

## **EFFECT OF ENVIRONMENTAL MYCOBACTERIA (*Mycobacterium avium*) ON IMMUNITY INDUCED BY A DNA VACCINE (DNAhsp65) AGAINST TUBERCULOSIS**

**Thesis:** D. R. Martins submitted this dissertation for his Masters in Science at the Department of Tropical Diseases, Botucatu School of Medicine, São Paulo State University, UNESP, Botucatu, São Paulo, Brazil, 2006.

**Advisor:** Professor Alexandrina Sartori

**ABSTRACT:** The efficacy of BCG vaccine (attenuated *Mycobacterium bovis*) against pulmonary tuberculosis varies enormously among different populations. The prevailing hypothesis attributes this variation to interactions between the vaccine and mycobacteria common in the environment. Studies have revealed that most protective antigens expressed by the antituberculous vaccine are conserved in *M. avium*, supporting the hypothesis that exposure to environmental mycobacteria generates a cross-reactive immune response that interferes with BCG efficacy. In this study we investigated the effect of a prior exposure to heat-killed *M. avium* on the immune response and the protective efficacy induced by a genetic vaccine pVAXhsp65 (hsp65 gene from *M. leprae* inserted in pVAX vector) against experimental tuberculosis. To evaluate the effect on the immune response, female BALB/c mice were initially injected with distinct doses ( $0.08 \times 10^6$ ,  $4 \times 10^6$ , and  $200 \times 10^6$ ) of heat-killed *M. avium* by subcutaneous route. Three weeks later, the animals were immunized with 3 doses of DNAhsp65 by intramuscular route ( $100 \mu\text{g}/15$  days apart). Control groups received only *M. avium*, vaccine (pVAXhsp65), vector (pVAX) or saline solution. Cytokine production and antibody levels were determined by ELISA. To evaluate the effect on the protective efficacy, animals were initially sensitized with  $200 \times 10^6$  heat-killed CFU of *M. avium* by subcutaneous route and then immunized with 3 doses of pVAXhsp65 ( $100 \mu\text{g}/15$  days apart) by intramuscular route. Control groups were injected with saline, pVAX (4 doses), pVAXhsp65 (4 doses), *M. avium* or *M. avium* plus pVAX (3 doses). Fifteen days after last DNA dose, the animals were infected with  $1 \times 10^4$  viable CFU of H37Rv *M. tuberculosis* by intratracheal route. Thirty days after challenge, the animals were sacrificed and the bacterial burden was determined by counting the number of CFU in the lungs. Lung histological sections were also analyzed. Splenic cells from primed animals produced more IL-5 but less IFN-gamma than non-primed ones. Also, prior contact with *M. avium* determined higher production of IgG1 and IgG2a anti-hsp65 antibodies in comparison to control groups. However, this higher immune response did not decrease the bacterial burden in the lungs. In addition, prior sensitization with *M. avium* decreased the parenchyma preservation observed in the group immunized only with pVaxhsp65. These results indicate that environmental mycobacteria can interfere with immunity and protective efficacy induced by DNAhsp65.

**KEY WORDS:** hsp65, mycobacterium, *Mycobacterium avium*, tuberculosis, genetic vaccine.

### **CORRESPONDENCE TO:**

DOUGLAS RODRIGUES MARTINS, Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu, 18618-970, Botucatu, SP, Brazil. Phones: +55 (14) 3811 6058 and +55 (14) 3811 6240. Email: [douglasgta@yahoo.com.br](mailto:douglasgta@yahoo.com.br).