# Characterisation of MAIT cells in the antifungal immune response in a microfluidic “invasive aspergillosis-on-chip” disease model

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## Introduction

Invasive pulmonary aspergillosis is a serious threat to immunocompromised patients because treatment options are limited and only successful upon early diagnosis which leads to high mortality rates. Infectious agents are conidia of the mould *Aspergillus fumigatus* that enter the lung alveoli routinely but, in immunocompetent humans, are cleared by innate immune cells such as macrophages and neutrophils. However, in immunocompromised patients, conidia are able to germinate and grow into filamentous bodies (hyphae) leading to tissue destruction and invasion of blood vessels. Recently, an innate-like subset of T cells, mucosal-associated invariant T (MAIT) cells that recognize riboflavin metabolites presented by an antigen-presenting cell (e.g., macrophages) were identified as a further player in the immune response against moulds[1,2]. To elucidate the antifungal potential of MAIT cells, we employed the novel microfluidic “invasive aspergillosis-on-chip” (IAC) model[3,4].

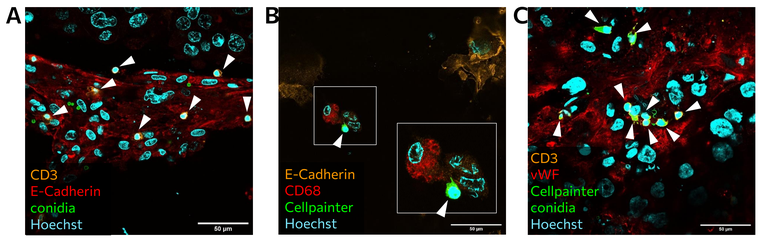


**Fig. 2 Integration of MAIT cells into the IAC model.** A + B) MAIT cells were seeded on the lung side or C) perfused in the blood side. After infection with *A. fumigatus* conidia the IAC model was subjected to fluorescence microscopy. Lung side was stained A) for MAIT cells with CD3 (orange), epithelial cells with E-Cadherin (red) and *A. fumigatus* conidia (green) or B) for epithelial cells with E-Cadherin (orange), macrophages with CD68 (red) and MAIT cells with CellpainterGreen. C) Blood side was stained for endothelial cells with von Willebrand factor (red) and MAIT cells with CD3 (orange) and CellpainterGreen. Nuclei were always stained with Hoechst33258 in cyan blue. MAIT cells are indicated with white arrows. Scale bar = 50µm.

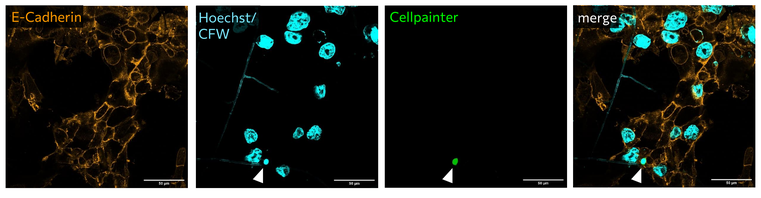


**Fig. 3 MAIT cell migration within the IAC model.** Prelabelled MAIT cells were perfused in the blood side for 5h. After infection the IAC model was stained and analysed with fluorescence microscopy. MAIT cells were also detected on the lung side which was stained for epithelial cells with E-Cadherin (orange), MAIT cells with CellpainterGreen and for nuclei with Hoechst33258 (cyan blue) and hyphae with Calcofluor White (cyan blue). White arrows indicate MAIT cells. Scale bar = 50µm.

