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

Bertuzzi M.[1,2], Howell G.J[2,3]., Fortune-Grant R. [1,2], Du X.[1,2], Smith J.[1,2], Thomson D.D.[1,2], Gregson L.[1], Greser B.A. [1,2], Motsi N.C. [1,2], Van Rhijn N. [1,2], Demirbag M.[1], Bignell E.M.[1, 2]

1. Manchester Fungal Infection Group, Institute for Inflammation and Repair, University of Manchester, CTF Building, Grafton Street, Manchester M13 9NT

2. Lydia Becker Institute of Immunology and Inflammation, Biology, Medicine and Health. The University of Manchester, Manchester Academic Health Science Centre

3. Manchester Collaborative Centre for Inflammation Research, University of Manchester, Manchester, UK

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 internalise spores.

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.

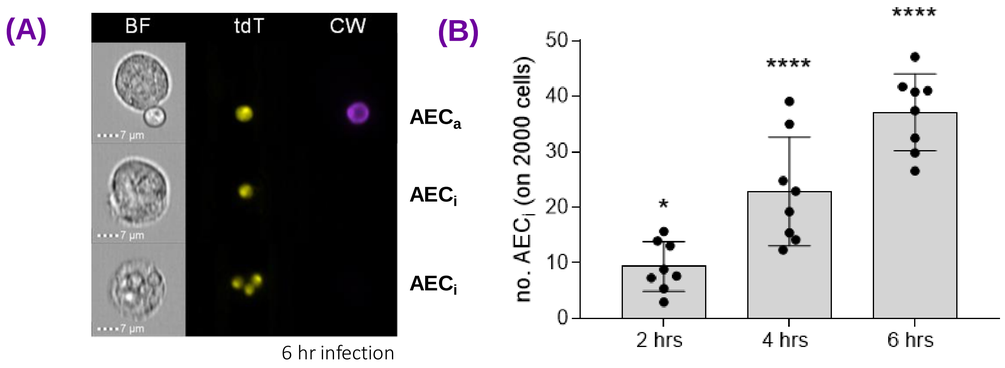


Fig. 1: Differential fluorescence-assisted imaging flow cytometry (IFC) permits quantitation of spore uptake *in vitro*. (A) IFC after infection with 10^5 spores/mL for 6 hr. **Yellow** = intracellular red fluorescent *Af* (tdT), **Purple** = extracellular *Af* stained with Calcofluor White (CW). **(B)** Number of internalising AECs (AEC$_i$) on 2000 total cell number analysed (n. = 8).

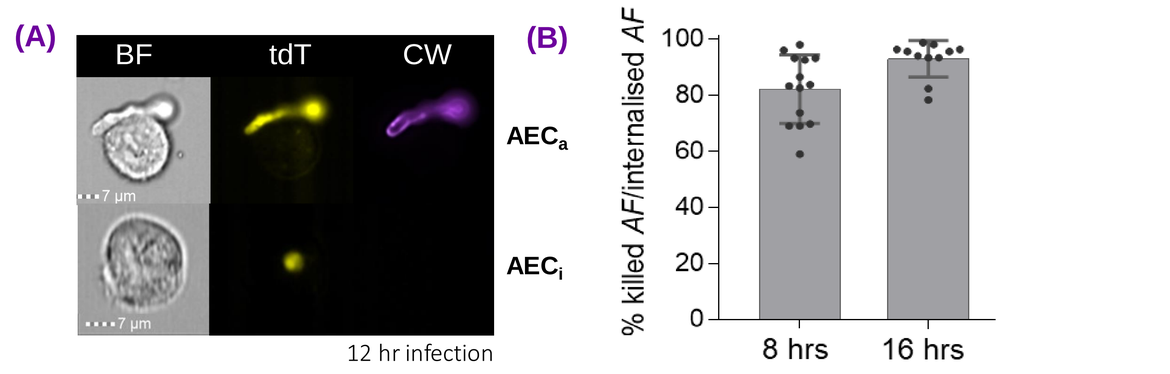


Fig. 2: Fungal spores are killed rapidly following uptake. (A) IFC after infection with 10^5 spores/mL for 12 hr show delay of germination of internalised *Af*. **(B)** Number of internalising AECs on 2000 total cell number analysed (n. = 8).

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)**.

