Title

Persisting *Cryptococcus* Yeast Species *Vishniacozyma victoriae* and *Cryptococcus neoformans* Elicit Unique Airway Inflammation in Mice Following Repeated Exposure

Introduction

Allergic airway diseases are a growing concern in industrialized nations and can be influenced by fungal exposures. Pathogenic yeast species such as *Cryptococcus neoformans* are known to exacerbate allergic airway disease. Recent indoor assessments have identified Basidiomycota yeasts, including non-pathogenic species such as *Vishniacozyma victoriae* (syn. *Cryptococcus victoriae*), to be prevalent and potentially associated with asthma. However, the pulmonary immune response to repeated *V. victoriae* exposure was previously unexplored. This study aimed to compare the immunological impact of repeated pulmonary exposure to pathogenic and non-pathogenic *Cryptococcus* yeasts. Mice were repeatedly exposed to either *C. neoformans*, *V. victoriae*, or PBS control, and the immune responses were analyzed by measuring histopathological scores, and quantifications of immune cells in the bronchoalveolar lavage fluid or lung via flow cytometry, and cytokine concentrations in the lung. These findings highlight the importance of *in vivo* characterizations of exposures to frequently detected fungal organisms.

Methods

- Fungal cultivation and sample preparation
    - *Vishniacozyma victoriae* (ATCC MYA-305) was grown in Potato Dextrose Broth at 15°C.
    - *Cryptococcus neoformans* (ATCC 32045) was grown in Yeast Mold Broth at 37°C.
    - Once cultures reached logarithmic growth, cells were collected, washed, and resuspended in specific concentrations in PBS to be used for exposures.
    - Cells were prepared fresh for each exposure.

- Animal Exposures
    - Mice were repeatedly exposed every other day for a total of six exposures via oropharyngeal aspiration.
    - Mice were exposed to either PBS, *V. victoriae*, or *C. neoformans*.
    - One group of mice was euthanized 1-day post final exposure and another 21 days post final exposure to examine potential recovery or exacerbation of exposure-induced immune responses over time.
- Histopathological analysis
    - Lungs designated for histopathological analyses were inflated with formalin, tied off, embedded in paraffin, sectioned, and stained for routine evaluation by a histopathologist.
    - Slides were stained with either hematoxylin and eosin, periodic acid Schiff, or Grocott's methenamine silver stain.
- Flow cytometry
    - Bronchoalveolar lavage fluid (2 mL) was collected from mice designated for flow cytometry analyses.
    - BAL-depleted lung was homogenized, and cells were harvested for flow cytometry.
    - Cells were stained with myeloid and lymphoid surface marker panels.
    - Total cell concentrations per sample (either 2mL BALF or lung) were calculated with counting beads.
    - Flow cytometry was analyzed in Flow Jo version 10.6.
- Cytokine multiplex
    - Frozen lung tissue was homogenized via bead beating and the supernatant was collected.
    - Supernatant was run on a custom ProCartaPlex from Invitrogen for quantification of IL-4, IL-5, IL-13, IL-33, and eotaxin.
    - In-software analysis was performed, and quantifications were extrapolated by comparing to standard curves.
    - All samples were run in duplicate and averaged.

Citations

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