How do macrophages deal with multiple infection challenges by the lethal fungus Cryptococcus neoformans?

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Introduction

Cryptococcus neoformans is an opportunistic pathogen that is able to infect and cause disease in both humans and mammals via the airways.

The lethal fungus is present in a number of environmental sources including soil, avian excreta, decaying wood from trees and can also be found in amoebae. Environmental exposure and inhalation of Cryptococcus can establish a pulmonary infection mainly in those with a weakened immune system.

Alveolar macrophages are thee initial major phagocytic innate cells that the pathogen encounters in the lungs thus, being the first line of defence. Following initial infection, *C. neoformans* can persist in the host in a latent phase within these macrophages.

Exposure to *C. neoformans* is probable, yet disease is uncommon. Majority of immunocompetent individuals usually develop an asymptomatic primary infection in the lungs and subsequently develop specific adaptive immunity against the pathogen in early childhood

However, in some immunocompromised individuals *C. neoformans* has the tendency to become reactivated and disseminate from the lungs, cross the blood-brain barrier and cause cryptococcal meningitis. The brain is the main organ that is frequently affected in *C. neoformans* dissemination although the pathogen can affect other organs such as the skin, liver, kidney and bones.

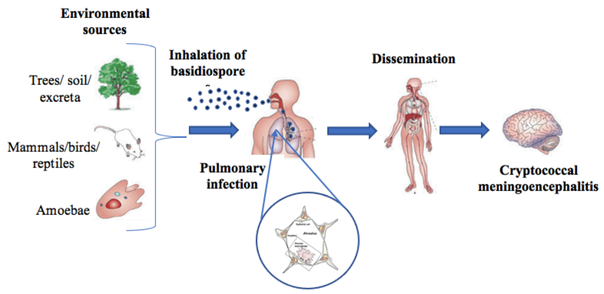


Figure 1: Disease causing pathway of *C. neoformans*.

Aim and Objective

The interaction between alveolar macrophages and *C. neoformans* give rise to whether the fungal pathogen will be;

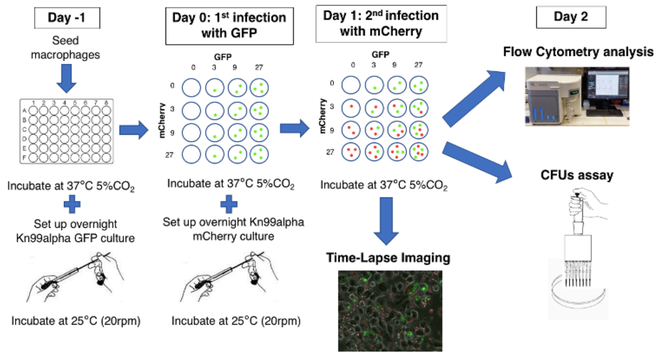
- cleared by macrophage response and the harsh phagosomal environment,
- enter a state of latency and potentially become reactivated and cause disease at a later stage
- thrive and overcome the innate response and thus, establish an infection.

The outcome depends on the hosts immune status and virulence strategies of *C. neoformans.* The literature on a single infection by C.neoformans is extensive however, repeated challenges by the lethal fungus has not been investigated.

- The aim is to understand if the macrophages respond differently to C.neoformans when they are repeatedly challenged, rather than encountering the pathogen for the first time. To address the question we expose J774 macrophages (a murine alveolar macrophage cell line) to sequential infections by **Kn99alpha GFP** and **Kn99alpha mCherry.**

We hypothesised that multiple infections challenges by *C.neoformans* will make it more virulent due to the first infection with the fungal pathogen and make macrophages more aware and therefore more efficient in dealing with the infection. In turn, the data will provide us with the initial recognition into how repeated infections challenges by a fatal fungal pathogen impact subsequent immune responses within the lungs.

Methods



Day-1: Seeding macrophages in a 48-well plate and overnight set up of Kn99alpha-GFP.

Day 0: 1st infection with Kn99alpha-GFP and overnight set up of Kn99alpha-mCherry.

Day 1: 2nd infection with mCherry.

Day 2: Flow cytometry analysis, CFUs assay and Time-Lapse imaging.

