The cloning and expression of the Rift Valley Fever G genes for the development of a DNA vaccine.

by

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Dedicated to my Heavenly Father, Herman and my parents.

I am grateful for undeserved mercy and support.

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SUMMARY

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Rift Valley fever is an acute or peracute zoonotic disease of domestic ruminants and it is caused by a single serotype of the Rift Valley fever virus, a member of the Bunyaviridae family of the *Phlebovirus* genus. Vaccines that are currently used against this disease include a live attenuated virus (the Smithburn vaccine strain) and an inactivated virus vaccine. Several disadvantages are associated with both vaccines. For the attenuated vaccine, abortions in pregnant ewes, anatomical abnormalities in neonatal lambs and low antibody response in cattle are observed. In the case of the inactivated vaccine, it is necessary to produce large quantities of the virus antigen and only short term immunity can be obtained after vaccination. Third generation vaccines include DNA vaccines that offer numerous advantages above the currently used vaccines. These advantages include the possibility of long term and even lifelong immunity as well as eliciting of humoral and cellular immune responses after vaccination. Problems

encountered with the vaccines in use for Rift Valley fever can potentially be addressed by investigating a DNA vaccination strategy.

A mammalian expression vector backbone (pCI) was used in the construction of the DNA vaccine plasmid construct pCI-G2G1-EGFP. This construct includes the protective, immunogenic Rift Valley fever virus glycoprotein genes, G2 and G1 as well as the Enhanced Green Fluorescent Protein (EGFP) gene, both under the control of a Cytomegalovirus (CMV) enhancer-promoter, the latter acting as a marker gene during the construction, development and evaluation of the DNA vaccine. The insertion site of the glycoprotein gene fragment as well as the transcriptional start site of the G2 gene localized at the 5' end of the fragment was shown to be correct using chain terminating sequencing. Expression of the cloned genes was shown in BSC 40 cells with the use of indirect immunofluorescence to detect glycoproteins and fluorescent microscopy to detect EGFP. The plasmid construct was subsequently tested in a mouse model, where the mice were vaccinated intramuscularly with the DNA vaccine. Due to the delayed deaths observed in the groups vaccinated with DNA vaccine in comparison to mice in the non-vaccinated negative control group, we decided to use this construct in a sheep model. In the DNA vaccination trial the sheep were vaccinated either intradermally or intramuscularly with the pCI-G2G1-EGFP plasmid construct with or without adjuvant. Higher titres of neutralising antibodies were seen in the neutralising antibody assays of the animals vaccinated with the DNA vaccine than the negative control animals. However, clinical signs and symptoms (temperatures and liver enzyme levels) of DNA vaccinated animals after challenge with a virulent strain of Rift Valley fever virus indicated viremia. In comparison to animals in the negative control group, clinical signs and symptoms of the group vaccinated with the DNA vaccine were not significantly different indicating no detectable levels of protection after vaccination with the DNA vaccine.

Several of the diagnostic tests for Rift Valley fever are time-consuming, expensive, complicated and require handling of whole virus in antigen preparation. The replacement of the whole virus as antigen by a recombinant protein would eliminate this risk. The glycoprotein gene fragment has been subcloned into a bacterial expression vector and the bacterial vector system was evaluated for the expression of the glycoproteins. A product could only be obtained after truncation of the G2 gene. Diagnostic methods that limit

health risk by eliminating handling of the virus, save time, are less expensive and would be useful in assisting the diagnosis of RVF. The RT-PCR method would conform to the requirements and it is sensitive and specific. A one-step RT-PCR specific for Rift Valley fever virus was developed evaluated and validated. Primers were designed to anneal in the glycoprotein gene G2 of the virus at the 3' end of the G2 gene and the non-translated region between the G2 and G1 genes upstream of the G1 gene. It is foreseen that the RT-PCR could be used as a rapid diagnostic test and as a method for the determination of the viral load during production of classical vaccines in the future.

