Quantitative analysis reveals internalization of Cryptococcus neoformans by brain endothelial cells in vivo

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Abstract

Migration of Cryptococcus neoformans from the blood to the brain parenchyma is crucial to cause fatal meningoencephalitis. Although mechanisms involved in brain migration of C. neoformans have been widely studied in vitro, less is known about how the fungus crosses the blood-brain barrier (BBB) in vivo. This is in part because of the lack of an approach to quantitatively analyze the dynamics of fungal transmigration into the brain across the BBB in vivo. In this study, we report a novel approach to quantitatively analyze the interactions between C. neoformans and brain endothelial cells in vivo, a mouse model using flow cytometry. Using this system, we show that C. neoformans was internalized by brain endothelial cells in vivo and that mice infected with acapsular or heat-killed C. neoformans yeast cells displayed a lower frequency of endothelial cells containing the yeast cell compared to mice infected with wild-type or viable yeast cells, respectively. We further demonstrate that brain endothelial cells were invaded by serotype A strain (H99 strain) at a higher rate compared to serotype D strain (520 strain). Our experiments established that internalization of C. neoformans by brain endothelial cells occurred in vivo and offered a powerful approach to quantitatively analyze fungal migration into the brain.

Introduction

Cryptococcus neoformans (C. neoformans) is an encapsulated fungal yeast (FIG. 1) that was first described in 1894. It is a dimorphic fungus, existing as yeast cells in the environment and as a mold at higher moisture levels (1). The fungal cells exist in the environment and when they are inhaled into the lungs, they will initially induce lung infection. In immunocompetent individuals, the fungal cells are usually cleared by the immune cells or establish a latent infection in the lung. However, in immunocompromised individuals including AIDS patients, the organisms can disseminate from the lung to the brain, causing meningitis (1, 2). Cryptococcal meningitis is often fatal without treatment, and even with treatment two-thirds of patients die within a few weeks of diagnosis. Worldwide, fatalities due to cryptococcal meningitis were estimated to be more than 181,100 cases each year (5). It is believed that crossing of the blood-brain barrier (BBB) by C. neoformans is a critical step to cause meningococcal meningitis (6). As such, the mechanism(s) of BBB crossing by C. neoformans is fundamental for understanding cryptococcal pathogenesis. There are several well-established pathways contributing to C. neoformans invasion to the brain, including transcytosis, paracellular pathway, causing tissue damages and trojan horse. Previous studies regarding the fungal invasion were mainly performed in vitro using endothelial cell lines mimicking the in vivo condition. However, relatively less is known about how C. neoformans transmigrates into the brain across the BBB in vivo, mainly because of technical challenges characterizing dynamic events of fungal BBB crossing in vivo. In vivo studies are urgently needed for solving the brain invasion problems.

Project Goal

To establish the novel strategy using flow cytometry to quantitatively analyze Cryptococcus neoformans transmigration rate to brain endothelial cells in vivo.

Internalization of C. neoformans by brain endothelial cells occurs in vivo

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Introduction

Cryptococcus neoformans (C. neoformans) is an encapsulated fungal yeast (FIG. 1) found worldwide. The original site of C. neoformans infection is in the lung, healthy individuals control fungal growth while fungus disseminate through blood stream and cross blood-brain-barrier causing fatal meningoencephalitis (2). Cryptococcosis is caused by encapsulated fungal pathogen Cryptococcus neoformans (1). The fungal cells exist in the environment and when they are inhaled into the lung, they will initially induce lung infection. In immunocompetent individuals, the fungal cells are usually cleared by the immune cells or establish a latent infection in the lung. However, in immunocompromised individuals including AIDS patients, the organisms can disseminate from the lung to the brain, causing meningitis (1, 2). Cryptococcal meningitis is often fatal without treatment, and even with treatment two-thirds of patients die within a few weeks of diagnosis (3, 4). Worldwide, fatalities due to cryptococcal meningitis were estimated to be more than 181,100 cases each year (5). It is believed that crossing of the blood-brain barrier (BBB) by C. neoformans is a critical step to cause meningococcal meningitis (6). As such, the mechanism(s) of BBB crossing by C. neoformans is fundamental for understanding cryptococcal pathogenesis. There are several well-established pathways contributing to C. neoformans invasion to the brain, including transcytosis, paracellular pathway, causing tissue damages and trojan horse. Previous studies regarding the fungal invasion were mainly performed in vitro using endothelial cell lines mimicking the in vivo condition. However, relatively less is known about how C. neoformans transmigrates into the brain across the BBB in vivo, mainly because of technical challenges characterizing dynamic events of fungal BBB crossing in vivo. In vivo studies are urgently needed for solving the brain invasion problems.

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