measurements of virulence.

Another limitation of lethality-based studies of virulence is the need to hold either the microbe (e.g., inoculum) or host (e.g., species and immune status) variables constant. For organisms such as *C. albicans,* the relative virulence of strains is usually determined by comparing survival times of lethally infected mice [63]. These measurements are influenced by the inoculum size, the mouse strain used, and even the medium used to grow the organism [63]. Current methods to assess virulence are inadequate for evaluating the pathogenicity of microorganisms that cause disease only in the presence of other microbial species (pathogenic synergism) [64] or for microbes for which no animal model exists [65]. Moreover, because the majority of natural infections do not kill the host, and because the host damage induced as a result of host-microbe relationships is a continuous variable, the physiological relevance of the use of LD_{50} determinations to measure virulence can be called into question.

Damage as the Operational Construct to Define Virulence and Immunity

In an effort to develop a system that acknowledges the contribution of both host and microbe to microbial pathogenesis, we proposed the damage framework in which the outcome of host-microbe interaction is characterized by the quantity and quality of host damage [3]. According to the damage framework, virulence is defined as the relative capacity of a microbe to cause damage in a host [3, 7]. The word *relative* is commonly used in definitions of virulence [3, 32] because there is no ab-

solute measure for virulence (see above). Furthermore, the word *relative* conveys the fact that there are differences among microbes in their capacity to cause disease. The concept that virulence is predicated on the variable nature and outcome of host-microbe interaction, rather than on either microbe- or host-based characteristics [3], is consistent with both the damage framework and the classical attributes of virulence described above. Toxicity is encompassed by this definition, because it was used to denote the production of toxins that directly could damage host tissues; aggressiveness and invasiveness both contribute directly to host damage by promoting replication in host tissue and avoidance of host defense mechanisms; hypersensitivity is essential for explaining the virulence of such organisms as *M. tuberculosis* and is a form of tissue damage that results from intense immunological responses; and adherence promotes damage by allowing for microbial contact with host cells and is a prerequisite for persistence and replication in the host. Conversely, despite the ability of commensal microbes to adhere to host cells and to replicate in a human host, the interaction of these microbes with their hosts does not result in any known host damage [7]. Hence, the lack of association of commensal infection with damage is fully consistent with the universally accepted view that these microbes are avirulent in immunologically intact hosts.

Interestingly, a central role for damage also has been put forth in a new general theory of the immune response: the danger hypothesis, which suggests that the immune system responds to danger signals caused by microbes in promoting host immunity to microbial pathogens [66, 67]. Although this hypothesis is controversial [68, 69], we note that the damage framework of microbial pathogenesis and the danger hypothesis can be reconciled, because the damage induced in the course of host-microbe interaction is considered to be a form of danger. Notably, by use of the common denominator of host damage, the damage framework has the flexibility to describe commonalities and the continuous variables expressed by microorganisms [7]. Consistent with the view that virulence is a functional expression of pathogenicity, virulence also is a clinically useful term, because it conveys the fact that host damage translates into disease. It is our hope that, as new approaches become available to quantify damage and to characterize qualitative characteristics of virulence, its mechanistic underpinnings will be unraveled, and novel concepts, such as the danger hypothesis, will be validated or rejected.

The Need for Terminology that Can Describe an Interaction

The terms "pathogen" and "virulence" may be obsolete for describing microbial pathogenesis, because they are unable to convey the outcome of an interaction or to accommodate changes in host or pathogen that translate into changes in pathogenicity. Problems with the current terminology are compounded by the use of the term "virulence" in phrases such as "virulence genes," "virulence factors," and "avirulence genes." Clearly, the terms "pathogen" and "virulence" are so widely used and form such a central part of the lexicon of microbial pathogenesis that they cannot be replaced easily. Therefore, although the question "What is a pathogen?" is commonly asked, in essence, this question is not as relevant as "What is the outcome of the host-microbe interaction?" Thus we urge that these terms be used in the least ambiguous manner possible by defining virulence and pathogenicity on the basis of the damage framework as phenomena that reflect the host damage that results from host-microbe interaction.

The genetic uniqueness of each mammalian host, the genetic diversity among microbes, and the inherent variability in the complexity and types of host-microbe contact result in an immense and perhaps unfathomable array of different combinations with the potential to result in different outcomes vis-à-vis microbial virulence. Certainly, all outbred hosts, such as humans, bring different genetic backgrounds and immunological histories to the point of host-microbe contact. Even in laboratory conditions, where many variables can be controlled, including the genetic background of the host and the pathogen, there is often remarkable animal-to-animal variation in the outcome of infection that presumably reflects the influence of chance and other uncontrolled variables. Given the many variables that affect the outcome of each host-microbe interaction, one may conclude that each individual host-microbe relationship is unique. Although this may seem to raise the troubling question of whether microbial pathogenesis can ever be a predictive science, it is our view that as the intricacies of the human immune system and microbial virulence factors are better understood, high confidence predictions of the outcome of host-microbial interactions may be possible. In this regard, the introduction of new technologies that can measure gene expression of the pathogen in vivo and of the host inflammatory response to infection promise to shed new light on the nature of host-microbe interactions and their different outcomes [39, 40, 70–72].

Summary

The phenomenon of microbial virulence is almost universally accepted. However, whether virulence is solely a microbial characteristic, as well as the definition of what constitutes a virulence factor, remain controversial. In our view, the controversy arises, because, even if virulence is a microbial characteristic, it is only relevant in the context of an interaction with a susceptible host, making it a complex phenotype intrinsically dependent on both host and microbe. Thus, the concept that virulence factors distinguish pathogenic from nonpathogenic microbes cannot be universally applicable to all pathogens in all hosts, particularly

those host-microbe interactions that take place between traditionally avirulent microbes and immunocompromised hosts. The understanding of the determinants of virulence has been hampered by the absence of a general conceptual framework that incorporates the contributions of both host and microbe to the outcome of their interaction and by the lack of the tools necessary to measure the gradations and features of a continuous process. From the perspective of the human host, the type and amount of damage incurred is the relevant outcome of the microbe-host interaction. Hence, given that both microbial and host processes contribute to host damage, we believe that damage represents the common denominator for microbial virulence [3]. Investigations of virulence that focus on the interplay of host and microbial attributes, rather than on the independent contributions of either entity alone, by characterizing the qualitative and quantitative attributes of host damage, have the potential to bring about a new understanding of the underlying mechanisms and regulation of virulence.

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