Immune responses against recombinant poxvirus vaccines that express full-length lyssavirus glycoprotein genes

by

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I declare that the thesis, which I hereby submit for the degree Ph.D. at the University of Pretoria, Pretoria, is my own work and has not been submitted by me for a degree at another university

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SUMMARY

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Rabies is a fatal but preventable neurotropic disease of potentially all mammals. The disease is caused by lyssaviruses. Rabies is recognized as the 10th most common lethal infectious disease in the world, rendering it one of the most feared zoonotic diseases known to man. Nevertheless, rabies can be prevented by application of pre- or post exposure treatments. Rabies vaccines have been available since the time of Pasteur, more that one hundred years ago. Since, vaccine research focused on the development of safer and more effective vaccines. Topics of current interest in the field of rabies vaccinology were addressed in this study. A primary concern regarding the disease is human mortalities, in the range of 60 000, reported every year. Most of these are linked to exposure to rabid dogs. In addition, a great number of post exposure treatments are administered each year at great costs. Despite availability of efficacious biologics, several factors influence the optimal use and accessibility of these agents in the countries of interest, with cost and availability being the major contributing factors.

A proven approach is mass oral vaccination of target animals, such as dogs, which indirectly infers protection to susceptible hosts, including man. Currently available vaccines present several disadvantages of use though, including issues of safety or

doubtful stability. Safer but effective alternative vaccines that could be used in oral baits would be valuable. Here the use of two candidate host restricted poxvirus vaccine vectors were explored, particularly also in regard to oral innocuity. The construction, convenient isolation and use of a recombinant Lumpy skin disease virus (Neethling strain) expressing rabies virus glycoprotein in a mouse model were investigated. In addition, a recombinant Modified Vaccinia virus Ankara expressing rabies virus glycoprotein was prepared and tested as a vaccine in mice, dogs and raccoons. In both cases it was clear that the severe attenuation of these viruses did affect the efficacy of the recombinant vaccines in the non-permissive hosts. With the recombinant MVA a clear dosage effect could be shown, and equivalent humoral responses could only be attained at much higher titers of vaccine virus as with replication competent counterparts.

Secondly, the cross-protection of rabies vaccines across the spectrum of lyssaviruses was addressed. Lyssaviruses can be divided into two groups based on sequence analysis and pathogenesis. Viruses belonging to the so-called phylogroup II, are the Mokola, Lagos and West Caucasian Bat viruses. Classic rabies biologics fail to fully protect against the viruses attributed to a lack of cross-neutralization. Here, cross-protection and cross-reactive immune responses induced by recombinant vaccinia viruses expressing rabies, Mokola or West Caucasian Bat virus glycoproteins, in single or dual combinations, were investigated. As expected, there was a lack of cross-protection of rabies and Mokola glycoprotein vaccines. There was also a clear lack of cross-protection of West Caucasian Bat virus glycoprotein vaccine and rabies and Mokola viruses. The dual antigen expressing vaccines did not appear to offer any additional protective effect in the tested model. The Mokola virus glycoprotein vaccines induced neutralizing antibody responses that significantly cross-neutralized Lagos Bat virus.

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Part Four: Preparation of recombinant vaccinia virus expressing full-length Rabies and non-rabies lyssavirus glycoprotein genes and evaluation of immunogenicity and cross-protection in a mouse model

LIST OF ABBREVATIONS

A	Adenine
A_{260}/A_{280}	The ratio of absorption at 260nm and absorption at 280 nm
AD	*Anno Domini* of the Christian era/after Christ
ARC-OVI	Agricultural Research Council-Onderstepoort Veterinary Institute
AUG	Adenine Uracil Guanine (translation initiator codon)
BC	Before Christ
bp	Base pair
C	Cytosine
°C	Degrees Celsius
CDC	Centers for Disease Control and Prevention
CEC-32	Chicken embryo fibroblast cell line
CEF	Primary chick embryo fibroblasts
CO_2	Carbon dioxide
CPE	Cytopathic effect
CTL	Cytolytic T lymphocyte
CVS	Challenge virus standard
DALY	Disability-adjusted life year
DMEM	Dulbecco's Modified Eagle's Medium
DMEM/F12	Dulbecco's Modified Eagle's Medium with Ham's F12 medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
*Eco*gpt	*Escherichia coli* guaninephosphoribosyltransferase
EBLV-1	European Bat Lyssavirus 1
EGFP	*Escherichia coli* green fluorescent protein
ERA	Evelyn-Rockitniki-Abelseth strain of rabies virus
FBT	Primary fetal bovine testis
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
FFU	Focus forming units

γ	Gamma
G	Guanine
g	Gravitational force
Gs	Soluble glycoprotein
GFP	Green fluorescent protein
HA	Hemaggluttin
H_2O	Water
HIV	Human Immunodeficiency Virus
IACUC	Institutional animal care and use committee
i.e	That is to say
IFN	Interferon
IL	Interleukin
Kb	Kilobasepair
KCL	Potassium chloride
KH_2PO_4	Potassium phosphate
KS-1	Kenya sheep-1 strain
LB	Luria Bertani
LSDV	Lumpy Skin DiseaseVirus
LSDV-RG	Recombinant Lumpy Skin Disease Virus (Neethling vaccine strain) expressing a full length rabies virus glycoprotein gene
LSDV-SA	Lumpy Skin Disease Virus Neethling vaccine strain
MEM	Minimum essential medium
$MICLD_{50}$	Mouse intracranial lethal dose fifty
MOI	Multiplicity of infection
ml	Milliliter
mM	Millimolar
mRNA	Messenger ribonucleic acid
MPA	Mycophenolic acid
MVA	Modified Vaccinia Virus Ankara
n	Sample size
NaCL	Sodium chloride

$Na_2HPO_4.2H_2O$	Hydrated sodium phosphate
nm	Nanometer
ORF	Open reading frame
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEP	Post-exposure prophylaxis
PFU	Plaque forming units
PV	Pasteur Virus
P7.5K	Vaccinia virus early late promoter
P11K	Vaccinia virus late promoter
RFFIT	Rapid Fluorescent Focus Inhibition Test
RIG	Anti-rabies immunoglobulin
RG	Rabies virus glycoprotein
RNA	Ribonucleic acid
rpm	Rotations per minutes
s	Sedimentation coefficient
T	Thymine
TBE	Tris-Borate-Ethylenediaminetetraacetic acid buffer
TK	Thymidine Kinase
TKl	Thymidine Kinase gene left flanking region
TKr	Thymidine Kinase gene right flanking region
µ	Micro
U	Uracil
UGA	Uracil guanine adenine (translation termination codon)
USA	United States of America
UV	Ultraviolet
VNAb	Virus neutralizing antibody
V-RG	Vaccinia virus rabies glycoprotein recombinant vaccine
WCBV	West Caucasian Bat virus
WHO	World Health Organization
w/v	Weight per volume

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