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

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Purpose

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. C*urrent 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 $^{+/+}$), we evaluated the impact of iron lowering strategies on mitigating a Th-2 mediated cytokine response (IL-4, IL-5, IL-13).

Figures

Fig1: Iron induces expression of *Af* proteases, which can be reduced by iron chelation

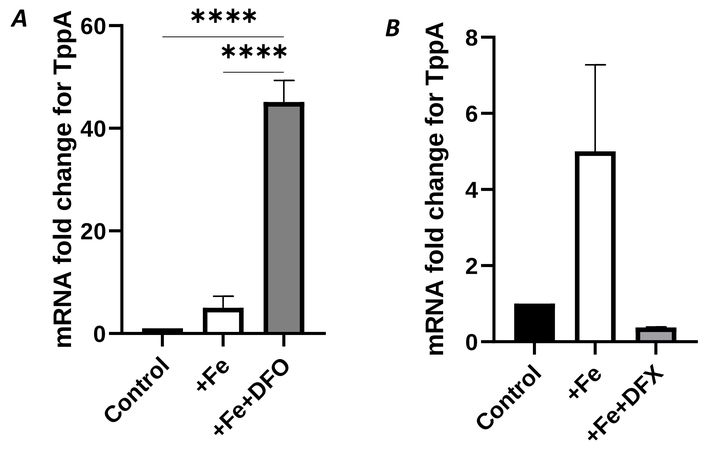


Fig 2: Iron does not induce *Af* protease transcription in *Af* iron metabolism or protease mutant

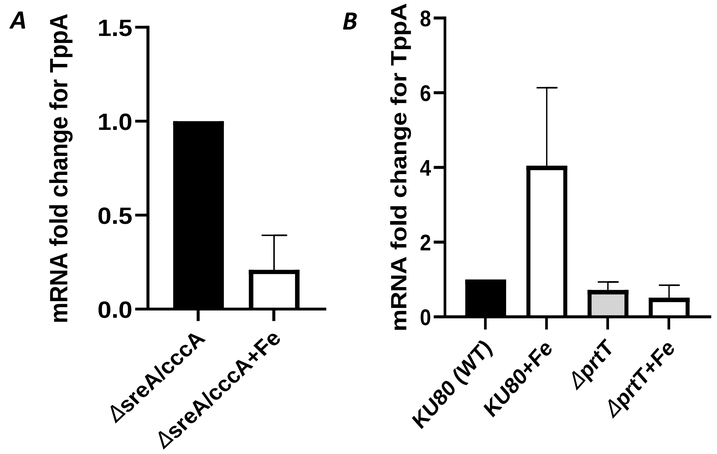


Fig 3: Treatment with bacteriophage Pf4 reduces *Af* protease expression

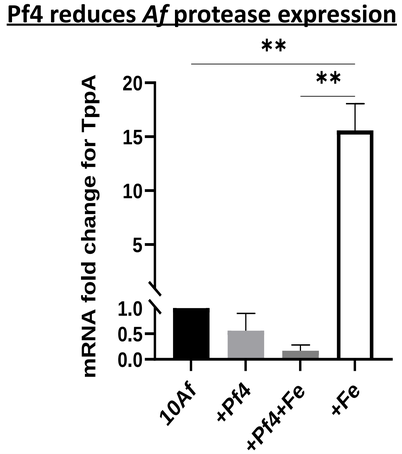


Fig 4: Qualitative analysis of *Af* protease activity in presence of iron

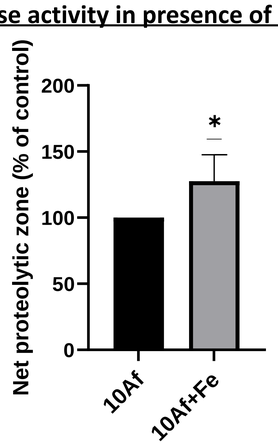
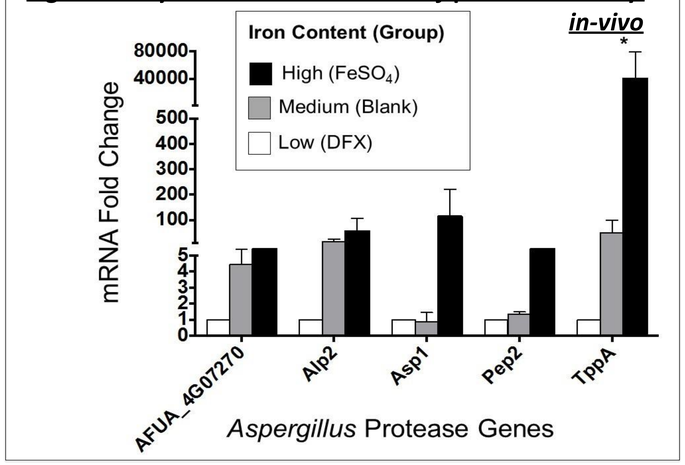


Fig 5: Airway iron overload induces *Af* protease activity *in-vivo*



Results

In-vitro, **supplemental iron increased *Af* protease production whereas protease expression decreased in cultures treated with DFX**. Expression of the *Af* serine protease TppA - 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 TppA 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 4x10^4-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.

Conclusion: Lowering airway iron may represent a novel treatment for ABPA in children with CF.

Acknowledgements

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