Establishment of single-cell and animal model to investigate pathogenesis of infection by Mycobacterium abscessus complex members in cystic fibrosis patients

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Background and rationale. M. abscessus (MA) is one of the most frequently isolated non tuberculous mycobacteria (NTM) in patients with cystic fibrosis (CF). Despite the increasing reports on prevalence of MA, including multidrug resistant strains, its pathogenic role is still controversial, due to the limitations of the available cellular and animal models used to study MA infection.

Hypothesis and objectives. Our hypothesis is that strains of MA isolated from patients with deteriorated lung functionality may differ in pathogenicity from the ones isolated from asymptomatic patients. For this reason we established a murine model of chronic lung infection to investigate the pathogenicity of the MA subspecies (sspp) and identify the patients who could benefit from antimicrobial treatment.

Methods. In this study the agar beads method, already established for other pathogens (e.g. P. aeruginosa), was adapted to MA sspp reference strains (MA sspp abscessus ATCC 19977, MA sspp bolletii ATCC 8156 and MA sspp massiliense ATCC 48898) to establish chronic infection in C57Bl/6NCr mice. Mice were monitored for body weight, mortality and lungs were processed for microbiological analysis, inflammatory response and histological evaluation.

Results. Our results demonstrated that MA modified agar beads preparation from the standard protocol was able to establish a chronic infection in mice up to 75 days with MA sspp abscessus, bolleti and massiliense, with a low rate of clearance. The analysis of the bacterial load in the total lung showed an high and stable number of colony forming unit (CFU) (~ $1X10^6$ -$1X10^7$) during the course of infection with minimal systemic spread and stable inflammatory response in broncoalveolar lavage fluid (BALF) up to 75 days of infection. Lung histopthological analysis revealed granulomatomatous response with lymphocytes and macrophage aggregation that are disseminated in the lung during the course of MA infection.

Spin-off for research and clinical purposes. The availability of a murine model for MA chronic infection will allow the identification of strains causing severe disease and open the door to further investigate the impact of MA pathogenesis and treatment.

References

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