Identifying potential host-directed therapies to enhance the innate immune response of macrophages to *Mycobacterium abscessus* infection

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INTRODUCTION

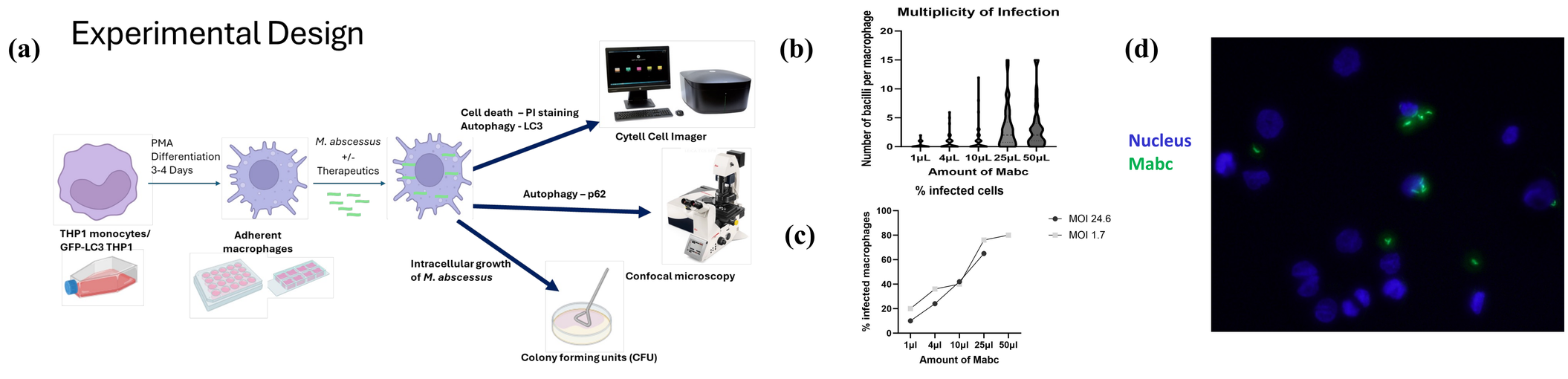
Mycobacterium abscessus (Mabc) is a multidrug-resistant nontuberculous mycobacterium (NTM) that presents a serious health risk to immunocompromised individuals and those with chronic lung diseases such as cystic fibrosis—a condition for which Ireland has the highest incidence rate in the world. Mabc infection poses significant therapeutic challenges due to its intrinsic antimicrobial resistance and ability to evade host immune responses like autophagy. Autophagy is a critical intracellular degradation and recycling pathway involved in pathogen clearance, but Mabc disrupts this process leading to its intracellular survival and persistence within macrophages. Host-directed therapies (HDT) aim to enhance host immune responses by targeting cellular pathways rather than directly targeting the pathogen as has been demonstrated for *M. tuberculosis* infection. HDT's offer a novel approach to overcome and circumvent Mabc's extensive antimicrobial resistance. Restoring autophagy through host-directed therapies is a promising adjunctive strategy to improve pharmacological treatment of Mabc infections.

AIM

To explore autophagy modulation as a strategy for identifying novel host-directed therapies for Mabc-associated disease

