Effect of iron on *Aspergillus* proteases: insight into a possible therapeutic target for allergic bronchopulmonary aspergillosis

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**Aspergillus fumigatus** (Af) is a ubiquitous mold that causes a severe allergic immune response known as allergic bronchopulmonary aspergillosis (ABPA) in 10-25% of children with cystic fibrosis (CF). Af secretes proteases, which result in a Th-2 mediated allergic immune response leading to progressive lung function decline. Despite the high morbidity, ABPA is not well characterized in children. Current therapies, including systemic and inhaled corticosteroids, do not modify disease progression and their use has long-term negative sequelae including growth retardation, diabetes, and osteoporosis. Thus, there is clear unmet need to better understand the CF (host) - Af (pathogen) interaction in order to develop new treatment strategies for ABPA and severe fungal asthma.

**Methods and Materials**

To better understand the role of iron in Af protease production, wild type Af (WT, 10AF) and the iron intolerant Af mutant strains (ΔsreA/cccA lacks relevant iron metabolism genes) and ΔprtT (lacks a transcription factor for Af protease expression) were cultured with iron dextran, the xenosiderophore (deferoxamine, DFO), or the iron chelator (deferasirox, DFX) and RT-PCR was used to measure Af protease transcription. The effect of an iron-avid *Pseudomonas* filamentous bacteriophage (PF4) on Af protease expression (RT-PCR) was assessed by co-culturing 10AF and PF4 in increasing iron concentrations. Protease activity was studied in Af cultures exposed to iron, using the skimmed milk substrate assay. To study the impact of iron in vivo, we used murine orthotopic tracheal transplant (OTT) model. The model allows for the delivery of iron or DFX in a nanoparticle solution at the time of transplant. Animals treated with a blank vehicle solution were studied in parallel (controls). Animals were inoculated intratracheally with Af and transcription of Af proteases in the murine trachea were measured using RT-PCR. Using an established ABPA model in CFTR deficient mice (CFTR 489X⁻, FABP-hCFTR 1/1), we evaluated the impact of iron lowering strategies on mitigating a Th-2 mediated cytokine response (IL-4, IL-5, IL-13).

**Results**

*In-vitro*, supplemental iron increased Af protease production whereas protease expression decreased in cultures treated with DFX. Expression of the Af serine protease Tppp - was induced by iron and DFO (P<0.001) (Fig 1A) and inhibited by DFX (Fig 1B). Iron did not upregulate TppA in ΔsreA/cccA strain (Fig 2A), or the ΔprtT strain compared with WT control (Fig 2B). We also found that co-culture of Af with PF4, a bacteriophage commonly found in CF patients, significantly mitigated Tppp expression in presence of iron (P<0.05) (Fig 3). Qualitative analysis of protease activity revealed greater zone of proteolysis in iron-treated Af compared to control (Fig 4). In OTT model, expression of TppA was 4x10⁻²-fold higher in the iron-treated compared to the DFX treated animals (P<0.05) (Fig 5).

**Conclusion**

Our study suggests that:

- Iron induces expression of Af proteases,
- iron chelation can mitigate the expression of iron-induced Af proteases.

**Acknowledgements**

We would like to thank the Division of Pulmonary, Allergy and Critical Care Medicine, and the National Institutes of Health for their support.

**Funding:** NIH/NHLBI K08HL122528-01A1 and R01HL157414-01