Tampere, Finland

28 May 2018

To the Editors,

On behalf of my colleagues and me, I hereby submit our revised manuscript entitled ‘*Mycobacterium marinum* infection in the adult zebrafish as a model of human tuberculosis’ to JoVE for consideration of publication. We have addressed the reviewers’ concerns and proposals and we feel that this has improved and clarified the manuscript.

One quarter of the world’s population has been estimated to be infected with *Mycobacterium tuberculosis*. According to the World Health Organization, tuberculosis caused1.7 million deaths in 2016 and is the leading cause of death by a single pathogen worldwide. A total of 10.4 million tuberculosis cases were reported along with an increasing number of multidrug resistant strains (WHO, 2017). As the current prevention and treatment regimens have proven insufficient, new treatment strategies are needed.

*M. tuberculosis* infection includes a wide range of disease outcomes with different pathologies in humans. The variation in the infection outcomes caused by this bacterium is hard to model experimentally. *Mycobacterium marinum* causes a very similar disease spectrum in adult zebrafish. By using this natural fish-pathogen, it possible to study tuberculosis in a vertebrate model with both the functional innate and adaptive immune systems, which is not possible in the commonly used zebrafish larva, since they do not have a fully functional adaptive immunity. In this study, we show how adult wild-type and *rag-/-* mutant zebrafish are infected with an intraperitoneal *M. marinum* injection and how the mycobacterial loads are measured with qPCR. In addition, the expression of *il4* and *ifnγ* are analyzed with RT-qPCR 4 weeks post infection. The results of increasing mycobacterial loads and limited induction of *il4* in *rag-/-* mutant fish compared to wild-type fish emphasize the importance of adaptive immunity in the control of mycobacterial infection.

Our work provides a valuable platform for studying tuberculosis. The understanding of the mechanisms leading to different disease outcomes will benefit the scientific community in the search of new vaccines and treatment regimens against tuberculosis. We wish to thank the reviewers for their valuable comments that have helped us improving the study. We hope that the revised manuscript is now suitable for publication.

Thank you for your consideration.

Hanna Luukinen