TITLE: NECROTIC PULMONARY PATHOLOGY IN TUBERCULOSIS MURINE MODEL: THE ROLE OF NEUTROPHILS, MATRIX METALLOPROTEINASES-8 AND -9 AND MYELOPEROXIDASE

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ABSTRACT:

Tuberculosis (TB) is a chronic infectious disease caused by the bacillus *Mycobacterium tuberculosis* (Mtb), which in severe cases induces irreversible necrosis in the lungs associated with exacerbated inflammation. The TB model in C57BL/6 mice is widely used in TB research. However, these mice present no necrotic pathology. The use of highly virulent Mtb clinical isolates for infection allowed us to induce necrotic pneumonia in these animals. This pathology was associated with the intense recruitment of neutrophils and mononuclear phagocytes accompanied by the necrotic death of the cells at the site of inflammation. In this study, we verified the role of neutrophils and matrix metalloproteinases (MMPs) produced by these cells in the necrotic pulmonary pathology induced in C57BL/6 mouse. For this, the mice were infected with the hypervirulent Mtb strain and treated with anti-Gr-1 monoclonal antibody for neutrophil immunodepletion. Pulmonary histopathology was evaluated on days 21 and 28 post-infection (p.i.). The influx of neutrophils into the lungs was monitored by flow cytometry. Immunohistochemistry assay (IHC) was used to verify the production of MMP-8, MMP-9 and MPO by the lung cells *in situ.* Additionally, the MMP activity was analyzed by zymography of bronchoalveolar lavage fluid or supernatants of the *ex vivo* lung cell culture. On day 28 p.i., the lung pathology was presented by extensive areas of pneumonia, with peripheral alveoli infiltrated by neutrophils and macrophages and central area composed of caseous necrosis with a large number of extracellular bacilli. The IHC analysis revealed labeling for MMP-8, MMP-9 and MPO in the area of necrosis and weak staining of the cells infiltrating alveoli. Treatment of the animals with anti-Gr-1 reduced the number of neutrophils in the lung. Typical granulomatous infiltration, predominantly by macrophages, without necrotic areas, was observed in these animals. Our results demonstrate that neutrophils, which release MMP-8, MMP-9 and MPO as a result of cell death at the site of infection, contribute to necrotic pathology observed in the C57BL/6 mice infected with hypervirulent Mtb strain.

Keywords: Tuberculosis, Neutrophils, Matrix metalloproteinases.

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