Study of the role of CD40L expressed as adjuvant by recombinant BCG in the activation of immune responses against paratuberculosis

Morgan Collin, Virginie Roupie, Mustapha Chamekh, Kris Huygen, Marc Govaerts
CODA-CERVA, Department of Bacterial Diseases, Brussels, Belgium; WIV-Pasteur Institute, Mycobacterial Immunology, Brussels, Belgium; ULB, Fac. de Médecine, Lab. de microbiologie, Brussels, Belgium;

CD40L, a co-stimulatory molecule preferentially expressed on activated CD4+ T cells, is the ligand of CD40 on dendritic and antigen presenting cells. CD40-CD40L interaction induces the production of IL-12 and the initiation of a Th1-type immune response. Several studies show that CD40L is required for the activation of macrophages and the maturation of DCs. Moreover, CD40L enhances the capacity of CD8+ T cells to produce IFN-γ and to lyse Mycobacterium tuberculosis-infected monocytes. In this study we attempt to improve existing Map vaccines with a recombinant BCG expressing CD40L.

We prepared the recombinant BCG strain expressing CD40L (rBCG2) by electroporation of BCG with a pGFM11/Ag85B signal sequence/CD40L extra-cellular domain construct, and another BCG recombinant strain (rBCG1) with the empty pGFM11 vector as a control. The expression of CD40L has been evaluated by Western blot. BALB/c mice were vaccinated with the live recombinant BCG vaccines. BCG persistence in vivo was determined by counting viable bacteria (CFU) in spleen and lungs. The immune response was evaluated by measuring Th1 type cytokine secretion (IFN-γ, IL-2) of splenocytes after in vitro restimulation with selected immunodominant antigens and peptides. Two months post vaccination, mice were challenged with Map and protection was evaluated by Map RLU measurement on spleen and liver.

Preliminary results show normal persistence of the two recombinant BCGs. Analysis of the immune response shows an effect of CD40L 2 weeks after vaccination but not at 4 and 8 weeks. rBCG2 seems to be more protective against paratuberculosis than rBCG1. Another vaccination experiment is in progress to confirm these results. The effects of BCG-CD40L on cultured DCs in vitro will further be explored.