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Variations in antibody response to Aspergillus fumigatus inhalation in mice

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Variations in antibody response to *Aspergillus fumigatus* inhalation in mice

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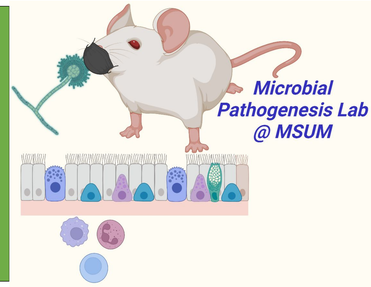

Background

Aspergillus fumigatus is an environmental mold linked with Allergic Bronchopulmonary Aspergillosis (ABPA) and Severe Asthma with Fungal Sensitization (SAFS). *A. fumigatus* exposure is ubiquitous in the environment, and is particularly relevant for farmers exposed to airborne moldy grain dust [1].

Figure 1. Pictures in correspondence from left to right demonstrate *Aspergilllus fumigatus* mold are acquired from dreamstime.com, moldbacteria.com, and gettyimages.com.

Asthma is a chronic inflammatory disorder of the respiratory tract, common in both men and women. *A. fumigatus* is an etiological agent in allergic asthma, therefore, understanding the effects of this mold on the respiratory system, in gender specific manner, would inform the design of advanced diagnosis and treatment options for patients with fungal asthma.

Immunoglobulin E (IgE) is an antibody that helps mediate allergy responses, including anaphylaxis and allergic asthma. This is due to the secretions of histamines due to high affinity of mast cells receptors [2]

***Gender disparities in asthma*:** Research has found the onset of asthma to be earlier in males, but with more severe symptoms and greater prevalence in females [3]. Investigating the effect of sex hormones that underlie these differences is an active area of investigation. In this study, we aimed to investigate the differences in inflammatory responses, in a gender specific manner, in *A. fumigatus* exposed C57BL/6J mice.

Age Disparities in Asthma: Shortly after birth, immune system shows weak bactericidal functions, poor responses to inflammatory stimuli, reduced adhesion to endothelial cells and diminished chemotaxis. THey also have impaired neutrophil functions, allowing a greater risk of bacterial infections. Reduced TLR4 expression, with impaired innate signalling pathways, resulting in diminished cytokine responses

Research Questions

Are there any sex-specific differences in inflammatory cells observed after repeated intranasal route of *A. fumigatus* in juvenile mice?

Rationale

Many studies have configured immunological responses to *Aspergillus fumigatus* in mice, leading to advanced understandings. However, the comparisons of the immune responses between juvenile (younger than 6 weeks) male and female C57BL/6J mice after *A. fumigatus* exposure, remains uninvestigated. At the end of the study, our data could indicate that sex differences could be an important factor in shaping the immune response of mice against *A. fumigatus* which could benefit the design of diagnostics and therapeutics for allergic asthma treatments.

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Methods

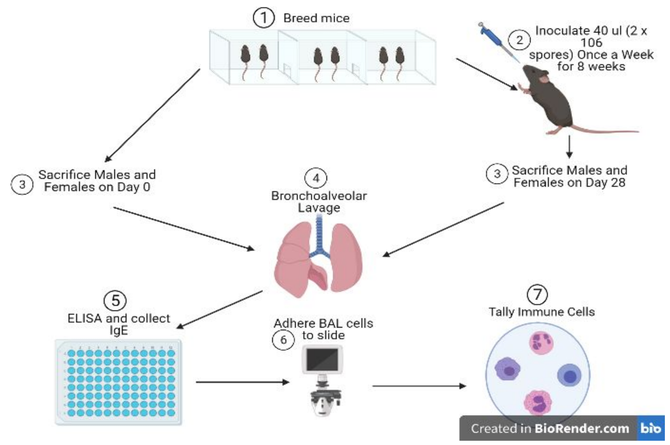

Figure 2. Methodology used to investigate the inflammatory response in C57BL/6J mice via intranasal route. Schematic was created using www.biorender.com

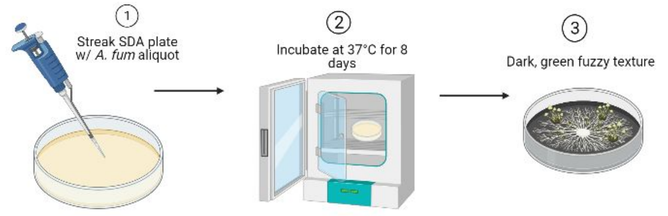

Figure 3. Methodology used to obtain live cultures of *Aspergillus fumigatus*. Schematic was created using www.biorender.com

