

TITLE: EFFECT OF *rv3405c* IN BCG MOREAU INTRACELLULAR LIFESTYLE

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**ABSTRACT:** *Mycobacterium bovis* BCG is the only available vaccine used in preventing tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis* and responsible for 1.8 million deaths in 2015. “BCG” comprises a family of different strains that show genetic differences such as the loss and duplication of genomic regions (RDs), small insertions/deletions and single nucleotide polymorphisms. BCG Moreau, the strain used in Brazil, lost a genomic region (RD16) leading to the truncation of the gene encoding a transcriptional regulator, *rv3405c*. To evaluate the impact of the loss of *rv3405c*, a BCG Moreau recombinant strain complemented with a copy of *rv3405c* from BCG Pasteur was constructed. BCG Moreau harboring the empty vector (M $\Delta$ 05c) and the complemented strain (M::05c) were used to infect THP-1 human monocytes in order to evaluate: the viability of the strains, the pattern of secreted cytokines (TNF- $\alpha$ , MIP-1 $\beta$ , IL-1 $\beta$ , IL-6 and IL-8) and viability of the host cells. THP-1 cells were differentiated into macrophages with PMA and infected at a moi of 10:1 and 1:1 (bacteria:cell). All the analyzed variables were observed in defined time points of 6, 24, 48, 72 and 96 hours after infection. To follow the intracellular viability of BCG, bacilli were recovered after lysis of cells and plated on 7H10 supplemented with ADC and colonies were counted after 30 days. Cytokine secretion was measured on culture supernatants by flow cytometry and macrophage viability evaluated using PrestoBlue®. Our results suggest that the viability of M $\Delta$ 05c is higher than that of M::05c in the intracellular environment. On the other hand, the profile of secreted cytokines seems to be the same between the different strains, both showing a significant difference for IL-8 and MIP-1B after 6h and 24h of infection, respectively, when compared to non-infected cells. No difference in macrophage viability was observed upon infection with the 2 rBCGs or when comparing different moi. These results suggest that the absence of *rv3405c* in itself has no detectable effect on the intracellular lifestyle of BCG, under the conditions tested, and contribute to a better understanding the behavior of the brazilian vaccine strain, BCG Moreau.

Keywords: BCG Moreau, THP-1, Cytokines, Viability

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