TITLE: CHARACTERIZATION OF VIRULENCE OF *Mycobacterium kansasii* STRAINS ISOLATED IN BRASIL FROM PATIENTS WITH PULMONARY DISEASE

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ABSTRACT:
Pulmonary infections due to nontuberculous mycobacteria (NTM), including those caused by *Mycobacterium kansasii* (*Mkan*), demonstrate increasing prevalence in Brazil. Clinical signs of the disease caused by *Mkan* in significant proportion of patients are similar to those of tuberculosis, suggesting increased virulence of these bacteria. The aim of this study was to evaluate virulence of *Mkan* strains (n=10) isolated in Brazil from patients with lung disease in comparison with the virulence of reference *Mkan* ATCC strain 12478. Bacterial fitness was evaluated by monitoring of *Mkan* growth in the 7H9 Middlebrook broth. Ability of mycobacteria to intracellular growth and induction of necrotic death in macrophages was evaluated by infection of murine RAW 264.7 cells. Additionally, we studied mycobacteria-induced macrophage activation through the quantification of cytokines (TNF-α, IL-1β, IFN-γ and IL-10) and nitric oxide in the cell culture supernatants by sandwich ELISA and Griess assay, respectively. The strains, that exhibited the highest levels of growth in macrophages and were able to induce necrotic death in cells infected at a multiplicity of infection, MOI, of 10:1, were considered highly virulent (the strains 8835, 8839 and 10953). Additionally, these strains displayed increased fitness. In contrast, the strains 3657, 7287 and 7439 showed low levels of cell toxicity in macrophage cultures infected either in low dose (MOI 10:1) or high dose of infection (MOI 50:1), suggesting low virulence. Other strains, including the reference *Mkan* strain 12478, exhibited intermediate levels of virulence. Highly virulent strains, in contrast to the less virulent strains, induced in macrophages high levels of TNF-α production, but no IL-1β or IFN-γ. To validate the results of *in vitro* testing, the virulence of selected *Mkan* strains (strains 8835, 4404 and 12478) was additionally evaluated in C57BL/6 mice infected intratracheally with 50.000 bacteria/mice. As expected, virulence of the strain 8835 was significantly higher. This strain induced rapidly progressive disease associated with extensive granulomatous lung inflammation leading to animal death within 40 days after infection, whereas other bacteria caused mild lung inflammation leading to chronic disease. A considerable difference in virulence of *Mkan* clinical isolates was observed. The majority of the isolates exhibited higher level of virulence than that of the reference 12478 strain.

Keywords: *Mycobacterium kansasii*, virulence, bacterial growth, macrophages, C58BL/6 mice

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