

ABSTRACT**Study on the Mechanism of Nematode *Heligmosomoides polygyrus* Sequential Co-infection Influence to *Mycobacterium tuberculosis* Infection****Immunocellular and Histochemistry Study in Balb/c Mice**

Objective: To evaluate whether there is any influence of *Heligmosomoides polygyrus* nematode co-infection on the histopathology of *Mycobacterium tuberculosis* infection, and to explore its immunocellular mechanisms.

Methods: Male Balb/c mice were randomly assigned into 7 different groups of intervention; i.e. single infection with *M. tuberculosis* or *H. polygyrus*, each for 8 or 16 weeks, or co-infection with *M. tuberculosis* and *H. polygyrus* in either sequential order, each for 8 weeks. The activity (the percentage) of Th1, Th2 and Tregs lymphocytes were measured by flow cytometry technique in lung, intestinal tissues, and peripheral blood. The production of Th1, Th2 and Tregs specific cytokines (IFN- γ , IL-4, IL-10, and TGF- β respectively) were measured by ELISA method. Histopathological changes in lung tissues were measured by Dormans score and *M. tuberculosis* colony count after Ziehl Neelsen staining.

Results: *H. polygyrus* and *M. tuberculosis* co-infection caused statistically significant changes in the activity of Th1, Th2, and Tregs lymphocytes in the intestinal, peripheral blood and lung tissues, but those immunocellular changes caused no detectable influence on the histopathologic changes of the lung tissues observed at the end of the (16 weeks) study. There were significant correlations between the levels of IFN- γ , IL-4, IL-10, and TGF- β in peripheral blood with the respective activity of Th1, Th2, and Tregs lymphocytes in peripheral blood and lung tissues. The levels of IL-4 and the activity of Th2 lymphocytes inversely correlated with IFN- γ levels and Th1 lymphocytes activity. The expression of Arginase1 showed significant inverse correlation with iNOS expression by lung macrophages. There were no significant difference in the histopathological changes and *M. tuberculosis* colony count in either single 8 weeks or 16 weeks *M. tuberculosis* infection or either sequential order of *H. polygyrus* and *M. tuberculosis* co-infection observed at the end of the study.

Conclusions: *H. polygyrus* co-infection to *M. tuberculosis* induces the activation of Tregs, which via the secretion of IL-10 (in particular) determine the balance of Th1 and Th2 activity in peripheral blood and lung tissues. The balance of Th1 and Th2 activity in turn determine the balance of iNOS or Arginase1 expressions by lung macrophages, which reflects their polarization. The expression of iNOS by lung macrophages determine the *M. tuberculosis* colony count, which in turn determine the scale of lung histopathologic changes measured by Dormans score. There were no significant influences of *H. polygyrus* nematode co-infection on the histopathology of *M. tuberculosis* infection observed at the end of 16 weeks study. It seems that 16 weeks observation did not allow enough time to detect any changes in the evolution of TB lung histopathology (granuloma formation).

Keywords: *M. tuberculosis*, *H. polygyrus*, co-infection, macrophage, Th1, Th2, T regulator, iNOS, Arginase1, Dormans score