Mouse pathway biology models: Investigating the biology of Alternaria alternata induced lung inflammation in the mouse - role of ILC2 and Th2 cytokines

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*Alternaria alternata* (A. alternate) is a fungal allergen associated with exacerbations and death in asthmatic patients. To gain a better understanding of the role of immunomodulatory mechanisms in Th2 mediated inflammation, we established a murine model of *A. alternata* induced lung inflammation.

Intratracheal instillation of *A. alternata* induces IL-33 release from lung epithelium, triggering activation of innate lymphoid cells (ILC2s), and subsequent release of the potent Th2 cytokines IL-5 and IL-13 eventually leading to BALf eosinophilia.

In this study, we established a time course for BALf eosinophilia following *A. alternata* challenge and examined the effects of neutralizing antibodies on Th2 mediated inflammation. We demonstrated that anti-IL-5 and anti-IL-13 antibodies inhibit eosinophilic inflammation (91% ± 2 and 65% ± 6; mean ± SEM, respectively), while anti-IL-4 and anti-TSLP treatment had no significant effect on eosinophil levels. In addition, using flow cytometry, we detected a significant increase in ILC2s presence in the lung, following *A. alternata* challenge. Finally, our mRNA expression profiling indicate a time dependent induction for a range of immunomodulators including PD-1, PD-L1, PD-L2 and TIGIT, with levels reaching peak at 72 to 96 hours post challenge.

The data obtained suggest that using human relevant allergen in rodent settings may provide useful tool to study the role of ILC2s and immunomodulatory targets following *A. alternata* challenge.

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Sex- and gene-specific differences in the acute in vivo effects of SP-A on the Mouse Alveolar Macrophage Proteome

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Surfactant protein-A (SP-A) is involved in lung innate immunity and affects many alveolar macrophage (AM) functions. We previously demonstrated that SP-A knockout (KO) mice, particularly males, are more susceptible to pneumonia with *Klebsiella pneumoniae*. Humans have two closely related SP-A genes, SFTPA1 and SFTPA2, each with several variants. We examined the in vivo effects of treatment with specific SP-A variants on the AM proteome from SP-A KO mice. We hypothesized that the AM proteome is differentially affected by SP-A1 and SP-A2 in each sex. To test this hypothesis male and female KO mice received either SP-A1 or SP-A2, 18h later the AM were collected, and their proteomes examined by 2D-DIGE and mass spectrometry. We identified 90 proteins and categorized them as related to actin/cytoskeleton, oxidative stress, protease balance/chaperones, regulation of inflammation, and regulatory/developmental processes. SP-A1 and SP-A2 had different effects on the AM proteome and these effects differed between sexes. In males vs females more changes occurred in oxidative stress, protease/chaperones, and inflammation groups with SP-A2 treatment than with SP-A1. In females vs males more SP-A1-induced changes were in actin/cytoskeletal and oxidative stress groups. We conclude that after acute SP-A1 and SP-A2 treatment, sex-specific differences were observed in the AM proteomes from KO mice, and that these sex differences differ in response to SP-A1 and SP-A2. These effects may be responsible for sex differences in lung disease susceptibility. These observations demonstrate the therapeutic potential of exogenous SP-A and illustrate that sex- and gene-specific differences exist in the response to SP-A.

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