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ABBREVIATIONS

A	-	adenine
ALT	-	alanine aminotransferase
APC	-	antigen presenting cells
AST	-	aspartate amino-transferase
ATP	-	adenosine tri-phosphate
AU	-	absorbancy units
bp	-	base pairs
BSA	-	bovine serum albumine
BSC-40	-	fibroblast like African Green Monkey kidney cells
C	-	cytosine
°C	-	degrees Celsius
CAT	-	chloramphenicol acetyltransferase
CD	-	clusters of differentiation
CF	-	complement fixation
CIAP	-	calf intestinal alkaline phosphatase
CMI	-	cell mediated immunity
CMV	-	Cytomegalovirus
CPE	-	cytopathic effect
CpG	-	cytosine –phosphodiester linkage-guanine
CTL	-	cytotoxic T-lymphocyte
DEPC	-	Diethyl pirocarbonate
DEAE	-	Diethylaminoethane
DMEM	-	Dulbecco's modified Eagle's medium
DMSO	-	Dimethylsulphoxide
DNA	-	Deoxyribonucleicacid
dNTP	-	deoxynucleotide triphosphate
ds	-	double-stranded
DTT	-	Dithiotreitol
EDTA	-	Ethylene diaminetetraacetic acid

EGFP	-	Enhanced Green Fluorescent Protein
ELISA	-	Enzyme linked immunosorbent assay
et al.	-	et alii (and others)
FCS	-	foetal calf serum
FITC	-	Fluorescein isothiocyanate
G	-	guanine
GFP	-	Green Fluorescent Protein
GGT	-	gamma-glutamyl transferase
GLDH	-	glutamate dehydrogenase
GMT	-	geometric mean titre
h	-	hour
HAI	-	haemagglutination inhibition
hGH	-	human growth hormone
HPRI	-	Human placental ribonuclase inhibitor
HRP	-	horseradish peroxidase
i.d.	-	intradermal
ID	-	immunodiffusion
IF	-	immunofluorescence
IFNγ	-	interferon gamma
Ig	-	immunoglobulin
IL	-	interleukin
i.p.	-	intraperitoneal
IP	-	immunoperoxidase
IPTG	-	Isopropyl - β-D-thiogalactopyranoside
kb	-	kilobases
kDa	-	kiloDalton
lac Z	-	β - galactosidase gene
LB	-	Luria-Bertani medium
LD_{50}	-	lethal dose 50
LFT's	-	lamb fetal testis cells
M	-	molar

mAbs	-	monoclonal antibodies
MCS	-	multiple cloning site
MDBK	-	Madin-Darby bovine kidney cells
mg	-	milligram
MHC	-	major histocompatibility complex
min.	-	minutes
ml	-	millilitre
mM	-	millimolar
m.o.i.	-	multiplicity of infection
mRNA	-	messenger RNA
MW	-	molecular weight
NaAc	-	Sodium acetate
ng	-	nanogram
NK	-	natural killer cells
nm	-	nanometer
Ns	-	non-structural
OD_{590}	-	optical density at a wavelength of 590 nm
OIE	-	Office International des Epizooties
PAGE	-	Polyacrylamide gelelectrophoresis
p.i.	-	post infection
PBS	-	phosphate buffered saline
pCMV	-	Cytomegalovirus promoter
PCR	-	polymerase chain reaction
PEG	-	Polyethylene glycol
pfu	-	plaque forming units
poly(A)	-	poly adenylation
PRNT	-	plaque reduction neutralisation test
Pu	-	purine
Py	-	pyrimidine
RIA	-	radio-immunoassay
rpm	-	revolutions per minute

RNA - ribonucleic acid
RT-PCR - reverse transciption PCR
RVF - Rift Valley fever
RVFV - Rift Valley fever virus
SDH - sorbitol dehydrogenase
SPF - specific pathogen free
SDS - Sodium dodecyl sulphate
ssRNA - single-stranded RNA
T - tyrosine
TAE - Tris-acetate-EDTA
$TCID_{50}$ - 50% Tissue culture infective dose
TE - Tris-EDTA
Th - T-helper cells
Tris - Tris(hydroxymethyl)aminomethane
U - units
µl - microlitre
µg - microgram
UV - ultra violet
X-gal - 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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