CELL-MEDIATED IMMUNE RESPONSES TO THE 18 KILODALTON PROTEIN OF *MYCOBACTERIUM LEPRAE*

Björn Thomas Bäckström, BSc(Hons)

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Department of Molecular Medicine
University of Auckland
New Zealand
September 1991

LOGICAL THINKING CANNOT YIELD US
ANY KNOWLEDGE OF THE EMPIRICAL WORLD:
ALL KNOWLEDGE OF REALITY STARTS
FROM EXPERIENCE AND ENDS IN IT.
PROPOSITIONS ARRIVED BY PURELY LOGICAL
MEANS ARE COMPLETELY EMPTY OF REALITY.

ALBERT EINSTEIN

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I thank my supervisor Professor Jim Watson for encouraging me to come all the way from Sweden to New Zealand and providing me with the opportunity to undertake post-graduate studies in the Department of Molecular Medicine. I also thank Jim for his excellent guidance and his concern for my welfare. I thank my assistant supervisor Professor John Marbrook for all the beneficial scientific discussions we have had and teaching me how to think and understand science. I also would like to express a very special appreciation to John for his invaluable assistance in the preparation of this thesis and personal generosity.

I am grateful to all the members of the department for their helpful assistance and friendly manner. In particular, I thank Dr Ross Prestidge for his "never-ending-always-smiling-helping" attitude and contribution toward my research. Dr Roger Booth has been very helpful throughout my three and a half years studies, by providing useful advice and constructive criticism of my research, especially during the preparation of this thesis. I am grateful to Prudence Grandison and Liz Doolin for their technical skills, producing recombinant 18 kDa protein and managing with my always increasing demand for more. I have appreciated and benefitted from discussions with Drs Phil Crosier, John Fraser, Paul Tan and Margot Skinner. I express my gratitude to Drs Kathy and Phil Crosier for critical reading of this thesis. I also thank Louise Moffatt for linguistic help in preparation of the thesis and for putting up with me in her office during that time. I owe Louise and Tui Harfield a great deal for keeping the mice in a very good condition and breeding all possible combinations of "high and low responder mice". I thank my golf-partner Euphemia Leung for teaching me the methods of molecular biology her way: "there are no short cuts". I thank Dr Hanxiu Liu for looking after my "pets" (T cell lines) during the last four of months of this study. I would also like to thank Kuljeet Singh for supporting and helping during the home stretch of preparing this thesis.

I am appreciative to The Leprosy Mission, particularly the Director Mr Gordon Coombs, for the financial support and continued interest of my research and welfare.

Finally, I am very grateful for the love and support from all my friends, here (New Zealand) and there (the rest of the World, mainly Sweden), and from my family, especially my mother Birgitta, always caring and supporting me "whatever I do, wherever I do it".

ABSTRACT

The cell-mediated immune system plays an important role in protective immunity against *Mycobacterium leprae*. The aim of work presented in this thesis was to study various T cell responses in mice to one protein of *M. leprae*, designated the 18 kDa protein.

Mice were immunised with recombinant 18 kDa protein and lymph node cells tested later in proliferative T cell assays. The *in vitro* response was specific for the 18 kDa protein and proliferation dependent on the immunising dose as well as *in vitro* antigen concentration. The proliferative response required CD4+ T cells, which recognised the antigen in the context of major histocompatibility class II molecules. The T cell response was tested in various recombinant and congenic pairs of mouse strains. BALB/cJ (H-2^d), BALB.B (H-2^b), B10.BR (H-2^k), and B10.M (H-2^f) mice were all high responder strains, while the C57BL/10J (H-2^b) mouse strain was a low responder. By comparing the genetic characteristics of these high and low responding strains, it was deduced that both H-2 and non-H-2 gene(s) contributed to the magnitude of responsiveness. F_1 progeny mice from high and low responder parents responded in a way that showed that high responsiveness was inherited in a dominant manner. Limiting dilution assays indicated that the frequency of cells proliferating in response to the 18 kDa protein correlated with high and low responsiveness of cells from secondary lymphoid tissue. The low frequency of C57BL10/J responding lymphocytes to the 18 kDa protein probably accounted for the reduced *in vitro* IL-2 production observed in this mouse strain.

The T cell response of mice immunised with either the 18 kDa or *M. leprae* antigens revealed the following. The 18 kDa protein-primed lymph node cells responded to both *M. leprae* and *M. tuberculosis*, suggesting a shared 18 kDa protein T cell epitope by these pathogens. The *M. leprae*-primed lymph node cells showed no significant response to the 18 kDa protein, but responded similarly to *M. leprae* and *M. tuberculosis* antigens. The results were obtained using both bulk cultures and limiting dilution experiments. This indicated that the 18 kDa protein was probably not a dominant antigen in primary murine immune responses against *M. leprae*.