## Invasive aspergillosis-on-chip model

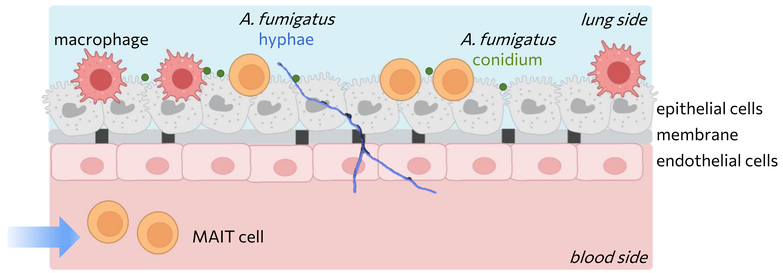


**Fig. 1 Setup of the invasive aspergillosis-on-chip (IAC) model** includes human alveolar epithelial cells and human monocyte-derived macrophages at an air-liquid-interface separated by a porous membrane from medium-perfused human endothelial cells. We included sorted human MAIT cells from peripheral blood in the alveolar epithelium and / or perfused them in the endothelial cell compartment. Following overnight incubation with pre-labelled *A. fumigatus* conidia on the epithelium, growth of hyphae was analysed by confocal laser scanning microscope to obtain z-stack images which were quantified by advanced automated image analysis (IMARIS® software). MAIT cells were analysed by flow cytotmetry.




**Fig. 4 Activation of MAIT cells in the IAC model.** A) Expression of the activation marker CD69 on MAIT cells perfused for 5h on the blood side of IAC model. Supernatants from blood and lung side before infection and after 14h co-incubation with *A. fumigatus* conidia (after infection) were analysed by flow cytometry. Statistical analysis was performed using Mann-Whitney test, *p<0.05, n ≥ 8. B) IAC models without or with MAIT cells on the blood side, the lung side or both sides were incubated with fungal conidia. After infection supernatants of the lung (B) and blood side (C) were analysed for MCP-1 by flow cytometry using a bead-based immunoassay. n ≥ 4.

## Conclusion

Taken together, MAIT cells could be successfully integrated into the *in vivo*-like IAC model and responded to *A. fumigatus* infection with increased activation marker expression and cytokine release but most importantly with inhibition of fungal growth. However, whether this effect is genuinely antifungal and / or supportive of the innate antifungal immune response requires further elucidation. In the long run, isolated and ex vivo expanded and stimulated autologous MAIT cells might be a promising tool of immunotherapy for aspergillosis-prone immuno-suppressed patients.

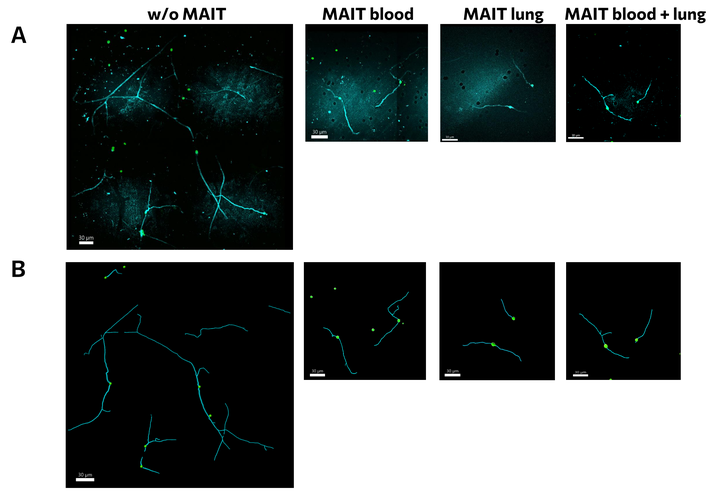


**Fig 5. Inhibition of *A. fumigatus* growth by human MAIT cells.** The IAC model containing no MAIT cells or MAIT cells seeded on the lung or blood or on both sides was infected with FITC labelleled *A. fumigatus* conidia. After infection the lung side was stained for fungal hyphae with Calcofluor White (cyan blue) and imaged by confocal microscopy. A) Representative 3D confocal laser scanning microscopy images of hyphae of the different IAC models. B) Corresponding reconstructed hyphae by IMARIS. C) Quantification of hyphal length and number of branches. Each dot represents an individual hypha from three or four independent experiments (n = 17 – 39). Statistical analysis was performed using Mann-Whitney test, ***p<0.001, *p<0.05.

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**References**

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