

**CHARACTERISING THE RELATIONSHIP BETWEEN FOWLPOX
VIRUS AND THE MAMMALIAN IMMUNE SYSTEM**

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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-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.

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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.

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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	Carboxyfluorescein 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-1 β -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 κ B
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