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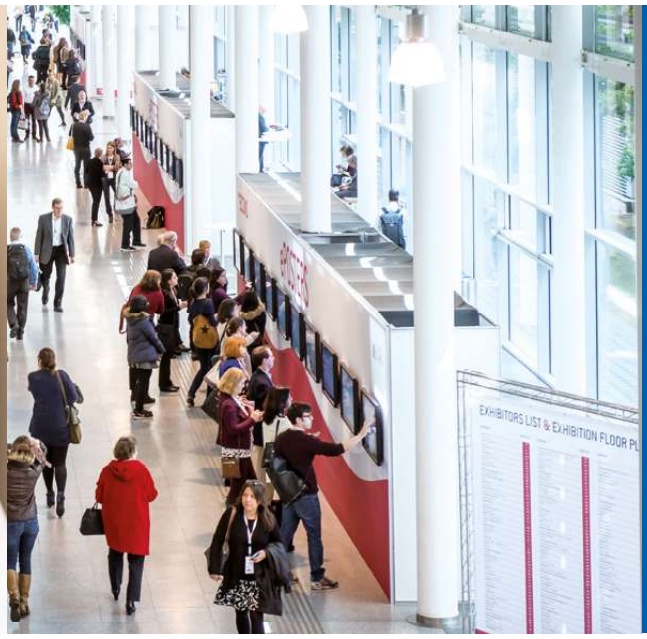

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Abstract 2507

On non-tuberculous mycobacteria in human alveolar macrophages

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Background: Despite growing relevance of nontuberculous mycobacteria (NTM) infections, namely pulmonary infections, accurate data on incidence and pathogenesis is lacking. Here, we evaluated the intracellular persistence of three NTM species in a human alveolar macrophages model in order to get insight on the contribution of innate immune mechanisms to mycobacteria intracellular fate. The NTM genome diversity was also explored.

Materials/methods: *M.smegmatis* mc^{2}155, reference strains (*M.avium* ATCC25291; *M.fortuitum* ATCC6841) and clinical isolates (*M.avium* 60/08; *M. fortuitum* 747/08) were used. Mycobacteria intracellular persistence was assessed via: 4x10^4 THP-1 cells platted/well and incubated for 72h with 100nM PMA. Then fresh medium without PMA was added and the cells incubated for further 24h. The cells were infected for 1h (fast) or 3h (slow growers). Intracellular persistence was evaluated by CFU enumeration at different time points. NO production was evaluated using the Griess reagent, phagosome acidification and apoptosis was followed by confocal microscopy. Persistence ability of mycobacteria at different pHs was evaluated using BACTEC-MGIT960. NTMs virulence factors were characterized by whole-genome sequencing.

Results: NTMs experienced different intracellular fates: *M.smegmatis* and *M.fortuitum* ATCC6841 were cleared within 24h; whereas the remaining strains replicated. Still, unexpectedlyy, high percentages of acidified phagosomes were found harbouring Rab7, but not CD63. All NTM were able to survive *in vitro* at acidic pHs, with the exception of *M.smegmatis*. Our data further suggest a minor role for NO in intracellular persistence and that apoptosis mediated by caspase 8 and 3/7, but not necrosis, is triggered during NTM infection. Insights on the genomic backbone corroborated the virulence potential of *M.avium* and *M.fortuitum*.

Conclusions: Phagosome maturation arrest plays a secondary role in intracellular survival of *M.avium* and *M.fortuitum* in comparison to *M.tuberculosis*. NO seems to play a minor role in NTM intracellular persistence. Contrarily, apoptosis mediated by the caspase 8- caspase 3/7 pathway influences survival probably by promoting mycobacteria spread to neighboring cells. Our insights on the genomic traits underlying virulence further emphasized the importance of carrying out multidisciplinary studies in the NTM field.

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