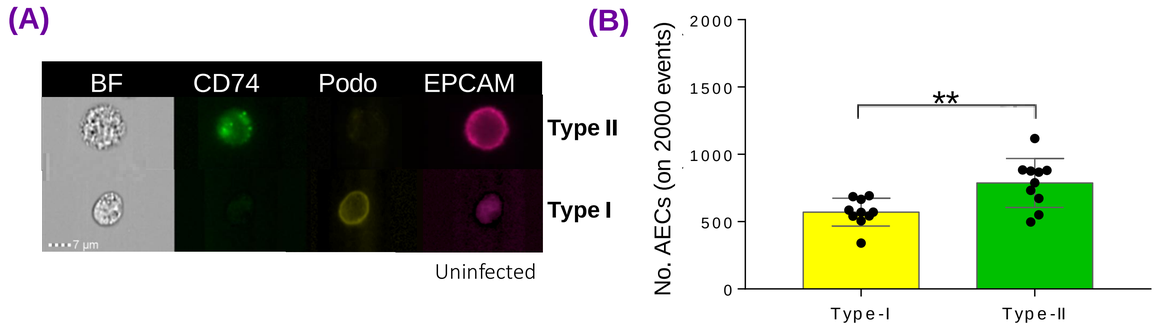


Fig. 3: Extraction and typing of AECs from murine lungs. (A) Murine type-I (Podoplanin$^+$ EPCAM$^+$) and II (CD74$^+$ EPCAM$^+$) AECs harvested directly from leukopenic mice. **(B)** Number of murine type I and II AECs harvested directly from leukopenic mice (on 2000 cells surveyed).

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)**.

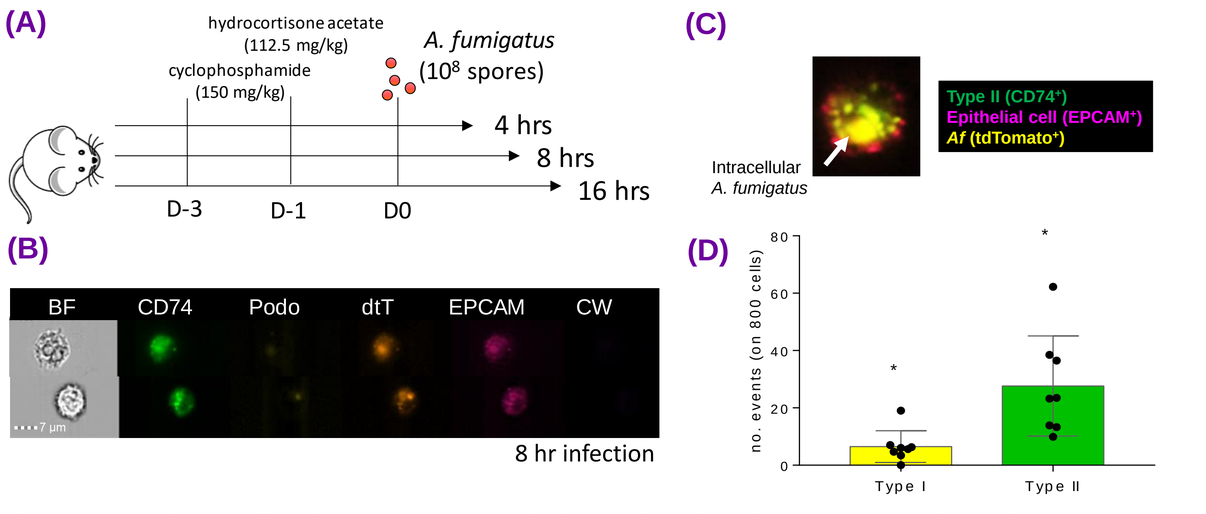
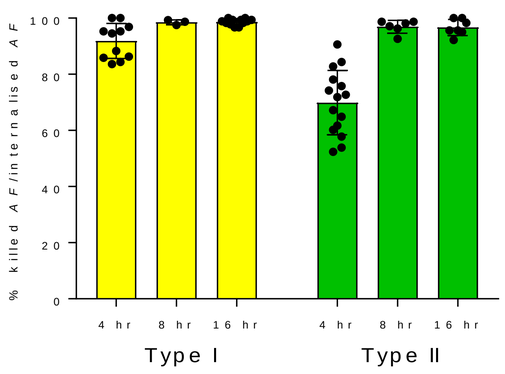


Fig. 4: AECs internalise fungal spores in leukopenic mice. (A) Experimental pipeline to harvest *Af*-interacting AECs harvested directly from leukopenic mice. **(B)** Flow cytometric profiling of type-II (CD74$^+$ EPCAM$^+$) AECs internalising *Af* 8 hrs post-infection. **(C)** Example of type-II (CD74$^+$ EPCAM$^+$) AECs internalising *Af.* **(D)** Number of murine type-I and -II AECs harvested directly from leukopenic mice and internalising *Af* (on 800 cells surveyed). Significance (*) indicated relative to uninfected mice.

