Dynamic Virulence: Real-Time Assessment of Intracellular Pathogenesis Links Cryptococcus neoformans Phenotype with Clinical Outcome

Michael K. Mansour, a Jatin M. Vyas, a and Stuart M. Levitz b

Department of Medicine, Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts, USA a, and Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical Center, Worcester, Massachusetts, USA b

Abstract While a myriad of studies have examined host factors that predispose persons to infection with the opportunistic fungal pathogen Cryptococcus neoformans, comparatively little has been done to examine how virulence factor differences among cryptococcal isolates may impact outcome. In the recent report by Alanio et al. A. Alanio, M. Desnos-Ollivier, and F. Dromer, mBio 2:e00158-11, 2011), novel flow cytometry-based techniques were employed to demonstrate an association between the phenotype of Cryptococcus neoformans—macrophage interactions, as measured by phagocytosis and intracellular replication, and patient outcomes, as determined by positive cultures on therapy and survival. These experiments establish that the prognosis of patients with cryptococcosis is influenced by the phenotypic properties of the infecting fungal isolate.

Cryptococcus neoformans is an encapsulated opportunistic yeast which is responsible for approximately 1 million infections and over 600,000 deaths per year worldwide (1). The patient populations most affected are those with adaptive immunity dysfunction, specifically T-cell defects. Individuals suffering from AIDS or lymphoma or recipients of chronic immunosuppressive medication are at highest risk for developing cryptococcal infection (2). C. neoformans is ubiquitous, but a higher rate of infection is observed in sub-Saharan countries, which suggests that either there are host factors that result in a more susceptible phenotype or there are differences in the virulence of the fungal strains found in these niches. More recently, hypervirulent strains of the closely related species Cryptococcus gattii have caused epidemic infections in predominantly immunocompetent individuals (3).

C. neoformans can exist as a haploid or diploid organism and divides using either an asexual budding cycle or a sexual stage with conidial forms. Cryptococcus has a complete and intact life cycle in the environment but has clearly found a niche as a mammalian pathogen. The ability of C. neoformans to survive in the human phagocyte may have evolved through the interactions of the fungal cells with free-living amoebae (4).

Exposure to Cryptococcus is thought to typically occur following inhalation of airborne organisms. Once the organisms are in the lungs, professional phagocyte populations (e.g., dendritic cells, macrophages, and polymorphonuclear leukocytes) clear the majority of the organism burden and potently influence the nature and outcome of adaptive immune responses. The strong association of CD4+ T-cell depletion or dysfunction with cryptococcosis is testimony to the particular importance of this immune cell to cryptococcal host defenses. For these reasons, studies aimed at understanding the interaction between professional antigen-presenting cells such as macrophages and Cryptococcus will help define the steps leading to lasting immunity and correlate clinical outcome.

In a recent issue of mBio, Alanio et al. (5) show that the interaction of Cryptococcus with host innate immunity is more complex than previously thought. Using a large panel of C. neoformans organisms isolated from cerebrospinal fluid (CSF) of patients with cryptococcal meningoencephalitis, clinical outcome was shown to rely not only on host immune factors but also on specific virulence properties of the organism. To accomplish this task, Alanio et al. (5) devised an ingenious flow cytometry-based standardized macrophage assay that allowed quantification of both C. neoformans phagocytosis and intracellular replication. Using a reference strain and a macrophage-like cell line, indices were then generated reflecting rates of phagocytosis and intracellular proliferation. Remarkably, based on these two metrics, the authors were able to segregate the cryptococcal isolates into distinct macrophage phenotypes that correlated with clinical and microbiological outcomes (Fig. 1). Patients with isolates that had both a high phagocytic index and high intracellular proliferation experienced a 5-fold-increased risk of death. On the other hand, patients with isolates exhibiting both a low phagocytic index and low intracellular proliferation had a 15-fold-increased risk of having positive CSF cultures after 2 weeks of antifungal therapy. Interestingly, phenotypic characteristics that have been associated with virulence in animal studies (including capsule size, growth rate, chitin content, and urease and laccase activities) did not correlate with clinical outcome.

It is important to remember that because all the isolates in the study by Alanio et al. (5) came from patients who had cryptococcal meningitis, they possess some degree of virulence. Thus, rather than looking at virulence in the traditional sense, the authors have taken a fresh approach by looking at how the interaction of C. neoformans with macrophages in vitro correlates with outcomes in patients who are already infected and receiving antifungal drugs. The results suggest that a dynamic interplay between host innate cells and Cryptococcus continues well after phagocytosis. In support of this concept, Alanio et al. (5) also demonstrated that Cryptococcus
tococcus changes its gene expression profile within the macrophage phagolysosome. Similarly to other intracellular pathogens, C. neoformans undergoes phenotypic and perhaps genotypic change as it adapts to life within the cell. One implication of the work of Alanio et al. (5) is that these intracellular changes have significant clinical ramifications. The receptor-ligand interactions leading to phagocytosis, antifungal activity, and cytokine responses have been well characterized (6). However, we are just beginning to understand the subsequent intracellular events occurring in the phagolysosomal compartment and the consequences of these events. It is clear that this period is not quiescent, and evidence is mounting that there is continued sampling of the pathogens by the innate immune cell. Recent studies have shown that the toll-like receptors (TLR), such as TLR 9, continue to engage the fungal phagolysosome well beyond the initial surface events leading to phagocytosis (7). Data also suggest that the fungal surface evolves within the phagosomal compartment, presenting new and distinct antigens. This fact was highlighted in a study of Aspergillus fumigatus conidia and hyphae where each morphotype was found to elicit a unique immune response (8).

How can we frame these data? The study by Alanio et al. (5) suggests that fungal pathogenesis is dependent not only on host response but also on the dynamic adaptations by the pathogen. Other studies have also supported fungus-specific intracellular virulence factors. C. gattii isolated from the Vancouver Island outbreak has enhanced intracellular survival and proliferation in macrophages. In this example, microarray analysis identified the majority of the gene expression differences, as compared to control, to be centered on mitochondrial genes, which are thought to promote a better-fit state of survival in a phagolysosome (9). Our ability to understand how fungi sense and adapt to intracellular compartments will be important for deciphering the nature of the host-pathogen interaction. In order to answer these questions, new techniques will need to be developed to assess the activities within the phagosomal compartments of professional phagocytes. The work by Alanio et al. (5) is an excellent example of the ingenuity required to advance the field of fungal pathogenesis. Moreover, the authors’ successful ability to correlate the macrophage phenotype of clinical isolates with patient outcomes has “to-the-bedside” implications. In the future, therapy for cryptococcosis may very well be individualized based on the phenotypic and genotypic characteristics of both the host and pathogen.

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**FIG 1** The intrinsic virulence of clinical isolates of Cryptococcus neoformans was assessed by flow cytometry following interaction with macrophages. Patients with isolates that had high phagocytic indices and increased cellular division had significantly increased mortality at 3 months. Conversely, patients with isolates exhibiting low phagocytic indices and reduced cellular division were more likely to fail to sterilize their cerebrospinal fluid at 2 weeks.


