Epithelial uptake of *Aspergillus fumigatus* spores drives efficient fungal clearance *in vivo* and is aberrant in Chronic Obstructive Pulmonary Disease (COPD) patients

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**Airway epithelial cells (AECs) efficiently extinguish *Aspergillus fumigatus* (Af) spores in vitro**

Upon inhalation of *A. fumigatus* (Af) conidia, the contact with airway epithelial cells (AECs) is instant and extensive. AECs internalise Af spores immediately upon *in vitro* infection (Fig. 1), and at 6 hrs post-infection 3% of A549 cells surveyed internalised spores.

![Fig 1: Differential fluorescence-assisted imaging flow cytometry (IFC) permits quantization of spore uptake in vitro. (A) IFC after infection with 10⁵ spores/mL for 6 hr. Yellow = intracellular red fluorescent Af (tdT). Purple = extracellular Af stained with Calceflour White (CW). (B) Number of internalising A549 (AECs) on 2000 total cell number analysed (n = 8).](image)

Af spore uptake by AECs *in vitro* efficiently limits spore germination and by 8 hrs, > 80% of the internalised Af is killed (Fig. 2). However, the wider relevance of Af uptake by AECs during infection remains unknown.

![Fig 2: Fungal spores are killed rapidly following uptake. (A) IFC after infection with 10⁵ spores/mL for 12 hr show delay of germination of internalised Af. (B) Number of internalising AECs on 2000 total cell number analysed (n = 8).](image)

**AECs internalise fungal spores during infection in mice**

In order to test if Af spores are internalised by AECs during mammalian infection, we optimized the isolation and antibody-mediated typing of AECs from mice (Fig. 3A). Using this experimental pipeline we were able to extract and identify both type-I and type-II alveolar epithelial cells, in a 40:60 ratio, which reflects the alveolar composition (Fig. 3B).

![Fig 3: Extraction and typing of AECs from murine lungs. (A) Murine type I (Podoplanin-EP CAM+) and II (CD74+ EP CAM+) AECs harvested directly from leukopenic mice. (B) Number of murine type I and II AECs harvested directly from leukopenic mice (on 2000 cells surveyed). AECs interacting and internalizing Af during infection of leukopenic mice were identified and quantified, obtaining the first ever demonstration that, in whole animals, Af is internalised by AECs (Fig. 4A-C). Respectively 1% and 3.5% of the murine type I and and type-II AECs were found to internalise Af (Fig. 4D).](image)

**Lung disease significantly impacts uptake of fungal spores**

To measure altered capacity of diseased epithelia to uptake spores and/or killing of internalised spores, commercially sourced primary human AECs were used (Fig. 6). Primary human AECs internalise Af and AECs from a chronic obstructive pulmonary disease (COPD) donor show a significant increase in the internalisation index.

![Fig 6: Phagocytic index in COPD donors is 3X higher than healthy donors. Number of internalising primary human AECs after infection with 10⁵ spores/mL for 6 hr, n=9000 sampled events, 3 biological reps with 3 technical reps, 1 donor for each group. Purity of primary human AECs was assessed by flow cytometry and ~97% were AECs Type I (Podo-PE) and ~1% AECs Type II (CD74-FITC)).](image)

**Isolation and culture of primary human AECs allows to assess donor-dependent variance of *A. fumigatus*-AEC interactions**

Single cell analysis with locally isolated primary AECs confirmed that AECs from COPD donors show a significant increase in the internalisation index which was 3X higher that observed for AECs from healthy donors (Fig. 7).

![Fig 7: Primary human AECs internalise spores, a process altered by lung disease. (A) Tissue is sourced via a collaboration with the Collaborative Centre for Inflammation Research (MCCIR, University of Manchester) and the University Hospital South Manchester. ~ 5 grams of resected tissue are processed. Purity of extracted primary human AECs was assessed by flow cytometry and ~90% cells were AECs Type I (Podo-PE) and ~1% AECs Type II (CD74-FITC). (B) Number of internalising primary human AECs after infection with 10⁵ spores/mL for 6 hr, n=1000 sampled events, 1 biological rep 3 technical reps, 1 donor for each group.](image)

**Conclusions**

The integration of the data obtained demonstrates that uptake and killing of Af spores by AECs can provide a potent means of pathogen control during infection. Our findings further suggest that such mechanisms of fungal control become compromised in patients with COPD, leading to heightened susceptibility to fungal infection.