TITLE: CARACTERIZATION OF P2X7 EXPRESSION IN $CD4^+$ T CELLS FROM LUNG PARENCHYMA AND VASCULATURE DURING SEVERE EXPERIMENTAL TUBERCULOSIS

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ABSTRACT:

Tuberculosis (TB) continues to be a major cause of morbidity and mortality worldwide. The severe disease is associated with a deleterious inflammatory response, leading to pulmonary necrosis and release of damage signals, such as ATP. Activation of the P2X7 receptor by extracellular ATP can induce cell activation or death. It is known that P2X7 signaling contributes to the development of experimental severe TB. However, it is still unclear whether this receptor is involved in the $CD4^+$ T cell response during infection. In the lungs of mice infected with virulent H37Rv mycobacteria, intravascular $CD4^+$ T cells produce comparatively more IFN-γ, but parenchymal $CD4^+$ T cells show greater protective activity. To evaluate P2X7 expression in lung $CD4^+$ T cells during severe TB, C57BL/6 mice were infected with the hypervirulent MP287/03 *Mycobacterium bovis* (100CFU). The $CD4^+$ T cells located in lung parenchyma and vasculature were distinguished by intravascular (iv) staining with anti-CD45 antibody. At 21 days post-infection, approximately 70% of $CD44^+CD4^+$ cells were present in the parenchyma (CD45iv-), while only 13.2% were in the vasculature ($CD45iv^+$). The parenchymal and intravascular $CD4^+$ T cells were then evaluated for expression of activation markers. The intravascular $CD44^+CD4^+$ population had high expression of KLRG1, a molecule associated with terminal differentiation into effector cells, and low expression of CD69 and PD-1. In contrast, the parenchymal $CD44^+CD4^+$ population had low KLRG1 levels, while a proportion of them showed increased CD69 and PD-1 expression. After the phenotypic characterization of these populations, we evaluated P2X7 expression. We observed low expression of this receptor in intravascular $CD44^+CD4^+$ cells and high expression in parenchymal $CD44^+CD4^+$ cells. Furthermore, among parenchymal $CD44^+CD4^+$ cells, $CD69^+PD\text{-}1^+$ cells expressed higher levels of P2X7 than $CD69^-PD\text{-}1^-$ cells. Further studies are in progress to elucidate the role of P2X7 in the parenchymal $CD4^+$ T cell response during severe TB.

Keywords: Tuberculosis, CD4 T cells, ATP, P2X7

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