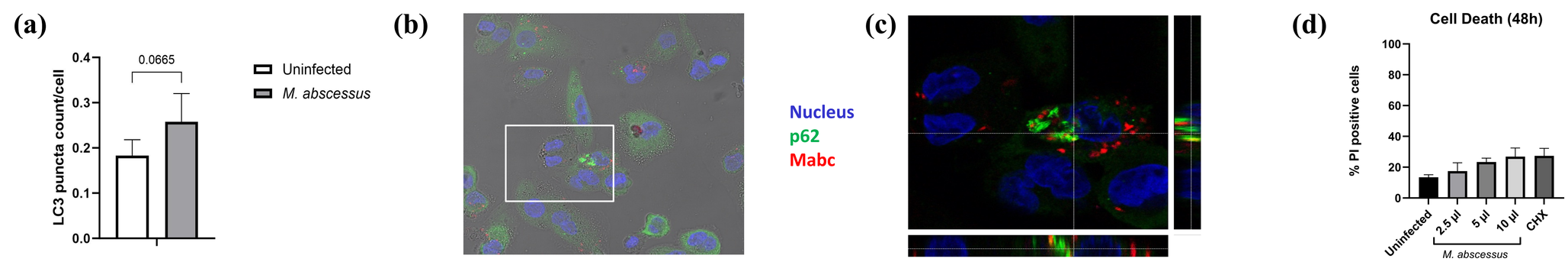
RESULTS

1. In vitro infection model of human macrophages with *Mycobacterium abscessus*



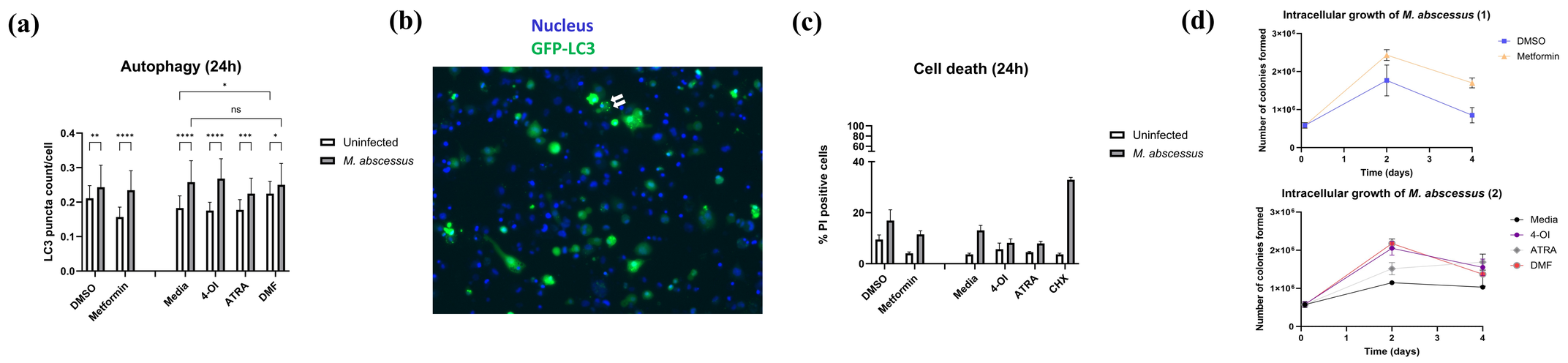
(a) Schematic diagram of experimental setup. Briefly, THP1 macrophages in chamber slides were infected with the indicated volumes of the Mabc reference strain (*M. abscessus* subsp. *abscessus*, ATCC 19977) for 2h. The multiplicity of infection (MOI) and **(b)** percentage of infected macrophages **(c)** was determined by **(d)** acid fast staining with auramine-O (green). Macrophage nuclei were counter stained with Hoechst 33342 (blue) and imaged using an inverted fluorescent microscope (Olympus IX51). In subsequent experiments macrophages were infected at the required MOI for 2h, washed and treated with amikacin to kill extracellular mycobacteria and incubated for the indicated times before fixation, staining and analysis for autophagy markers and cell death or determination of intracellular growth of Mabc by enumeration of CFU on agar plates.

2. *Mycobacterium abscessus* induces low levels of autophagy and cell death in human macrophages



(a) THP1 cells expressing GFP-tagged LC3 were infected with Mabc at an MOI of 1-10 in 48 well plates and autophagy was assessed by determining the number of LC3 puncta per cell 24h post infection (p.i.) using the Cytell Cell Imager. Results are shown as mean +/- standard error of the mean (SEM) for 2 independent experiments. Data were analysed using GraphPad Prism 10 and a paired t-test. (b) Macrophages in glass chamber slides were infected with Mabc (stained with Alexa Fluor 594) at a MOI of 1 - 10. At 2h and 24h p.i. the cells were fixed and indirect immunofluorescence was performed using a mouse antibody to human p62 followed by goat anti mouse antibody conjugated to Alexa Fluor 488. A representative image of infected macrophages 24 h p.i. taken using a Leica SP8 scanning confocal microscope and 63X objective is shown. (c) Orthogonal views of confocal Z-stacks taken of the area indicated in (b) and imaged using 63X objective with 3X optical zoom. (d) Dose dependent increase in cell death at 48h p.i. with Mabc (one experiment with 3 replicate wells per condition).

3. DMF-induced autophagy is inhibited by *Mycobacterium abscessus* infection while Metformin reduces cell death



Macrophages were infected at an MOI of 1 - 10 and treated with metformin (5mM), 4-octyl itaconate (4-OI, 50 µM), all-trans retinoic acid (ATRA, 10 µM) or dimethyl fumarate (DMF, 20 µM) for 24 h. (a) LC3 puncta per cell were enunerated using the Cytell Cell Imager. LC3 results shown are mean and SEM of 2 independent experiments with 2 and 3 replicates respectively. Data were analysed by 2-way repeated measures ANOVA, * p< 0.05. ** p< 0.01, *** p< 0.001, **** p< 0.0001, ns - not significant. (b) Representative image from Cytell Cell Imager showing LC3-GFP expressing (green) macrophages infected with Mabc at MOI 1 - 10. Nuclei are counter stained with Hoechst 33342 (blue). (c) Cell death (one experiment, 3 replicates per condition) was assessed as above. (d) and (e) Treated and control macrophages were infected with a MOI of 1 – 2. Cells were lysed at 2h, 2 days and 4 days p.i. and 10 fold dilutions were spread in triplicate on Middlebrook agar plates. Colonies were enumerated after 4 days incubation at 37°C.

SUMMARY and CONCLUSIONS

- DMF significantly increased autophagy in uninfected macrophages. However, there was no difference in LC3 puncta formation between untreated, Mabc-infected macrophages and those treated with DMF, suggesting that Mabc may inhibit DMF-induced autophagy.
- Treatment with Metformin, 4-OI and ATRA inhibited Mabc-induced cell death.
- None of the treatments tested reduced intracellular growth of Mabc.

These preliminary data indicate that Mabc, unlike *M. tuberculosis*, may be resistant to the effects of these host-directed therapies on its intracellular growth. Nevertheless, an adjunctive therapy that increases autophagy and/or decreases host cell death could have a beneficial effect on therapeutic outcomes by boosting the innate immune response to infection.

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