Iron overload decreases macrophage lysosomal acidification, impairing the clearance of Aspergillus fumigatus conidia

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INTRODUCTION
Aspergillus fumigatus (A. fumigatus) is an increasing cause of pulmonary disease after lung transplantation.

More research is needed to define modifiable risk factors.

In a murine orthotopic tracheal transplant (OTT) model transplant-associated tissue iron promoted Aspergillus invasion.

Purpose: In this study we sought to determine the effect of iron on macrophage function in murine and human transplant recipients.

HYPOTHESIS
Hypothosis: We hypothesized that iron-laden macrophages have a decreased ability to kill A. fumigatus.

METHODS
Characterization and quantification of iron overloaded macrophages at sites of A. fumigatus invasion

- A GFP expressing A. fumigatus (GFP-A. fumigatus) was used to infect alveoli and excised tracheas were stained for iron (ferritin) and macrophages

- Day 12 alveoli and syntransplants were studied by flow cytometry gating for macrophages (F4/80+ MHC-II+ CD11b+) and these were then re-gated to differentiate between M1 (pro-inflammatory, iNOS+, TNF-α+ and M2 (anti-inflammatory, Arg2+, CD206+) subpopulations.

Conidial phagocytosis and killing

- Primary murine macrophages were exposed to increasing iron concentrations (0.04, 0.1, 0.4 mg/ml) and co-cultured with DeRed-expressing (FLARE)-Af fumigatus

Lysosomal acidification and leakage

- Lung macrophages were seeded on sterile coverslips.

- Lysosomal acidification was detected using LysoSensor Green DND-189 probe.

- LysoSensor reagents exhibit a pH-dependent increase in median fluorescence intensity (MFI) upon acidification.

- The ratio of yellow/blue fluorescence was compared to a standard curve generated by exposing macrophages to pH 1.8-8.0.

- Macrophage iron-induced lysosomal leakage was determined using a FITC-dextran/TRITC-dextran probe.

Deposition of macrophages in mouse transplant recipient

- Flow cytometry was used to verify macrophage deposition in recipient mice treated with clodronate liposomes at day 8 post-transplantation.

- The level of A. fumigatus invasion in clodronate liposome treated transplant recipients were compared to those transplant recipients treated with liposomes containing PBS (controls).

- Invasion was graded on histological 0-4 scale (Figure 1).

- Figure 2 depicts experimental design.

RESULTS

- Figure 3. Iron overloaded macrophages do not clear A. fumigatus conidia. (A) Representative IF staining of lung macrophages pre-treated with increasing concentrations of iron (0.04, 0.1, and 0.4 mg/ml) and co-cultured with A. fumigatus conidia, live (orange – DsRed), A. fumigatus and dead (yellow – A. fumigatus only).

- (B) Percentage of A. fumigatus phagocytosed by macrophages pretreated with iron and co-incubated with A. fumigatus for 24 hours (n=3/group). (C) Percentage of viable A. fumigatus phagocytosed within macrophages pretreated with iron and co-incubated with A. fumigatus (n=3/group).

- Figure 4. Iron decreases macrophage mediated A. fumigatus killing. (A) Representative IF staining of lung macrophages pre-treated with increasing concentrations of iron (0.04, 0.1, and 0.4 mg/ml) and co-cultured with A. fumigatus conidia, live (orange – DsRed), A. fumigatus and dead (yellow – A. fumigatus only).

- (B) Percentage of A. fumigatus conidia killed by macrophages pretreated with iron and co-incubated with A. fumigatus for 24 hours (n=3/group). (C) Percentage of viable A. fumigatus conidia killed within macrophages pretreated with iron and co-incubated with A. fumigatus (n=3/group).

- Figure 5. Lysosomal acidification is defective in iron overloaded macrophages due to lysosomal leakage. (A) Representative IF staining of lung macrophages pre-treated with increasing concentrations of iron (0.04, 0.1, and 0.4 mg/ml) and co-cultured with A. fumigatus conidia, live (orange – DsRed), A. fumigatus and dead (yellow – A. fumigatus only).

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CONCLUSIONS

- Iron-laden macrophages in the murine OTT model do not clear A. fumigatus conidia.

- Lysosomal acidification is defective in iron-laden macrophages, potentially through increased lysosomal acidification.

- Transplant recipient macrophages depletion is protective against A. fumigatus invasion in the murine OTT model.

- These findings were recapitulated in a pilot study in human lung transplant recipients.

IMPLICATIONS

- Macrophage dysregulation may play a central role in transplant (host-A. fumigatus) pathogenesis.

- What happens when macrophages are completely ablated?

- Iron-laden macrophages have a decreased ability to kill ingested A. fumigatus.

- Lysosome acidification is defective in iron-laden macrophages, which may be associated with decreased macrophage acidification.

FUTURE STUDIES

- Studies a more complete ablation of macrophages in the murine OTT model.

- Cytometry by Time of Flight (CyTOF) to characterize differences in macrophage phenotype and signaling responses.

References
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Figure 1. Histologic scale of fungal invasion. Abbreviations: TL: tracheal lumen, C: cartilaginous ring, EL: extra luminal.

Figure 2. Experimental design of macrophage depletion experiments of alveolartransplants treated with clodronate liposomes or PBS- liposomes (control).

Figure 3. Depletion of iron laden macrophage recipients decreases the risk of A. fumigatus infection in the murine OTT model. (A) Depth of fungal invasion (u.s.) semi-quantitatively grade (0-4) histologically using a Grocott’s Methenamine silver stain for A. fumigatus based on depth of invasion in tracheal transplant in clodronate treated and control animals (n=8/group). Data are mean ± SEM as analyzed by non-parametric Mann-Whitney U test. (B) Representative flow cytometry analysis of macrophages (CD11b+, F4/80+), neutrophils (CD11b+, Ly6G), and dendritic cells (CD11c+, CD86+) in CTR and CTR clodronate treated with clodronate liposomes or PBS-liposomes (controls).

Figure 4. Human lung transplant recipients have increased iron levels, and a decreased capacity to kill A. fumigatus conidia. (A) Representative Prussian blue iron staining of bronchial epithelial biopsy samples from recipient (upper panel) and transplanted (lower panels) airways from lung transplant patients, red arrows denote iron laden macrophages. (B) Mean number of iron-laden macrophages in recipient (control) and transplanted endobronchial biopsy samples (n=5). (C) Percentage of A. fumigatus phagocytosed by alveolar macrophages (AM) isolated from a lung transplant recipient (n=11). (D) Percentage of phagocytosed conidia that remained viable after 6 hours as determined by flow cytometry. E) Representative dot plot of fungal killing analyzed by flow cytometry. Flow cytometry data are mean ± SEM and analyzed by Student’s t-test. (P<0.05)

Figure 5. Lysosomal acidification is defective in iron overloaded macrophages due to lysosomal leakage at sites of A. fumigatus invasion. (A) Representative IF staining of lung macrophages pre-treated with increasing concentrations of iron (0.04, 0.1, and 0.4 mg/ml) and co-cultured with A. fumigatus conidia, live (orange – DsRed), A. fumigatus and dead (yellow – A. fumigatus only).

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Figure 6. Lysosomal acidification is defective in iron overloaded macrophages due to lysosomal leakage at sites of A. fumigatus invasion. (A) Representative IF staining of lung macrophages pre-treated with increasing concentrations of iron (0.04, 0.1, and 0.4 mg/ml) and co-cultured with A. fumigatus conidia, live (orange – DsRed), A. fumigatus and dead (yellow – A. fumigatus only).

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