bolic stimuli and responding accordingly. The recent studies described herein support an important role for SCD1 in the metabolic response of these tissues and the development of obesity and insulin resistance. The mechanism for how SCD1 or its product, MUFAs, modulate metabolism is unknown. However, the studies by Gutiérrez-Juárez et al. highlight that increased hepatic insulin sensitivity due to liver-specific inhibition of SCD1 may exist independent of body weight and paradoxically in the presence of increased liver TG and long-chain fatty acyl-CoAs (10).

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Cryptococcal virulence: beyond the usual suspects

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In this issue of the *JCI*, the observation of the altered pathogenicity of a *Cryptococcus neoformans* glucosylceramide (GlcCer) mutant shines new light on the initiation of cryptococcal infection. Rittershaus and colleagues demonstrate that the cell surface glycosphingolipid GlcCer is essential for the fungus to grow in the extracellular environments of the host bloodstream and alveolar spaces of the lung, which, in contrast to the acidic intracellular environment of macrophages, are characterized by a neutral pH (see the related article beginning on page 1651). Their findings establish an unexpected connection between this glycosphingolipid and the fungal responses to physiological CO_2 and pH. They also focus new attention on the therapeutic potential of anti-GlcCer antibodies found in convalescent sera.

Cryptococcus neoformans is among the few fungal pathogens with well-defined virulence factors, including a polysaccharide capsule and a melanin coat (1). The recent past has witnessed the identification of many new genes that impact *C. neoformans* virulence, and in most cases the new genes ultimately govern those known virulence factors. A report from Rittershaus, Del Poeta, and colleagues in this issue of the *JCI* (2) is thus surprising in that it establishes that a new virulence regulator does not act through any previously known virulence traits, but through a connection between lipid-mediated signaling and the pathogen's response to the CO_2 levels and pH of host tissue. The report provides intriguing new insight into the natural infection process and points to the potential therapeutic significance of an antifungal antibody response.

Nonstandard abbreviations used: Gcs; glucosylceramide synthase; GlcCer, glucosylceramide.

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The connection between GlcCer and *C. neoformans* virulence

C. neoformans is an opportunistic pathogen that causes disseminated infection and meningoencephalitis in immunocompromised hosts, especially those with AIDS (1). Its close relative, *Cryptococcus gattii*, is a primary pathogen that caused an outbreak recently on Vancouver Island (3, 4). Infection begins with inhalation of airborne spores or yeast cells. The organism is eventually phagocytosed by macrophages, in which it survives as an intracellular pathogen (5). Rittershaus and colleagues show that the poorly understood events that occur between inhalation and macrophage phagocytosis depend upon cryptococcal synthesis of the sphingolipid glucosylceramide (GlcCer) (2).

GlcCer is found at the surface of *C. neoformans* cells and accumulates at the neck between the mother cell and the emerging daughter cell. In order to determine the function of GlcCer, the authors created a mutant *C. neoformans* strain lacking GlcCer synthase 1 (Gcs1), which they rigorously show to be encoded by the *gcs1* gene (2). This Δ*gcs1* mutant had an unusual phenotype: it was completely avirulent in mice following nasal inhalation, yet caused lethal infection when delivered through intravenous injection (Figure 1). The inhaled organisms

