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Determinants of virulence in the pathogenic fungi

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Why are fungi pathogenic? In considering this profound question it is necessary to have functional definitions for virulence and pathogenicity. Although these terms are often used synonymously there are subtle differences in their definitions that make them non synonymous (Casadevall & Pirofski, 1999). For the purposes of this essay I will used definitions of virulence and pathogenicity that were proposed some time ago (Casadevall & Pirofski, 1999). Pathogenicity was defined as the capacity of a microbe to cause damage in a host, while virulence is viewed to be a relative term and defined as the relative capacity of a microbe to cause damage in a host (Casadevall & Pirofski, 1999). In this formulation, a pathogenic microbe causes disease only when the damage incurred in the host is sufficient to affect homeostasis. Importantly, host damage can occur as a result of direct microbial action on tissues, as a result of immune response to the microbe or both. An example of direct fungal damage on tissue may tissue damage from the tremendous force of hyphal extension into cells (MacDonald et al., 2002) or the secretion of fungal toxins such as the gliotoxin produced by *Aspergillus* spp (Kamei & Watanabe, 2005). In contrast, some fungal diseases such as allergic bronchopulmonary aspergillosis and allergic fungal sinusitis are caused by the immune response to the fungus in tissue (Schubert, 2006). In fact, a major difference between fungal pathogenesis of plants and animals is that vertebrates have adaptive immune systems that can respond to fungal antigens. The adaptive immune system can mediate protection but also produce responses that contribute to disease. These definitions simplify the approach to pathogenicity by allowing us to view the multitude of host-fungal interactions in the context of whether damage is incurred by the host. Similarly, the states of microbial pathogenesis, commensalism, colonization, persistence and disease are considered to be continuous and differ from one another only in the amount of damage incurred by the host (Casadevall & Pirofski, 2000).

A determinant of pathogenicity is a virulence factor. The damage-response framework defines a virulence factor as a microbial component that damages the host (Casadevall & Pirofski, 1999). This simple definition provides great freedom for it encompasses microbial products that are directly toxic to the host as well as antigens that elicit harmful immune responses. Virulence factors are of great interest in microbial pathogenesis because they are often the target of the immune response and responses that neutralize the action of virulence factors are often protective. For example, the capsule of *Cryptococcus neoformans* is an essential virulence factor and vaccines that elicit antibody responses to the capsular polysaccharide are protective (Pirofski, 2001).

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In considering the determinants of virulence for animal pathogenic fungi it is important to note the huge diversity of animal life that is susceptible to fungal diseases. Fungi are major pathogens of both vertebrates and invertebrates. In this regard, insects are particularly vulnerable to many species of fungi. Vertebrate animals can be divided into ectothermic and endothermic species. Ectothermic animals (eg 'cold-blooded) maintain body temperature by absorbing heat from the environment and consequently have core temperatures not too different from ambient temperatures. In contrast, endothermic animals (eg 'warm-blooded) spend an enormous metabolic cost to regulate and maintain body temperature. Amphibians and reptiles, like insects, are ectothermic and are vulnerable to many fungal diseases. An extreme example of this vulnerability is provided the catastrophic drop in world amphibian populations, which are being decimated by the chytrid fungus *Batrachochytrium dendrobatidis* (Retallick et al., 2004). The seriousness of this infectious disease is underscored by the fact that several frog species have become extinct. In contrast, mammals and birds are endothermic and use high metabolic basal rates to maintain elevated body temperatures. Interestingly, mammals are relatively resistant to fungal diseases in contrast to bacterial and parasitic diseases. Birds are also resistant although some species are susceptible to disease with thermophilic fungi such as *Aspergillus* spp. The association between high temperature and relatively low susceptibility to fungal diseases and the fact that most fungi grow best at ambient temperatures led to the suggestion that endothermy provides a thermal barrier that excludes the possibility that most fungal species are pathogenic for endothermic animals (Casadevall, 2005).

Among the animal pathogenic fungi the most extensively studied have been those that cause disease in humans. Although the human experience is certainly a very small subset of the larger world of fungal-animal interactions it provides a point of comparison for which considerable information is available. Hence, this essay will examine the origin of virulence in human pathogenic fungi and will attempt to make some generalizations that could be tested in other pathogenic fungi. When surveying the human pathogenic fungi it is immediately apparent that humans are highly resistant to fungal diseases. Most human fungal diseases were described in the past 100 years, an observation that stands in contradistinction to such classical infectious diseases as tuberculosis, plague and smallpox that have been known in one form or another since recorded history. In contrast to viral, bacterial, and parasitic diseases, fungal diseases are sporadic and observed primarily in individuals with an immune deficiency, an alteration in ecology, or an exposure to a disproportionably high inoculum. Although hundreds of fungal species have been described to have the capacity for causing disease in humans in isolated case reports, the overwhelming majority of life-threatening human fungal diseases are caused by less than a dozen fungi that include *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp, *Histoplasma capsulatum*, *Coccidioides* spp, *Blastomyces immitis*, and *Paracoccidioides brasiliensis*. The relative paucity of human-pathogenic fungi among the 1.5 million estimated fungal species is a testament to the efficacy of human defenses.

