Immune Activation by IFN-γ–Producing Cryptococcus neoformans Vaccine Candidates in Monocyte-Derived Macrophages

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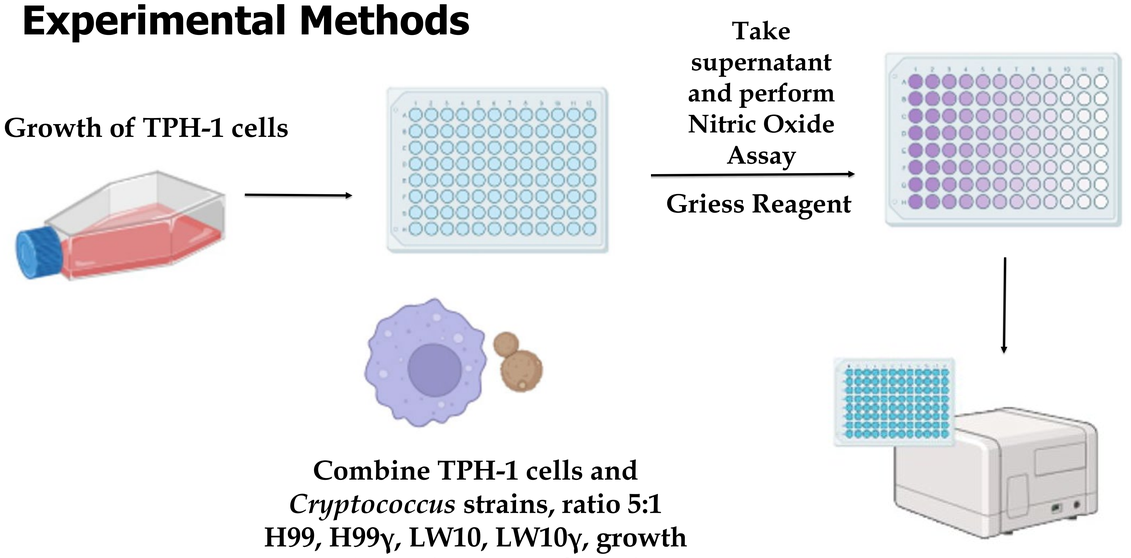
Abstract

Cryptococcus neoformans is a pathogenic fungus that can cause cryptococcosis, affecting the lungs and central nervous system with potentially morbid consequences. This pathogen is particularly aggressive in individuals with impaired T-cell function, such as those with AIDS or on immunosuppressive medications. There are currently no vaccines available for this pathogen, and a limited arsenal of antifungals is available. Our lab has developed a *C. neoformans* strain that produces mouse IFN-ɣ, called H99ɣ, that induces protective immunity against subsequent infection with wild-type *C. neoformans* in mouse models of cryptococcosis. We aim to use variants of this strain by knocking out genes previously known to induce protection in mice, to create a robust vaccine candidate, and better understand the immune response against *Cryptococcus* and develop new therapies. In this study, our goal is to evaluate the efficacy of various newly developed *C. neoformans* vaccine mutants to induce protective immune responses in macrophages against *C. neoformans*. We will expose TPH-1 monocytes to the different *C. neoformans* strains: H99 (wild type), H99ɣ, LW10, LW10ɣ, *sre1Δ*LW10ɣ, u*ra5 Δ*LW10ɣ, and s*gl1Δ*LW10ɣ; and examined the ability of the monocytes to produce nitric oxide, upregulate MHC-II production, and initiate protective immune responses.

Experimental Methods



Findings and Future Directions

Analysis:

- THP-1 cells infected with *C. neoformans* received the best protection in M1 path
- H99ɣ and LW10ɣ had higher levels of Nirate suggesting there are M1 activated
- H99 (wild type) and LW10 had similar or lower levels of Nirate than THP-1 cells alone
- LW10ɣ showed a significant increase between cells alone, LW10, and H99 suggesting LW10ɣ was more likely to activate M1 pathway

Next Steps:

- Analyze other vaccines candidates: *sre1Δ*LW10ɣ, u*ra5 Δ*LW10ɣ, and s*gl1Δ*LW10ɣ; and examine the NO levels produced
- Continue profiling macrophage activation with the different vaccine candidates, measuring production of reactive oxygen species, as well as measuring the production of cytokines like TNF-α, IFN-ɣ, among others.

Introduction

Cryptococcus is a fungal pathogen that mainly affects immunocompromised patients. Inhalation of desiccated spores in the susceptible population can lead to pneumonia and, in some cases, meningoencephalitis.

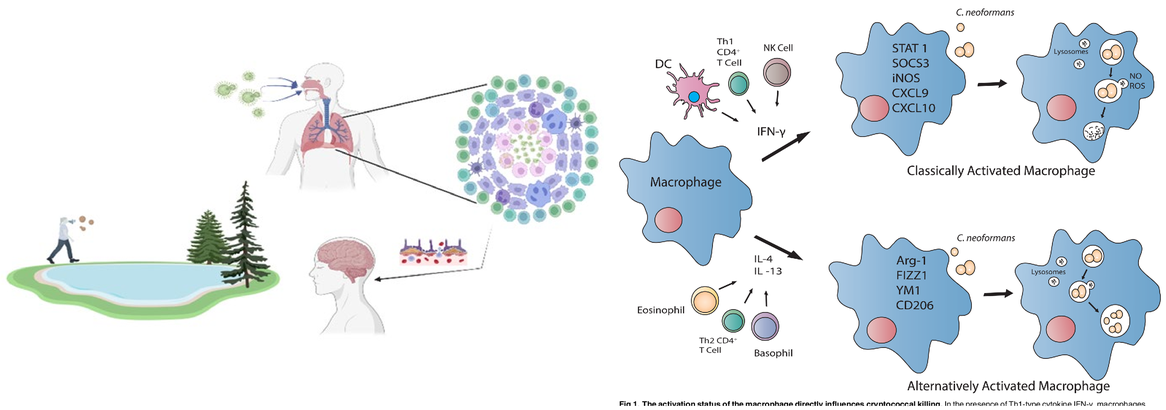
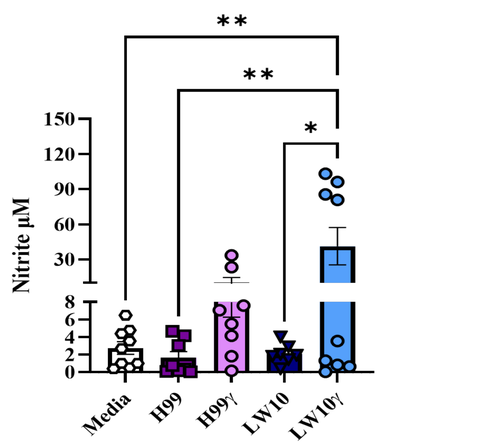


Fig 1. The activation status of the macrophage directly influences cryptococcal killing. In the presence of Th1-type cytokine IFN-γ, macrophages

Production of NO by THP-1 cells increase in the presence of LW10ɣ



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