Figure 2: Illustrative representation of the methods used

Results

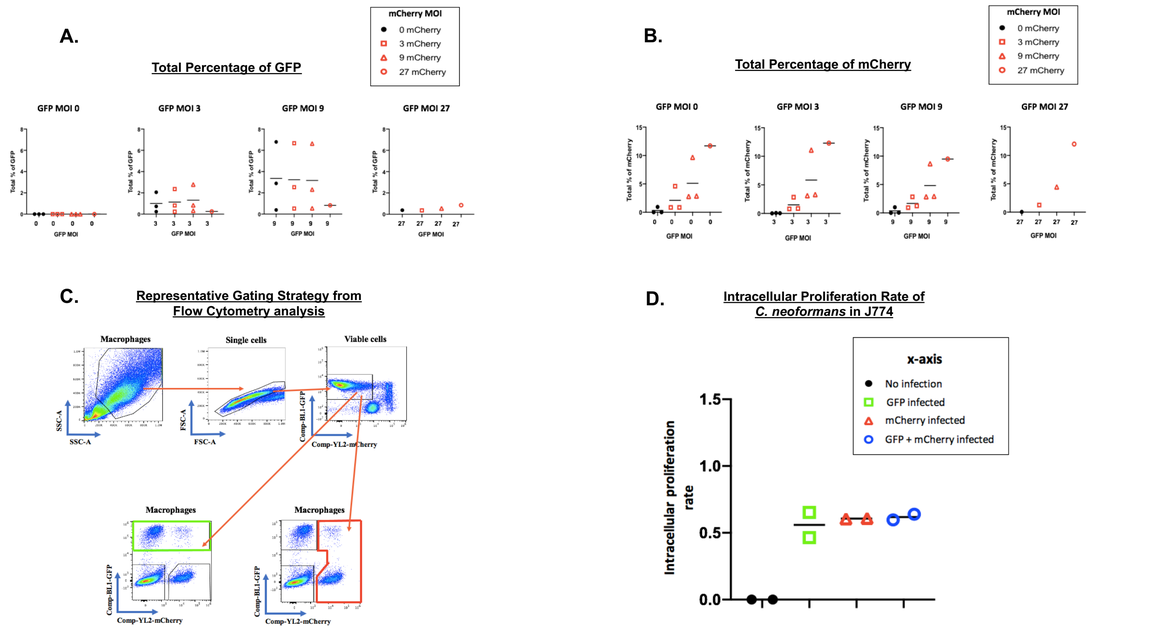


Figure 3A: The total percentage of Kn99alpha-GFP associated with the J774 macrophages. From the graph at GFP MOI 0 there was no GFP infection in the wells and therefore the percentage of GFP was zero. At GFP MOI 3, MOI 9 and MOI 27 there were minimal difference in the total percentage of GFP with increasing mCherry MOI.

Figure 3B: The total percentage of Kn99alpha-mCherry associated with the J774 macrophages. From the graph at GFP MOI 0 there was no mCherry infection in the wells and hence the percentage of mCherry was close to zero. The first infection with GFP regardless of the GFP MOI being 3, 9 or 27 did not influence the uptake of mCherry by the J774 macrophages. Overall, we observed an increase in the phagocytosis of mCherry that is dependent on the MOI of mCherry but this may not be statistically significant due to the variation from one sequential assay to another.

Figure 3C: A representative gating strategy used to identify a specific cell population. The fluorescence intensity of Kn99alpha GFP and mCherry were measured using flow cytometry. The macrophages population was measured for each experimental well and showed a different level of intensity for each condition in the matrix. The population of cells were identified from the side light scattered (SSC). The macrophage population were sub gated to show the fluorescence intensity of single cells which were identified using the forward light scattered (FSC). The singlets were further sub gated to reveal the number of alive cells present. The macrophage population were further sub gated to the level of total GFP (green gate) and total mCherry (red gate) present.

Figure 3D: Average number of total *C. neoformans* per infected J774 macrophage. From the time-lapse imaging two technical replicates were performed with MOI 27. The intracellular proliferation rate was based on a count done by eye and recorded to a maximum of 2 hours after the start of the time-lapse imaging analysis. The data showed similar levels of intracellular proliferation rate with the GFP infected wells, mCherry infected wells and the double infected wells. The mean of the results showed a slight increase in intracellular proliferation rate with double infected macrophages compared to GFP infected and mCherry infected macrophages.

Conclusion and Future Experiments

To summarise from this study, the first infection with Kn99alpha-GFP did not influence the uptake of Kn99alpha-mCherry by the macrophages. The second infection regardless of the ratio of infectious fungi to macrophage cells did not influence the amount GFP inside the macrophages. Overall, the results from the study suggest that the first infection with Kn99alpha-GFP and the second infection with Kn99alpha-mCherry do not have any influences on each other.

As many of the studies focus on a single infection with *C. neoformans* future experiments could give more focus towards several infections with the lethal pathogen. Sequential infections by *C. neoformans in vivo* (mice) could be a potential experiment to compare the results observed *in vitro*. Taken together the data will give the insights into how multiple challenges by *C. neoformans* influence the subsequent immune responses within the lungs.

References

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