In general, diseases caused by human-associated fungi like *Candida* spp. or the dermatophytes are associated with host disturbances. For example, candidiasis is associated with lesions in integument, placement of indwelling catheters and/or bacterial flora, while dermatophyte-related diseases such as athlete's foot frequently involve an alteration in foot environment by the wearing of shoes. In contrast, fungal diseases caused by fungi acquired from the environment are associated with new exposures or reactivation of latent infection in hosts who develop immunosuppression.

When considering similarities and differences between animal and plant pathogenic fungi it is instructive to view them from the context of the challenges inherent in establishing themselves in their respective hosts. Given that plant and environmentally-acquired human pathogenic fungi both have their primary ecologic niches in the environment they are the groups most suited for comparison. For human pathogenic fungi thermotolerance for mammalian

temperatures would seem to be a critical requirement not applicable to fungi that cause disease in plants, invertebrates, and ectothermic vertebrates (Casadevall, 2005). Since both plants and animals have powerful innate immune mechanisms, it is reasonable that both groups of pathogenic fungi possess the ability to withstand the ravages of innate immune systems that include, depending on the host, microbicidal peptides, oxidative bursts, phagocytic cells, and nutrient deprivation. Pathogenic fungi that attempt to establish themselves in vertebrate hosts must also survive the adoptive immune response. Interestingly, analysis of mechanisms of virulence of human pathogenic fungi has shown that these microbes have mechanism that can undermine the adaptive immune response. For example, *C. neoformans* sheds capsular polysaccharide in tissue that has profound adverse effects on many functions of innate and adoptive immune function (Vecchiarelli, 2000). Similarly, *B. dermatititis* has an immunodominant antigen known as BAD1 that serves as an adhesion and can subvert the immune response (Klein, 2000). Despite the apparent precision with which certain mechanisms in human pathogenic fungi disable the immune response it is unlikely that these determinants of virulence arose for the sole purpose of mammalian invasion since animal passage is not required for the survival or replication of pathogenic fungi acquired from the environment. In the cases mentioned above, the major role of the capsular polysaccharide of *C. neoformans* is protection in the environment from desiccation and amoeboid predators whereas the BAD1 protein of *B. dermatitis* is involved in Ca2+ metabolism (Brandhorst et al., 2005). Phytopathogens do not have to contend with an adaptive immune system but instead must possess powerful physical and enzymatic mechanisms for piercing the plant cell wall.

Both plant and animal pathogenic fungi that reside in soils and vegetation inhabit extreme environments where they must compete with other microbes, endure extremes of humidity and survive predation by amoeboid organisms and small animals such as nematodes. Hence, both share comparable risks and selection pressures. For several human pathogenic fungi it has been demonstrated that determinants of virulence needed for mammalian pathogenicity are also important for surviving predation by amoeba, slime molds, and nematodes (Steenbergen et al., 2001;Steenbergen et al., 2003;Steenbergen et al., 2004;Mylonakis et al., 2002). As a case in point, *C. neoformans* manifests a remarkably similar intracellular pathogenic strategy in mammalian macrophages and amoebae. For *C. neoformans* the well-established virulence factors of the polysaccharide capsule, melanin production, and phospholipase expression each promote survival in amoebae. Furthermore, the intracellular replication strategy of *C. neoformans* in amoebae and macrophages share uncanny similarities despite the enormous phylogenetic distance between protista and mammalian species. These associations have led to the suggestion that some of the determinants of virulence for human pathogenic fungi were originally selected as mechanisms for surviving against amoeboid cell predators (Casadevall et al., 2003). Hence, many of the virulence factors identified for the human pathogenic fungi appear to be ‘dual use’ determinants in the sense that they allow survival in the environment and establishment of the microbe in mammalian host. It is likely that this principle may extend to the phytogenic fungi whereby determinants of virulence for plants also function to protect the fungal cell from the rigors and dangers of soil environments.

The concept that many virulence determinants of human-pathogenic fungi are dual purpose traits selected for by environmental pressures independent of their function in pathogenicity has several important implications. Firstly, there are almost certainly multiple solutions to the problems posed selection pressures in the environment. For example, some soil fungi could resist ingestion by amoebae whereas others might adopt an intracellular survival strategy that subverts protozoal killing mechanisms. Secondly, the enormity of microbial species in the environment the number of potential microbe-microbe interactions that might lead to the selection of survival traits is staggering. Hence, each environmental microbe has a set of traits that may serve as virulence factors in some hosts. Whether an individual fungus is a pathogen of animals, plants, protozoa, or neither is likely to reflect its individual set of virulence traits.

A conceptual tool for understanding this problem is to consider each virulence characteristic as a ‘card’ in a metaphorical card game (Casadevall, 2006). For a microbe to be capable of virulence in an animal host it must have the appropriate card set. In this formulation the capacity for pathogenicity may be a stochastic process requiring the fortuitous accumulation of appropriate virulence cards to yield a winning ‘hand’ that would allow the microbe to establish itself in a susceptible host. A corollary of this view is that there is nothing special about individual virulence factors exhibited by pathogenic fungi and that it is their combination together with the appropriate host that results in the phenomenon of virulence. Hence, the difference between plant and animal pathogenic fungi may reflect only the cards at hand. In this view there is great value for comparative studies of animal and plant virulence since many determinants of pathogenicity will fall within common themes. Given that emerging diseases often reflect inter-species and kingdom jumps in host parasitism by microbial species knowledge of fungal virulence strategies in plants and animals could help prepare for future disease threats.

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