***A. fumigatus* spores harvest procedure**

1. Aliquot 200 mL of sterile PBS-Tween 80 (0.1% v/v).
2. Poke two layer of gauze into the top of a 50mL tube using a sterile serological pipet.
3. Cover SDA agar plate with *A. fumigatus* spores with PBS-Tween 80.
4. Using serological pipet, scrape the conidia from the agar surface into PBS-Tween 80.
5. Pour PBS-Tween 80 with *A. fumigatus* spore into guaze packed tube.
6. Centrifuge the tube to pellet down the spores at 4°C, 200 X g for 10 mins.
7. Resuspend spores in 1 ml PBS and count using hemocytometer.
8. Store *A. fumigatus* suspension at 4°C and dilute to desired concentration right before use.

Results

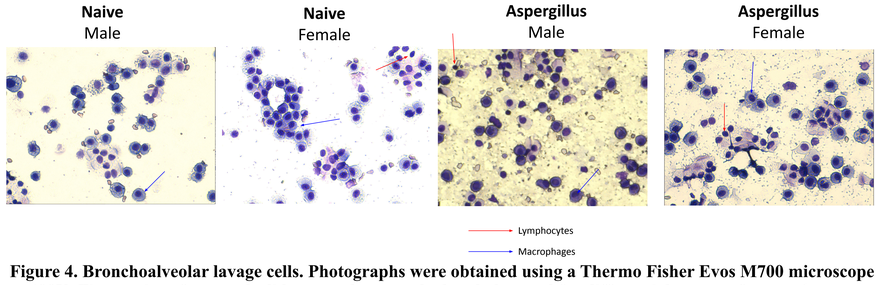

Figure 4. Bronchoalveolar lavage cells. Photographs were obtained using a Thermo Fisher Evos M700 microscope at 400X. Five sections from each slide were photographed and observed for differential counts of macrophages, lymphocytes, neutrophils and eosinophils.

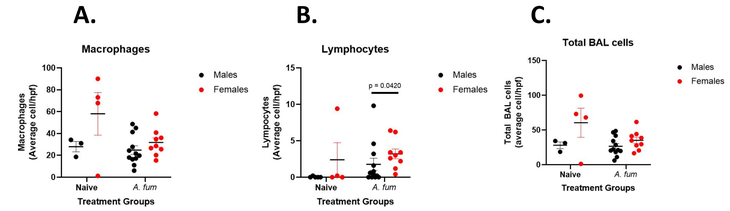

Figure 5. Leukocyte counts (Mean +/- SEM) observed in bronchoalveolar lavage (BAL) obtained from male and female mice at days 0 (Naive) or day 28 (treated) post eight A. fumigatus treatments. p-values ≤ 0.05 are indicated above the treatments comparing males and females and indicated with *** comparing naive and *A. fum* treated groups using Mann-Whitney test. Average cells per high power field (hpf) are demonstrated in Macrophages(A), lymphocytes(B), and total cell count(C) using GraphPad Prism.

Discussion

Each BAL slide was counted and the average of the specimens were calculated. Implementing the mean helped capture if the results were significantly different. No significant difference was calculated between the treatment group, thus rejecting the prediction. However, in support of the prediction, there is a dissimilarity between both sexes due the presence of a heightened lymphocyte count in females with *A. fumigatus* exposure. Elevated lymphocytes are a indicator for inflammatory or infections that are presented in the immune system. Macrophage are vital immune cells that facilitate phagocytosis and secrete inflammatory responses. Treated female mice with *A. fumigatus* are prone to environmental factors that will affect the gene expression. This will have an impact the production of leukocytes, lymphocytes, and macrophages.

In contrast, there is no decrease in macrophages that are present in the treated group in comparison to the naive group. This indicates that *A. fumigatus* does not play a role in causing a decline in macrophages in the treated group, nor between sexes. It can be concluded that *A. fumigatus* treated mice could undergo further analysis when designing diagnostic treatment due to the differing immune responses due to *A. fumigatus* exposure, in turn indicating allergic asthmatic symptoms. Future directions for the upcoming research to include the detection of IgE in both treatment groups in juvenile mice and adult mice, along with detected collagen and goblet cell levels. This would help better understand the role of IgE and airway remodeling in allergic response that would benefit allergic asthma treatments. Also, detecting IgE in both groups to recognize the significance in the role of the antibody and test if they are significantly different.

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Acknowledgments

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