Proliferative T cell epitopes on the 18 kDa protein were examined in several mouse strains. Each mouse strain responded to one dominant region after immunising with 18 kDa protein. The dominant T cell epitope was not identical in all strains. The peptide consisting of residues 106-125 of the 18 kDa protein was dominant in BALB/cJ and BALB.B mice, whereas the B10.BR mice responded to peptide 31-50 and C57BL/10J mice to peptide 16-35. Lymph node cells primed with individual synthetic peptides and challenged with 18 kDa protein *in vitro* revealed additional T cell epitopes which were not recognised following immunisation with 18 kDa protein.

Cytotoxic T cell epitopes on the 18 kDa molecule were investigated, using an *in vitro*-priming system. Only one region of the 18 kDa protein elicited cytotoxic T cell responses. This T cell epitope was restricted to mouse strains of H-2^b haplotype. The cytotoxic response was a feature of $CD8^+$ T cells and the lysis of target cells depended upon peptide concentration. Cells infected with a recombinant retrovirus carrying the 18 kDa gene were used to prime mice for the induction of cytotoxic T cells *in vivo*. Cytotoxic T cell responses were detected but none were specific for the 18 kDa protein.

Inflammatory T cell responses to the 18 kDa protein were investigated in a number of mouse strains, using a delayed-type hypersensitivity footpad assay. The C57BL/10J mouse strain elicited a similar or even greater response than other strains tested, in contrast to proliferative T cell responses where C57BL/10J was a low responder strain. The number of immunisations influenced the type of immune response elicited in mice. Multiple immunisations with the 18 kDa protein resulted in a reduced delayed-type hypersensitivity response concomitant with the appearance of antigen-specific IgG antibodies.

Several 18 kDa-specific T cell lines derived from BALB/cJ, B10.BR, and C57BL/10J mouse strains were generated. T cell lines from the BALB/cJ strain showed characteristics of T_H1 and T_H0 phenotypes and one line responded to peptide 46-65 of the 18 kDa protein. The B10.BR- and C57BL/10J-derived T cell lines responded to peptide 31-50 and 106-125 respectively and secreted IL-3 upon stimulation, but neither IL-2 nor IL-4 were detected.

Various subsets of T cells might play different roles in the immune response against pathogens. The T cell subsets involved in protection might not be identical to those eliciting a strong immunological reaction. T cell responses against intracellular parasites are discussed in relation to factors that govern protective immunity.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

a.a.	Amino acid(s)
APC	Antigen-presenting cell(s)
BCG	Bacille Calmette-Guérin
BM-MØ	Bone marrow-derived macrophage(s)
bp	base pair(s)
CFA	Complete Freund's adjuvant
Con A	Concanavalin A
Counts/min	Counts per minute
CTL	Cytotoxic T lymphocyte(s)
CWP	Cell wall protein(s)
DEAE	Diethylaminoethyl
DNA	Deoxyribonucleic acid
DTH	Delayed-type hypersensitivity
EDTA	Ethylenediamide tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FACS	Fluorescence-activated cell sorter
FITC	Fluorescein isothiocyanate
g	Acceleration of gravity
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HEL	Hen egg lysozyme
HLA	Human leukocyte antigen(s)
HPLC	High-performance liquid chromatography
HSP	Heat-shock protein(s)
IFA	Incomplete Freund's adjuvant
IFN	Interferon (e.g., IFN-γ)
Ig	Immunoglobulin(s)
Ii	Invariant chain
IL-	Interleukin (e.g., IL-2)
kDa	Kilodalton
KLH	Keyhole limpet haemocyanin
LDA	Limiting dilution analysis
LNC	Lymph node cell(s)

LPS	Lipopolysaccharide
LT	Lymphotoxin
mAb	Monoclonal antibod(y)(ies)
mIg	Membrane immunoglobulin(s)
MHC	Major histocompatibility complex(es)
mRNA	Messenger RNA
OVA	Ovalbumin
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
sB-cell	Purified B cell population(s)
SDS	Sodium dodecyl sulphate
SE	Standard error(s)
SI	Stimulation ind(ex)(ices)
TCL	T cell line(s)
TCR	T cell receptor(s)
[^{3}H]-TdR	Tritiated thymidine
T_H	T helper cell(s) (e.g. T_H1)
TNF	Tumour necrosis factor
Tris	2-amino-2 (hydroxymethyl) propane-1-3-diol