Optimization of Aspergillus versicolor culture and aerosolization in a murine model of inhalational fungal exposure_dataset

Introduction

Aspergillus versicolor is ubiquitous in the environment and is particularly abundant in damp indoor spaces. Exposure to Aspergillus species, as well as other environmental fungi, has been linked to adverse health outcomes including asthma, allergy, and even local or disseminated infection. However, the pulmonary immunological mechanisms associated with repeated exposure to *A. versicolor* have remained relatively uncharacterized. Here*, A. versicolor* was cultured and desiccated on rice, then placed in an acoustical generator system to achieve aerosolization. Mice were challenged with titrated doses of aerosolized conidia to examine deposition, lymphoproliferative properties, and then the immunotoxicological response to repeated inhalation exposures. The necessary dose to induce lymphoproliferation, but not infection-like pathology, was identified. Further, it was determined that the dose was able to initiate localized immune responses. The data presented in this study demonstrate an optimized and reproducible method for delivering *A. versicolor* conidia to rodents via nose-only inhalation. Additionally, the feasibility of a long-term repeated exposure study was established. This experimental protocol can be used in future studies to investigate physiological effects of repeated pulmonary exposure to fungal conidia utilizing a practical and relevant mode of delivery. In total, these data constitute an important foundation for subsequent research in the field.

Methods

- Fungal cultivation and sample preparation
    - *Aspergillus versicolor (Vuillemin) Tiraboschi,* ATCC 9577 was inoculated onto sterile Malt Extract Agar (MEA) using a sterile inoculating loop and incubated at 23 ˚C with 98% humidity for 7 days.
    - Conidia were recovered from the MEA with a sterile inoculating loop in sterile distilled water and diluted to a concentration of $2.5x10^6$ conidia/ml in 5 ml.
    - The suspension of conidia was then inoculated onto 10 g of autoclaved (132˚C for 30min) Mahatma white rice (Riviana Foods Inc., Houston, TX, USA) in 100mm petri dishes and grown at 23 ˚C, 98% humidity for 7-10 days.
    - Heat-inactivated *A. versicolor* conidia were used throughout these studies as a biological particle control. Rice cultures were baked in a laboratory oven (Thermo Fisher, Waltham, MA, USA) at 80 ˚C for 4 hours to produce heat-inactivated *A. versicolor* conidia.
    - Viability was tested by plating 100 viable or heat-inactivated *A. versicolor* spores on MEA plates and counting colony growth after 72 hours.

- Viable and heat-inactivated *A. versicolor* rice cultures were desiccated in vacuum-sealed chambers for 3-5 days or 7-10 days where noted.

- Animal Exposures
  - Animals were exposed in nose-only inhalation chambers, for up to an hour at a time. The total number of exposures and timeline of exposures was unique for each assay listed below.
    - Conidia deposition studies: C57BL/6 mice were exposed to either HEPA-filtered air, viable *A. versicolor* conidia resulting in an estimated ($1x10^5$), or heat-inactivated *A. versicolor* conidia ($3.4x10^4$) spores deposited in the alveolar region of the mouse lung over a single 60-minute duration exposure. Animals were euthanized immediately following the completion of the exposure, via administration of a final dose of 100-300 mg/kg pentobarbital via intraperitoneal injection.
    - Modified local lymph node assay: C57BL/6 mice were exposed to either HEPA-filtered air, viable *A. fumigatus* conidia ($1x10^5$), or viable *A. versicolor* conidia ($1x10^4$, $1x10^5$, $5x10^5$) for 60 minutes for three consecutive days, followed by two days with no exposure. Mice were then injected intravenously via the lateral tail vein with 20 µCi 3h-thymidine (Dupont NEN, Waltham, MA; specific activity 2 Ci/mmol) 48 hours following the final exposure, then euthanized via CO_2 inhalation after five hours.
    - Dosimetry study: C57BL/6 mice were exposed twice weekly for a total of 8 exposures, to doses per exposure of viable *A. versicolor*, or HEPA-filtered air. The per exposure doses included were $1x10^4$, $3x10^4$, $1x10^5$, or $5x10^5$ number of conidia deposited in the alveolar region of each mouse lung. Twenty-four hours following the final exposure, animals were humanely euthanized and dissected.

- 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 or Grocott's methenamine silver stain.
  - Pathological findings were assessed by external pathologists at HistoTox Labs

- Flow cytometry
  - Bronchoalveolar lavage fluid (2 mL) was collected from mice designated for flow cytometry analyses.
  - Cells were stained with myeloid and lymphoid surface marker panels.
  - Flow cytometry was analyzed in Flow Jo version 10.6.

- Serum immunoglobulin quantification
    - Serum was collected from whole blood following cardiac puncture.
    - Serum concentrations of total IgG, IgA, IgM, and IgE were quantified using Invitrogen ELISA kits, per the manufacturer's instructions (Thermo Fisher).

Citations

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