Identification of an immunogenic 18 kDa protein of *Helicobacter pylori* using alkaline phosphatase gene fusions

Jane Oliaro

A thesis presented in partial fulfillment of the requirement for the Doctor of Philosophy in Molecular Microbiology

Massey University, Palmerston North, NZ

December, 1999

Abstract

Secreted or surface-associated proteins play an important role in the immunopathogenesis of *Helicobacter pylori* infection. The aim of this study was to identify, using a genetic approach, *H. pylori* exported proteins and assess their role in the host immune response to infection. As part of this work, an *H. pylori* expression library was constructed and screened with a monoclonal antibody raised to a component of outer membrane vesicles from *H. pylori*, identified and characterised in a separate study. The screening strategy identified a locus of the genome containing two genes encoding exported proteins. Subsequent expression studies identified the gene product detected by the antibody as Lpp20, which encodes a well characterised lipoprotein from *H. pylori*. In addition, the use of alkaline phosphatase (AP) gene fusion methodology enabled the identification of a large number of other *H. pylori* exported proteins. Immunoscreening of a selection of enzymatically active *H. pylori* AP fusion proteins was carried out by Western blot analysis with patient sera and lymphocyte proliferation assays using peripheral blood mononuclear cells from *H. pylori* infected individuals. These assays identified a novel *H. pylori* exported antigen which was recognised by antibody derived from *H. pylori* infected individuals. Southern blot analysis revealed that the gene encoding the protein was absent in other *Helicobacter* species tested and sequence analysis of the gene demonstrated that it is highly conserved among *H. pylori* isolates. In order to obtain pure recombinant protein, the gene encoding the protein was cloned and expressed as a Beta-galactosidase (β-gal) fusion in *Escherichia coli* and the protein purified by affinity chromatography. The size of the recombinant protein released (18 kDa) was consistent with the calculated molecular mass of the polypeptide deduced from the DNA sequence. In Western blot assays, the purified protein was recognised by 71% of sera taken from patients infected with *H. pylori*, but by only 16% of sera taken from patients with unrelated or with no gastrointestinal disease. These results indicated that the 18 kDa protein from *H. pylori* was immunogenic and expressed *in vivo*. In other experiments, it was found that oral administration of this antigen did not protect mice against *H. pylori* colonisation following challenge with *H. pylori*.

Acknowledgements

I wish to thank Professor Bill Manktelow, former Head of the Department of Veterinary Pathology, for allowing me the opportunity to undertake a postgraduate degree in this Institute. I am also grateful to my chief supervisor, Dr Alan Murray, not only for his support and advice during this PhD project, but also for having the faith to take me on as a new graduate and allowing me the opportunity to grow and learn in the world of academia. I am also like to thank my second supervisor, Dr Paul O'Toole, whose expertise in the field has been invaluable. A special mention goes to Dr. Richard Johnson, my supervisor at the start of the project, for his contribution to the work and friendship over the years.

I would like to acknowledge the outstanding contribution of our collaborators at the Wakefield Gastroenterology Research Unit, Wellington. I would like to thank Dr Vinton Chadwick, for his guidance and encouragement throughout the project, and whose knowledge and expertise always kept me on my toes. With sincere gratitude I acknowledge Dr Wangxue Chen and Dairu Shu for performing the lymphocytes proliferation assays detailed in Chapter five, and the mouse immunisation trial in Chapter six. A special thanks to Chen, not only for his excellent scientific advice, but also for his friendship and willingness to assist.

I would also like to acknowledge Brigitte Gicquel, of the Unite de Genetique Mycobacterienne., Pasteur Institute, Paris, France, for graciously providing the pJEM11 plasmid, without which this project would not have existed. I am also grateful to the The Lottery Health Board, The Palmerston North Medical Research Foundation, The Rose Catherine Hopkins Memorial Scholarship and The Health Research Council of New Zealand for funding this research.

A big thank you to my dear friends Dr Eamonn Gormley and Dr Suzanne Borich who are now many miles away, but never far from my thoughts. I couldn't have done it without you Eamonn, thank you for keeping me smiling and for all the good times we have shared over the years. Thanks also to Stan Fenwick, for providing me with numerous opportunities to unwind at his many memorable parties. I would like to thank to each of my lab coworkers, both past and present; Chris, Hassan, Jeremy, Laurie,

Linley, Liz, Sandy, and Miho, for their companionship and always helpful advice. Thank you also to the Virology group; Magda, Darelle, Joanne and Matthew for their friendly advice and help over the years. A special thank you to Dr John Lumsden, for his patient assistance in helping me unravel the mysteries of lymphocyte proliferation assays. I am also grateful to Andrew Rowatt, for his immediate attention to all my computer crises. So many people in the Institute deserve a special mention, too many to list individually, so I collectively thank you all for the friendships and memories I have gathered over the years.

Finally, a very special thank you to my family. To Carl, for his infinite patience and support, and to my little princess, Tamara, who has brought more joy to my life than I ever thought imaginable. It has been a long but truly unforgettable journey.

This thesis is dedicated to my parents.

Table of Contents

List of Figures

List of Tables