Differential profiles of mammalian and mosquito derived Ross River virus

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Abstract

Ross River virus (RRV) is a mosquito borne virus that results in polyarthritic disease with symptoms including rash, lethargy and myalgia. Currently, RRV is responsible for around 5000 infections each year in Australia and disease symptoms can persist for several months or years in RRV infected patients. Early interaction of the virus with the host immune system has been identified as critical to the resulting disease. In addition, it has been shown that there are differences between mammalian and mosquito derived virus in the N-linked glycans on envelope proteins, the ability of the virus to infect cells *in vitro* and the resulting cytokine expression. However, the mechanism behind these differences and their effect *in vivo* is yet to be elucidated. The work reported in this thesis aimed to test the hypothesis that mammalian and mosquito derived RRV differ in replication fitness *in vitro* and *in vivo*, and are associated with differences in host response and the subsequent clinical disease.

RRV-T48, the common laboratory strain of RRV, showed differences in replication *in vitro*. Mammalian derived RRV T48 (Mam-RRV-T48) showed a replication advantage in mammalian (Vero) cells and a disadvantage in mosquito (C6/36) cells. In contrast, mosquito derived RRV T48 (Mos-RRV-T48) demonstrated a replication advantage in C6/36 cells and a disadvantage in Vero cells. This is indicative of RRVs ability to adapt to different host cells and increase the infectivity in the host within one passage. Furthermore, Mos-RRV-T48 showed a replication advantage in Raw 264.7 cells. Previous studies have also showed that mosquito derived virus had increased binding affinity

with cell surface receptors such as DC-SIGN, resulting in an increased infectivity. This is likely due to high mannose N-linked glycans present on mosquito derived virus only. Glycosylation differences have been found to result in strong IFN induction for mammalian but not mosquito derived virus in DCs. We confirmed this occurs in RRV-T48 infection of Raw 264.7 and Jaws II cells.

The RRV-T48 studies were expanded further utilising RRV field isolates extracted from mosquitos in Western Australia. The aim of this study was to examine if the differential effect observed with RRV-T48 could also be observed using wild type circulating RRV isolates *in vitro*. The study confirmed that RRV isolates mimicked the same pattern of infection as seen in RRV-T48 study. Mam-RRV-isolates produced the highest titres in Vero cells while Mos-RRV isolates produced the highest titres in C6/36 cells. Furthermore, approximately half of all Mam-RRV isolates replicated to higher titres than their Mos-RRV counterparts in Raw 264.7 cells; indicating that binding affinity is not the only factor involved in RRV replication.

Subsequent *in vivo* studies showed a significant difference between Mam-RRV-T48 and Mos-RRV-T48. The i*n vivo* pilot study also revealed that disease progression was influenced by the gender of mice. While female mice showed almost no difference in disease when inoculated with Mam-RRV-T48 or Mos-RRV-T48, disease measures in male mice were significantly more severe. This has led to the development of a male only mouse model of RRV disease which provides more robust and reproducible results.

In the *in vivo* model, Mos-RRV-T48 infection induced less weight gain and higher clinical scores compared to Mam-RRV-T48 inoculated mice. Additionally, Mos-RRV-T48 infection resulted in more inflammatory infiltrates, greater tissue and bone destruction present in quadriceps and ankle samples compared to Mam-RRV-T48 inoculated mice. IFNβ induction was upregulated in Mam-RRV-T48 infected mice at peak disease. Isolates RRV-M and RRV-R showed similar disease patterns as seen with RRV-T48. This demonstrates that the differential induction of disease by Mam-RRV and Mos-RRV also occurs for wild type circulating isolates.

In addition, Barmah Forest and Dengue viruses also showed differential profiles between mammalian and mosquito derived viruses *in vitro*, similar to RRV-T48 findings. This suggests that the observations in RRV infection may be more widely applicable to other arboviruses and warrants investigation.

In conclusion, the cell culture findings in this thesis suggest that arboviruses should be cultured in mosquito cell lines, ensuring that the data generated is related to natural infection and host response. Furthermore, one of the most pivotal outcomes of this thesis is in the refinement of the current RRV mouse model which now takes into account gender dimorphism. The improvement of the RRV mouse model is an important step forward in more reproducible arbovirus research.

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[1] All images, unless otherwise referenced, were created by Thomas Townsend under instruction from the candidate – Jade Redfern.

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List of Abbreviations

ADE	Antibody-dependant enhancement
ATF	Activation Transcription Factor
BFV	Barmah Forest Virus
BHK	Baby Hamster Kidney Cells
CP	Capsid Protein
C3	Complement Component 3
CDC	Center for Disease Control
cDNA	Complementary DNA
ChikV	Chikungunya Virus
CR3	Complement Receptor 3
DC	Dendritic Cells
DC-SIGN	Dendritic Cell Specific Intercellular Adhesion Molecule-3-Grabbing Non-Integrin
DenV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DSS	Dengue Shock Syndrome
E	Envelope Protein
EEEV	Eastern Equine Encephalitis Virus
GAS	INF ɣ Activated Site
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HMG	High Mobility Group Protein
HS	Heparin Sulfate
IFN	Interferon
IFNAR	Interferon Alpha Receptor
IKK	Inhibitor of Kappa Beta Kinase
IL	Interleukin
IP	Interferon ɣ Induced Protein

IRF	Interferon Regulator Factor
ISGF	IFN-Stimulated Gene Factor
ISRE	IFN-Stimulated Response Element
Jak-STAT	Janus Kinase – Signal Transducer and Activator of Transcription
JEV	Japanese Encephalitis
kb	Kilobases
KV	Kunjin Virus
LPS	Lipopolysaccharide
L-SIGN	Liver/Lymph Node Intercellular Adhesion Molecule-3-Grabbing Non-Integrin
Mam	Mammalian Derived
MBL	Mannose Binding Lectin
MCP/CCL2	Monocyte Chemotactic Protein
MHC	Major Histocompatibility Complex
MIF	Macrophage Inhibitory Factor
MIG	Macrophage Induced Gene
MIP	Macrophage Inhibitor Complex
Mos	Mosquito Cell Derived
mRNA	Messenger RNA
MVE	Murray Valley Encephalitis
NF-KB	Nuclear Factor Kappa B
NK	Natural Killer cells
NO	Nitric Oxide
NsP	Non-Structural Protein
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PDC	Plasmacytoid Dendritic Cells
PKR	Serine Threonine Protein Kinase R
Ra	Receptor Antagonist
RdRp	RNA-dependant RNA Polymerase

RRV-T48	Ross River Virus T48
RVFV	Rift Valley Fever
SFV	Semliki Forest Virus
SinV	Sinbis Virus
SIRS	Systemic Inflammatory Response Syndrome
TBK	Threonine Protein Kinase
TC	T Lymphocyte Cytotoxic Cells CD8+
TGF	Transforming Growth Factor
TH	T Lymphocyte Helper Cells CD4+
TLR	Toll Like Receptor
TM	T Lymphocyte Memory Cell
TNF	Tumour Necrosis Factor
VEEV	Venezuelan equine encephalitis virus
WHO	World Health Organisation
WNV	West Nile Virus
Wt	Wild Type
YF	Yellow Fever