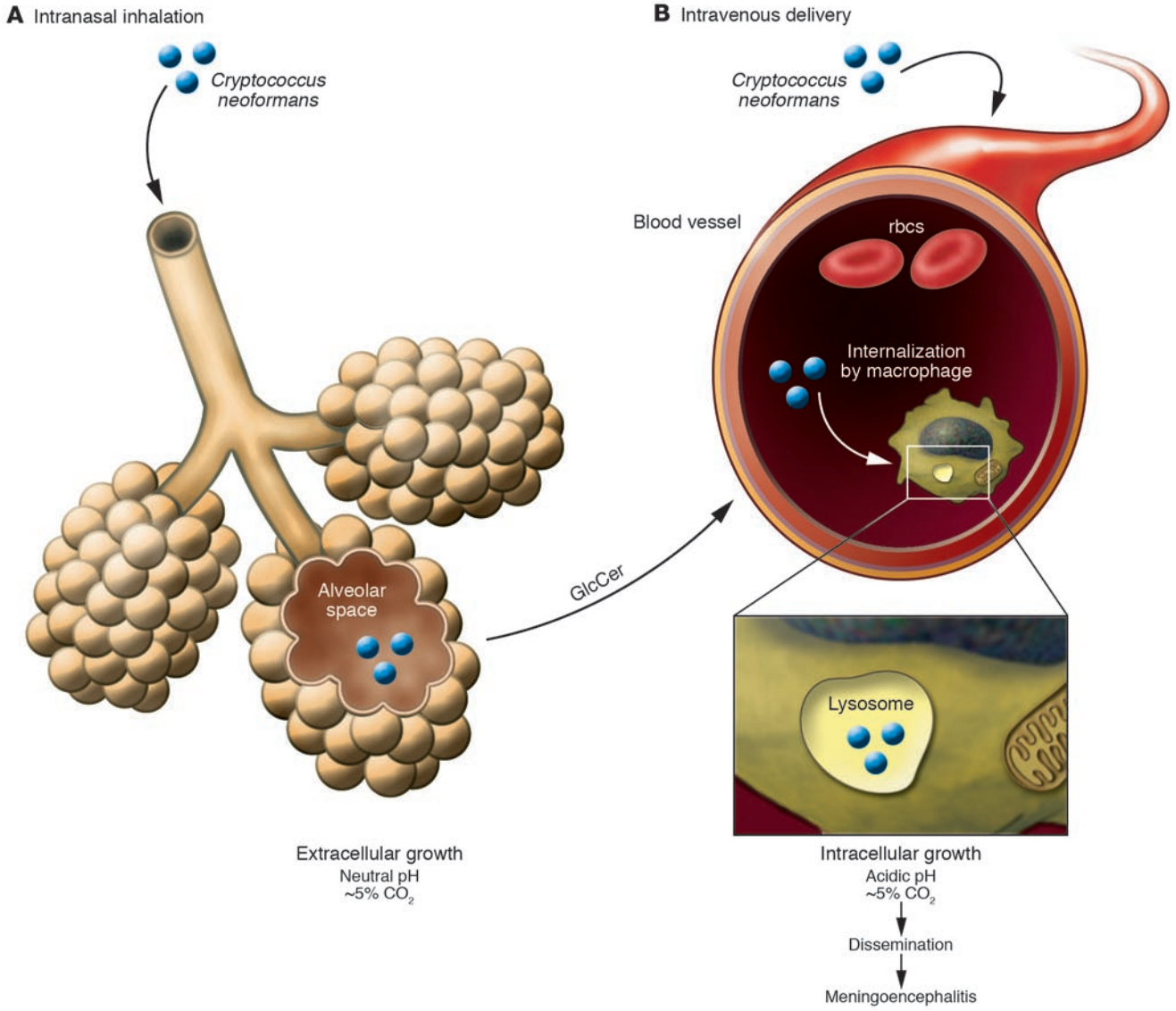

Figure 1

Natural *C. neoformans* infection is initiated after inhalation of airborne spores or cells. After reaching the lung alveoli, the organism is taken up by macrophages into their acidic lysosomes, where the organism divides and disseminates. The report from Rittershaus and colleagues in this issue of the *JCI* (2) shows that there is a critical extracellular growth phase after the organism reaches the lung, but before uptake by macrophages. In this phase, the organism encounters the neutral pH and physiological (~5%) CO_2 level that characterizes host tissues. Rittershaus et al. find that the fungal surface sphingolipid GlcCer is critical for growth under these specific conditions. Thus GlcCer is required for experimental murine infections that are initiated by inhalation (**A**), but not for infections initiated by injection (**B**).

were largely confined to the lung, where they were contained by surrounding layers of host defense cells in what is known as a granulomatous response. A battery of tests of virulence factor production and known attenuating defects failed to identify any reasonable cause of the virulence defect. The authors finally made what is believed to be a novel observation: growth of the Δ*gcs1* mutant was blocked specifically in the presence of high (5%) CO_2 levels, and only at neutral (as opposed to acidic) pH. The Δ*gcs1* mutant grows perfectly well within macrophages, where it is transported to the acidic lysosome. However, it is unable to traverse the tissue of the lung to reach that intracellular sanctuary.

Implications of GlcCer function

This work (2) establishes a connection among GlcCer function and 2 fungal responses that are intimately tied to virulence. The first is the response to neutral or alkaline pH. This response and its relationship to virulence are well established from studies of the fungi *Aspergillus, Saccharomyces,* and *Candida* (6, 7). The Rim101/PacC pH response pathway does not appear to be conserved in *C. neoformans*, but there are several other candidate pH response mediators that are conserved, notably calcineurin (8). The second relevant response is to CO_2. CO_2 levels are low (<0.04%) in the atmosphere but high (~5%) in tissue, and it has long been known that high CO_2 levels induce *C. neoformans* capsule synthesis (9). Our understanding of CO_2 sensing has been revolutionized recently through the work of Buck, Levin, and colleagues

(10), whose studies reveal that soluble adenylyl cyclase is an evolutionarily conserved CO_2 sensor. Muhlschlegel's lab has extended that work elegantly with the demonstration that *C. neoformans* adenylyl cyclase, though membrane associated, is nonetheless CO_2 responsive (11). Thus it is possible that GlcCer function is tied to adenylyl cyclase activity. But, as with any rapidly evolving field, there are many other equally plausible scenarios. Noteworthy in that context is the work of Bahn et al. (12), who showed that high CO_2 levels inhibit *C. neoformans* sexual development, and this inhibition requires the carbonic anhydrase specified by the *can2* gene. Although the signal transduction pathway that mediates sex inhibition is not known, the parallels raise a simple question: is the *can2* carbonic anhydrase also required for CO_2 inhibition of growth in the Δ*gcs1* mutant? These signal transduction–related questions will keep microbial molecular geneticists busy for some time to come.

A second fascinating aspect of this study is the insight it provides into the mechanism of establishment of infection. The path from inhalation to dissemination is dimly lit, and the last major insight into these events was the finding that *C. neoformans* is a facultative intracellular pathogen in vivo (5). With the present finding that the Δ*gcs1* mutant cannot effectively reach the macrophage (2), one can begin to ask what functions permit the wild-type organism to do so. Perhaps most important is to understand whether and how wild-type cryptococci actively inhibit the protective granulomatous response.

Finally, this study highlights a potential therapeutic strategy. Convalescent sera have been shown to contain fungus-specific anti-GlcCer antibodies (13). These antibodies bind to surface-accessible GlcCer and inhibit *C. neoformans* growth (13). The newly revealed novel properties of the Δ*gcs1* mutant suggest that this antibody response may be efficacious in blocking initiation of infection and that mutation of the fungus through loss of Gcs1 function would not cause a resistant infection.

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