CHARACTERISING THE RELATIONSHIP BETWEEN FOWLPOX VIRUS AND THE MAMMALIAN IMMUNE SYSTEM

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A thesis submitted for the degree of
Masters in Medical Science
School of Medicine
at The University of Adelaide
October 2008

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ABSTRACT

Fowlpox viruses (FPV) are attractive platform vaccine vector candidates because their capacity for insertion of multiple heterologous genes makes them favourable for genetic modification. They also have strong adjuvant activity in their own right. As FPV does not replicate in mammalian cells, there is significantly less opposition associated with their clinical application, with a number already in use. However, a thorough understanding of the immunological relationship between FPV and the mammalian immune system is still lacking.

The aim of this thesis was to construct a series of recombinant FPV vectors that co-expressed the nominal antigen chicken ovalbumin (OVA), (FPV_{OVA}), and/or murine interleukin-4 (mIL-4). These constructs were used for the characterisation of the relationship between FPV and the mammalian immune system and how this is altered by the co-expression of mIL-4. Immunisation with FPV_{OVA} resulted in rapid and highly localized OVA expression which induced strong $CD8^+$ cytotoxic T cell (CTL) activity but only weak $CD4^+$ T helper and antibody responses. In addition, presentation of FPV-derived antigen and the priming of antigen-specific CTL responses required a permissive bone marrow (BM)-derived cell as the antigen presenting cell (APC). Co-administration with $FPV_{mIL\text{-}4}$ resulted in a dramatic reduction in CTL activity that remained largely non-functional throughout the infection and a skewing of the T helper (Th) response towards Th2 with a reduction in interferon (IFN)-γ production by OVA-specific Th cells. These findings provide a sound basis for further characterization of how FPV interacts with the innate and adaptive arms of the immune system, how these can be manipulated via the co-administration of cytokines, and discovering if future rationally designed modifications result in FPV vectored vaccines that induce durable cellular and humoral immunity.

THESIS DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Emma Beukema

NOTE:
The comic strip is included on page 12 of the print copy of the thesis held in the University of Adelaide Library.

This Masters thesis is dedicated to my husband, John.

ACKNOWLEDGEMENTS

First of all I would like to thank my supervisors Dr John Hayball, Dr Michael Brown and Dr Paul Howley. Thank you to Dr John Hayball for all the advice, encouragement and opportunities you provided me with and Dr Michael Brown for giving me the opportunity to work in his laboratory. Special thanks to Dr Paul Howley for the advice, support and collaboration throughout my Masters.

Thank you to the members of the Experimental Therapeutics Laboratory at the Hanson Institute for their friendships and support throughout the time I spent there. In particular, thank you to Kerrilyn Diener, Cara Fraser and Erin Lousberg for the friendship, conversations and laughter that we shared and best wishes for your PhDs and your futures. Thank you to Jocelyn Darby for the friendship and technical assistance. Special thanks to Anastasia Yu for the friendship, help and collaboration and best wishes with your PhD. I would also like to acknowledge Sonia Tingay from Virax Holdings Ltd, who helped construct the recombinant fowlpox viruses and Lachlan Moldenhauer from the Research Centre for Reproductive Health for making the bone marrow chimaeric mice.

Finally, I would like to thank my friends and family for their unending love, support and encouragement, in particular, my parents Paul and Anne, and my husband John. Thank you for having faith in me and being confident of my success even when I wasn't. Words can not express the amount of gratitude and love I have for all of you. Mum and Dad, thank you for listening and taking such an interest in my research as well as all the comforting hugs and cups of tea. A big part of my success can be attributed to the wonderful job you have done as my parents, and the examples that you have set me throughout my life. To John, thank you for riding the rollercoaster that was my Masters with me. You have been my tower of strength, picking me up after the falls and giving me reality checks and reminders of what is important when I needed them. Thank you for the patience and understanding that you have shown me and for your unconditional love. I couldn't have done this without you and I feel that you have earned this Masters just as much as I have.

ABBREVIATIONS

ALB	Alkaline lysis buffer
ALVAC	Canarypox virus
ANK	Ankyrin repeat
APC	Antigen presenting cell
AT	Adoptive transfer
BHK	Baby hamster kidney
BM	Bone marrow
BP	Binding protein
bp	Base pair
BSA	Bovine serum albumin
CC	Chemokine
CEA	Carcinoembryonic antigen
CEF	Chicken embryonic fibroblast
CFSE	Carboxyfluoroscein succinimidyl ester
CLR	C-type lectin receptor
CMI	Cell-mediated immunity
CP	Circumsporozoite protein
CPV	Cowpox virus
crmA	Cytokine response modifier A
CRV	Crocodilepox virus
CT	Connective tissue
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
DNA	Deoxyribonucleic acid
Ds	Double stranded
EEV	Extracellular enveloped virus
EGFP	Enhanced green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay
EV	Ectromelia virus
FACS	Fluorescence-activated cell sorting
FCS	Foetal calf serum
FDA	Food and Drug Administration
FL	Fluorescence
FPV	Fowlpox virus
GM	Growth medium
GM-CSF	Granulocyte macrophage colony-stimulating factor
HAT	Hypoxanthine-aminopterin-thymidine
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
HSV	Herpes simplex virus
ICE	Interleukin-1β-converting enzyme
IFN	Interferon
IL	Interleukin

IMV	Intracellular mature virus
IND	Investigational New Drug Application
IRES	Internal ribosome entry site
LCMV	Lymphocytic choriomeningitis virus
LN	Lymph node
LTR	Long terminal repeat
LU	Lytic unit
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
MHC	Major histocompatibility complex
MM	Maintenance medium
MPA	Mycophenolic acid
MOI	Multiplicity of infection
MVA	Modified vaccinia Ankara
NF- κB	Nuclear factor κB
NHP	Non-human primate
NK	Natural killer
NLR	NOD-like receptor
NR	Neutral red
NTA	Nitrilotriacetic acid agarose
O/N	Overnight
ORF	Open reading frame
OVA	Ovalbumin
PAGE	Polyacrylamide gel electrophoresis
PAMP	Pathogen-associated molecular pattern
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pDC	Plasmacytoid dendritic cell
PFU	Plaque forming unit
PI	Proliferation index
p.i.	Post-infection
PRR	Pattern recognition receptor
PSA	Prostate-specific antigen
PVR	Polio virus receptor
RB	Roller bottle
RBC	Red blood cell
rFPV	Recombinant FPV
REV	Reticuloendotheliosis virus
RIG	Retinoic acid-inducible gene
RLR	RIG-like receptor
RT	Room temperature
SDS	Sodium dodecyl sulfate
Serpin	Serine protease inhibitor
TAA	Tumor-associated antigen
TAE	Tris-acetate-EDTA
TAP	Transporter associated with antigen presentation

TCR	T cell receptor
Th	T helper cell
T_{reg}	Regulatory T cell
TK	Thymidine kinase
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TRICOM	Triad of co-stimulatory molecules
UV	Ultra violet
VSS	Virus seed stock
VSV	Vesicular stomatitis virus
VV	Vaccinia virus