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**“NOVEL INSIGHTS ON MECHANISMS OF VACCINE-  
INDUCED PROTECTION AGAINST INVASIVE MYCOSES  
ELICITED BY A CRYPTOCOCCUS MUTANT STRAIN”**

By

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Infection, Immunity and Inflammation Track

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## ABSTRACT

Fungal pathogens are underappreciated causes of significant morbidity and mortality worldwide. In previous studies we determined that a heat-killed, *Cryptococcus neoformans* fbp1-deficient strain (HK-fbp1) is a potent vaccine candidate. We find that vaccination with HK-fbp1 confers protective immunity against lethal infection with the parental, virulent strain Cn-H99. We previously determined that vaccine-induced protection is dependent on adaptive immunity and interferon  $\gamma$  signaling (IFN $\gamma$ ). The goal of this study is to determine how IFN $\gamma$  orchestrates the protective immune response induced by HK-fbp1 vaccine, and to explore the potential of HK-fbp1 as a vaccine candidate to protect in different immunocompromised mouse models. To discover the mechanism of protection against homologous infection with H99, our studies aimed to identify relevant innate cell sources of IFN $\gamma$  as well as decipher the contributions of innate cell populations to vaccine-induced protection. We found that early IFN $\gamma$  production peaks at day 3 post vaccination and that neutralization of IFN $\gamma$  at day 3 results in impaired CCR2<sup>+</sup> monocyte maturation and reduced differentiation into monocyte-derived dendritic cells (Mo-DC). IFN $\gamma$  neutralization altered the balance of protective Th1 responses towards a harmful Th2 response after vaccination with HK-fbp1. We also find that monocytes and neutrophils are important sources of early production of IFN $\gamma$ , and that depletion of CCR2<sup>+</sup> cells during priming affects Th1 T cell activation and differentiation. STAT1 is required for IFN signaling, we thus hypothesize that relevant cellular targets of the protective effect of IFN $\gamma$  in our model can be identified by studying mice with targeted STAT1 removal. To test this hypothesis, we examined the impact of selected removal of STAT1 on CD11c<sup>+</sup> cells, which targets alveolar macrophages, Mo-DCs and monocyte-derived macrophages (Mo-Mac) on the activation of vaccine-induced protection. Strikingly, we found that HK-fbp1 immunization was unable to induce any protection in mice deficient in STAT1 expression in CD11c<sup>+</sup> cells. In contrast, control CD11c<sup>cre</sup> and STAT1<sup>fl/fl</sup> mice were responsive to vaccine-induced protection comparable to WT-B6 mice. Altogether, our aggregate findings suggest that monocyte and neutrophils are important innate cell orchestrators of vaccine-induced protection at least in part via regulation of IFN $\gamma$  responses. Our findings also suggest that CD11c<sup>+</sup> cells are important targets of the protective effects of IFN $\gamma$ . To explore the potential of HK-fbp1 as a broad-spectrum vaccine for immunocompromised individuals, we applied HK-fbp1 vaccine in multiple invasive mycoses murine models. We found that vaccine protection remains effective even in mice depleted of CD4<sup>+</sup> T cells. This finding is particularly important in the context of HIV/AIDS-induced immune deficiency. Moreover, we observed that vaccination with HK-fbp1 induces significant cross-protection against challenge with diverse invasive fungal pathogens, including *C. neoformans*, *C. gattii*, and *Aspergillus fumigatus*. Our data suggest that HK-fbp1 has the potential to be a suitable vaccine candidate against cryptococcosis and invasive aspergillosis in both immunocompetent and immunocompromised populations. Beyond the vaccine strategy, we found that HK-fbp1 can also be used as a therapeutic agent to treat early stage invasive *Cryptococcus* infection. Mice infected with H99 and then treated with HK-fbp1 showed significant reduction of fungal burden in the infected lung and no dissemination of fungal burden to the spleen and brain. Immune analysis revealed that early treatment with HK-fbp1 elicited a Th1-biased protective immune response that helps promote better host protection. All these data could significantly expand the utility of HK-fbp1 not only as a prophylactic vaccine but also as a novel therapy against cryptococcosis.