In vivo uptake of *A. fumigatus* leads to rapid killing



Rapid killing of the intracellular fungal population was measured, obtaining the **first demonstration that epithelial uptake of *Af* drives efficient fungal clearance during infection.** By 8 hrs, >98% of the internalised fungus was killed by AECs **(Fig. 5)**.

Fig. 5: Fungal killing amongst internalised *Af* by murine type-I and type-II during infection in the whole animal. n. = 3-5 pools of 100 cells, 3 mice per time-point.

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.

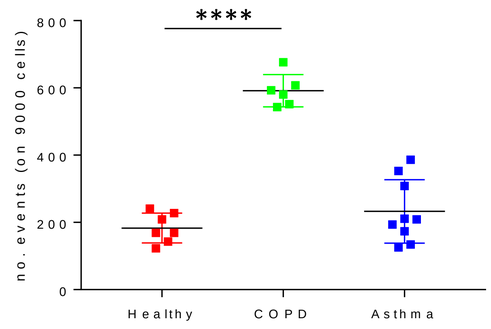


Fig. 6: Phagocytic index in COPD donors is 3X higher than healthy donors. Number of internalising primary human AECs after infection with 10^5 spores/mL for 6 hr, n= 9000 sampled events, 3 biological reps with 3 technical reps, 1 donor for each group. Purity of primary human AECs was assessed by flow cytometry and ~97% cells were AECs Type-I (Podo-PE$^+$) and ~1% AECs Type-II (CD74-FITC$^+$).

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)**.

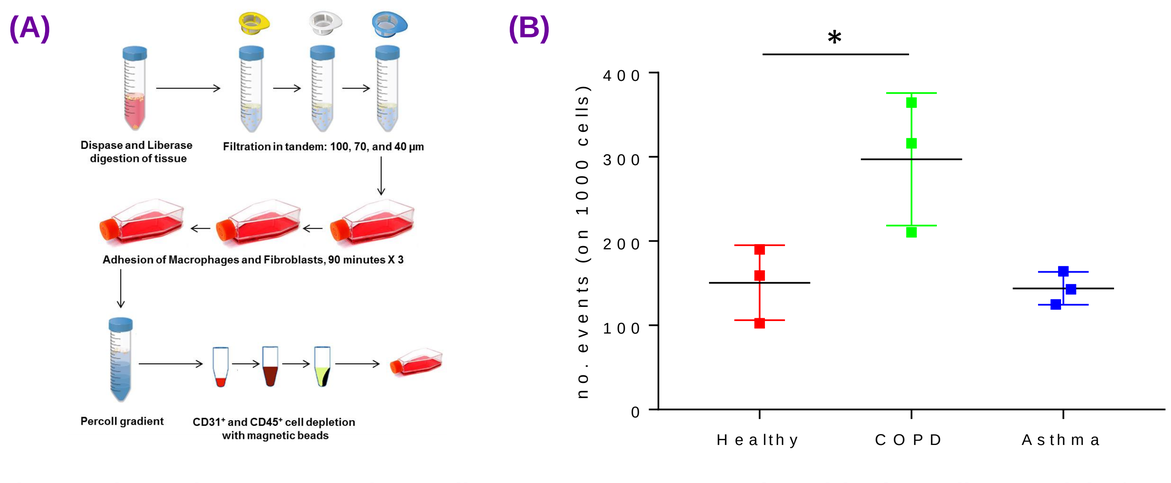


Fig. 7: Primary human AECs internalise spores, a process altered by lung disease. (A) Tissue is sourced via a collaboration with the Collaborative Centre for Inflammation Research (MCCIR, University of Manchester) and the University Hospital South Manchester. ~ 5 grams of resected tissue are processed. 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 10^5 spores/mL for 6 hr, n= 1000 sampled events, 1 biological rep with 3 technical reps, 1 donor for each group.

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.

MFIG
MRC Medical Research Council
MANCHESTER 1824 The University of Manchester
Fungal Infection Trust
BSMM British Society for Medical Mycology
Faculty of Biology, Medicine and Health