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Engineering of a chimeric SAT2 foot-and-mouth disease virus for vaccine production

by

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SUMMARY

Engineering of a chimeric SAT2 foot-and-mouth disease virus for vaccine production

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Foot-and-mouth disease virus (FMDV), a member of the *Picornaviridae*, causes a highly contagious disease affecting cloven-hoofed animals. In 2000, a SAT2 type virus, SAU/6/00, was introduced into the Middle East, causing a severe outbreak of foot-and-mouth disease (FMD) in Saudi Arabia. Although an inactivated vaccine containing the Saudi Arabian strain antigen is currently available, SAU/6/00 is not an ideal vaccine producing strain. This is due to a lack in consistent high antigen yield produced at a rate complying with good vaccine production practices. Towards the long-term goal of developing an alternative approach for producing the current inactivated SAT2/SAU/6/00 vaccine, the aim of this study was to engineer and characterize a chimeric FMDV.

To facilitate engineering of a chimeric SAT2 virus, the capsid (P1)-coding region of the SAU/6/00 strain was molecularly characterized. Comparison of the nucleotide and deduced amino acid sequence to that of different SAT2 type viruses indicated a high level of intratypic variation. The greatest variation was observed in the 1D protein, which forms part of the external capsid and contributes to the antigenicity of the virus. Hypervariable regions were identified in the SAU/6/00 capsid-coding region and found to correspond to known antigenic

sites of FMD viruses. Using a previously constructed genome-length cDNA clone derived from the SAT2 vaccine strain ZIM/7/83, a chimeric construct was engineered by replacing the external capsid-coding region (1B-1D) of ZIM/7/83 with that of SAU/6/00 in the SAT2 genome-length cDNA clone. *In vitro*-synthesized RNA transcripts derived from the chimeric pSAU6/SAT2 clone were subsequently used to transfect baby hamster kidney (BHK) cells and resulted in the recovery of a viable chimeric SAT2 virus.

The recovered chimeric virus vSAU6/SAT2 and parental SAU/6/00 vaccine strain were compared in terms of their growth properties, temperature stability and antigenic profile of the viral particles. The plaque morphologies of the respective viruses were similar on BHK and IB-RS-2 cells, indicating that the phenotypic characteristics of the parental virus were maintained in the chimera. In addition, the chimera exhibited improved growth properties in BHK cells and produced higher titres than the parental SAU/6/00 virus. A rapid growth rate in tissue culture, as well as high antigen yields, are desirable for vaccine strains. Investigation of antigen stability at high temperatures indicated that the chimera is not distinctly more heat-stable than the parental virus. With regards to their antigenic profile, both the chimera and parental virus displayed a similar profile in virus neutralization tests (VNT), suggesting that the necessary antigenic properties of the parental virus are most probably present in the chimera. *In vivo* testing of the SAT2 chimera would be necessary to evaluate the usefulness of the chimera in commercial vaccine production.

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LIST OF ABBREVIATIONS

aa	amino acid
BEA	bromoethylamine hydrobromide
BEI	binary ethyleneimine
BHK	baby hamster kidney
BME	Eagle's basal medium
BTY	bovine thyroid cells
°C	degrees Celsius
ca.	approximately
cDNA	complementary deoxyribonucleic acid
CHO	Chinese hamster ovary
CPE	cytopathic effect
cre	*cis*-acting replication element
CsCl	caesium chloride
DAPSA	DNA and Protein Sequence Analysis
D-MEM	Dulbeco's minimal essential medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside-5'-triphosphate
EDTA	ethylenediaminetetra-acetic acid
EIF	eukaryotic initiation factor
e.g.	for example
ELISA	enzyme-linked immunosorbant assay
EMCV	encephalomyocarditis virus
EtBr	ethidium bromide
FCS	fetal calf serum
Fig.	figure
FMDV	foot-and-mouth disease virus
g	gram
GuSCN	guanidinium thiocyanate
h	hour

IB-RS-2	Instituto Bioloica Rim Suino
ICAM	intercellular adhesion molecule
Ig	immunoglobulin
IPTG	isopropyl β-D-thiogalactosidase
IRES	internal ribosome entry site
kb	kilobase pair
KNP	Kruger National Park
LB medium	Luria-Bertani medium
M	molar
MBS	MES-buffered saline
MEGA	Molecular Evolutionary Genetics Analysis
MES	[*N*-morpholino]ethane-sulfonic acid
MHC	major histocompatibility complex
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
NEAA	non-essential amino acids
ng	nanogram
nt	nucleotide
OD	optical density
OIE	Office des Epizooties
ORF	open reading frame
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PD	protective dose
PEG	polyethylene glycol
PEO	polyethylene oxide
pfu	plaque forming units
PIADC	Plum Island Animal Disease Center
p.i.	post-infection
pmol	picomole

poly(C) tract	polycytidylate tract
PKs	pseudoknots
pSAT2	SAT2 genome-length cDNA clone
pSAU6/SAT2	recombinant construct (SAU/6/00 external capsid-coding region cloned into pSAT2)
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RPMI	Roswell Park Memorial Institute-1640 medium
RT-PCR	reverse transcriptase-polymerase chain reaction
s	second
S	Svedberg unit
SAT	South African Territories
SAU/6/00	Saudi Arabian outbreak strain
SDS	sodium dodecyl sulphate
SNT	serum neutralization antibody titre
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TCID	tissue culture infective dose
TE	Tris-EDTA
TPB	tryptose phosphate broth
Tris	Tris-hydroxymethyl-aminomethane
U	units
μg	microgram
μl	microlitre
μM	micromolar
UTR	untranslated region
VNT	virus neutralization test
VP_g	viral genome-linked protein
vSAT2	virus derived from the SAT2 genome-length cDNA clone
vSAU6/SAT2	chimeric virus derived from the pSAU6/SAT2 construct
v/v	volume per volume
w/v	weight per volume
X-Gal	5-bromo-4-chloro-3-indolyl β-D-galactopyranoside