Novel polyhydroxyalkanoate beads for use as a vaccine against tuberculosis

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Abstract

Tuberculosis was in 1993 declared as a re-emerging disease by the World Health Organization. The only vaccine currently available, BCG, an attenuated strain of *Mycobacterium bovis*, does not protect adults against the pulmonary disease, which is the form of transmission. New vaccine candidates are being developed to provide protection against tuberculosis. Subunit vaccines offer a safer alternative than whole cell preparations and provide the possibility of utilizing only the components that mediate protective immune responses. This thesis describes the production of bacterially derived polyhydroxyalkanoate (PHA) beads for use as a delivery system for *Mycobacterium tuberculosis* reverse vaccinology antigens and immune modulators.

In the first study, the immunogenicity of beads derived from an endotoxin-free host, *Clear coli*, displaying *M. tuberculosis* antigens Rv1626, Rv2032 and Rv1789 was evaluated in mice. Beads displaying Rv1626 were selected for further studies based on the magnitude and specificity of the immune response elicited. In a final study, the immune modulators Cpe30, CS.$T3_{378\text{-}395}$ and Flagellin were co-displayed with Rv1626 antigen on beads and the immunogenicity of these functionalised beads evaluated in mice. Vaccinations with Rv1626 beads and the immune modulators Cpe30 and CS. $T3_{378\text{-}395}$ induced a Th1/Th17 skewed immune response. These beads were then assessed for their ability to protect mice against aerosol challenge with *Mycobacterium bovis*. Rv1626 beads reduced the bacterial loads in 0.48 $\log_{10}$ compared with the negative control group but the inclusion of immune modulators did not enhance the immunogenicity or protection induced by Rv1626 beads.

This study has demonstrated the potential of PHA beads delivering a single reverse vaccinology antigen for protection against tuberculosis infection in mice. While the co-display of immune modulators did not improve the protection induced by the antigen, further studies are needed to determine optimal doses for delivery of immune modulators to enhance protective immunity.

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Preface

This thesis is written according to the Graduate Research School regulations for PhD thesis by publications. The list below presents the publication status of each chapter.

Chapter 1A

Basic concepts in immunology, vaccines and tuberculosis.

This chapter was written by Patricia Rubio Reyes as an introductory section of this thesis and is not intended for publication

Chapter 1B

Self-Assembled Protein-Coated Polyhydroxyalkanoate Beads: Properties and Biomedical Applications. Natalie A. Parlane, Sandeep K. Gupta, Patricia Rubio Reyes, Shuxiong Chen, Majela Gonzalez-Miro, D. Neil Wedlock and Bernd H. A. Rehm. Published in ACS Biomaterials Science & Engineering. Special Issue: PHA Biomaterials (2016).

This review was written by all the authors. Patricia Rubio Reyes made a contribution on the section describing biomedical applications of polyhydroxyalkanoate beads.

Chapter 2

Immunogenicity of antigens from *Mycobacterium tuberculosis* self-assembled as particulate vaccines. Patricia Rubio Reyes, Natalie A. Parlane, D. Neil Wedlock and Bernd H.A. Rehm. Published in International Journal of Medical Microbiology, 306 (2016) 624–632.

All experiments were carried out by Patricia Rubio Reyes except mice vaccinations and processing of mice samples that were co-carried out with Natalie A. Parlane.

Chapter 3

Immunological properties and protective efficacy of a single particulate mycobacterial antigen displayed on polyhydroxybutyrate beads. Patricia Rubio Reyes, Natalie A. Parlane, Bryce M. Buddle, D. Neil Wedlock, Bernd H.A. Rehm. Published in Microbial Biotechnology (2017).

All experiments were carried out by Patricia Rubio Reyes. Natalie A. Parlane helped with mice vaccinations and processing of mice samples and Bryce Buddle assisted with challenge experiment and lungs histology.

Chapter 4

Conclusions

This chapter was written by Patricia Rubio Reyes as conclusions of this thesis and it is not intended for publication

Appendix 4

***In vivo* polyester immobilized sortase for tagless protein purification.** Iain D. Hay, Jinping Du, Patricia Rubio Reyes and Bernd H. A. Rehm. Published in Microbial Cell Factories (2015) 14:190.

Patricia Rubio Reyes made a contribution on the preparation of the plasmid pET14:PhaC-SrtA-Rv1626, purification of Rv1626 antigen and in the demonstration of the functionality of PhaC-SrtA-MBP beads and only those parts are submitted for examination. The entire publication is included for a better understanding of the methods used.

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