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ORIGINAL ARTICLE

The role of host microfilaments and microtubules during opsonin-independent interactions of *Cryptococcus neoformans* with mammalian lung cells

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Abstract The purpose of this investigation was to characterise the interactions of *Cryptococcus neoformans* with mammalian host alveolar epithelial cells and alveolar macrophages, with emphasis on the roles of the cryptococcal capsule and the host cell cytoskeletons. The adherence and internalisation of *C. neoformans* into mammalian lung cells and the roles of host cell cytoskeletons in host–pathogen interactions were studied using in vitro models coupled with a differential fluorescence assay, fluorescence staining, immunofluorescence and drug inhibition of actin and microtubule polymerisation. Under conditions devoid of opsonin and macrophage activation, *C. neoformans* has a high affinity towards MH-S alveolar macrophages, yet associated poorly to A549 alveolar epithelial cells. Acapsular *C. neoformans* adhered to and internalised into the mammalian cells more effectively compared to encapsulated cryptococci. Acapsular *C. neoformans* induced prominent actin reorganisation at the host–pathogen interface in MH-S alveolar macrophages, but minimally affected actin reorganisation in A549 alveolar epithelial cells. Acapsular *C. neoformans* also induced localisation of microtubules to internalised cryptococci in MH-S cells. Drug inhibition of actin and microtubule polymerisation both reduced the association of acapsular *C. neoformans* to alveolar macrophages. The current study visualises and confirms the interactions of *C. neoformans* with mammalian alveolar cells during the establishment of infection in the lungs. The acapsular form of *C. neoformans* effectively adhered to and internalised into alveolar macrophages by inducing localised actin reorganisation, relying on the host's actin and microtubule activities.

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Introduction

Cryptococcus neoformans, the aetiological agent of cryptococcosis, gains entry into the mammalian host through the respiratory route and can disseminate to extrapulmonary sites, with its neurotropism leading to meningoencephalitis, which is universally fatal without treatment [1].

Adherence and subsequent internalisation within mammalian cells are common strategies used by many pathogenic microbes to establish a successful infection [2]. Phagocytosis plays an important role in host defences against invading pathogens, yet the process and host cytoskeleton pathways can be co-opted by some pathogens to promote survival and persistence within the host [3]. It was previously demonstrated that the internalisation of *C. neoformans* into human brain microvascular endothelial cells (HBMEC) and peritoneal macrophages is facilitated through actin reorganisation at the pathogen adherence site [4, 5].

Infectious particles of *C. neoformans* that arrive at the alveolar space encounter alveolar type I and type II epithelial cells, as well as alveolar macrophages [6]. The adherence to and internalisation of *C. neoformans* into mammalian alveolar epithelial cells [7–11], as well as macrophages (reviewed in [12]) have been previously documented. Most studies on cryptococcal–macrophage interactions were conducted using peritoneal macrophages or monocyte-derived macrophages as the host macrophage models in the presence of opsonin and macrophage activation [12]. However, macrophages obtained from distinct anatomical sites differ in functions and phenotypes

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