DEVELOPMENT OF RECOMBINANT VACCINES AGAINST FOOT-AND-MOUTH DISEASE

By

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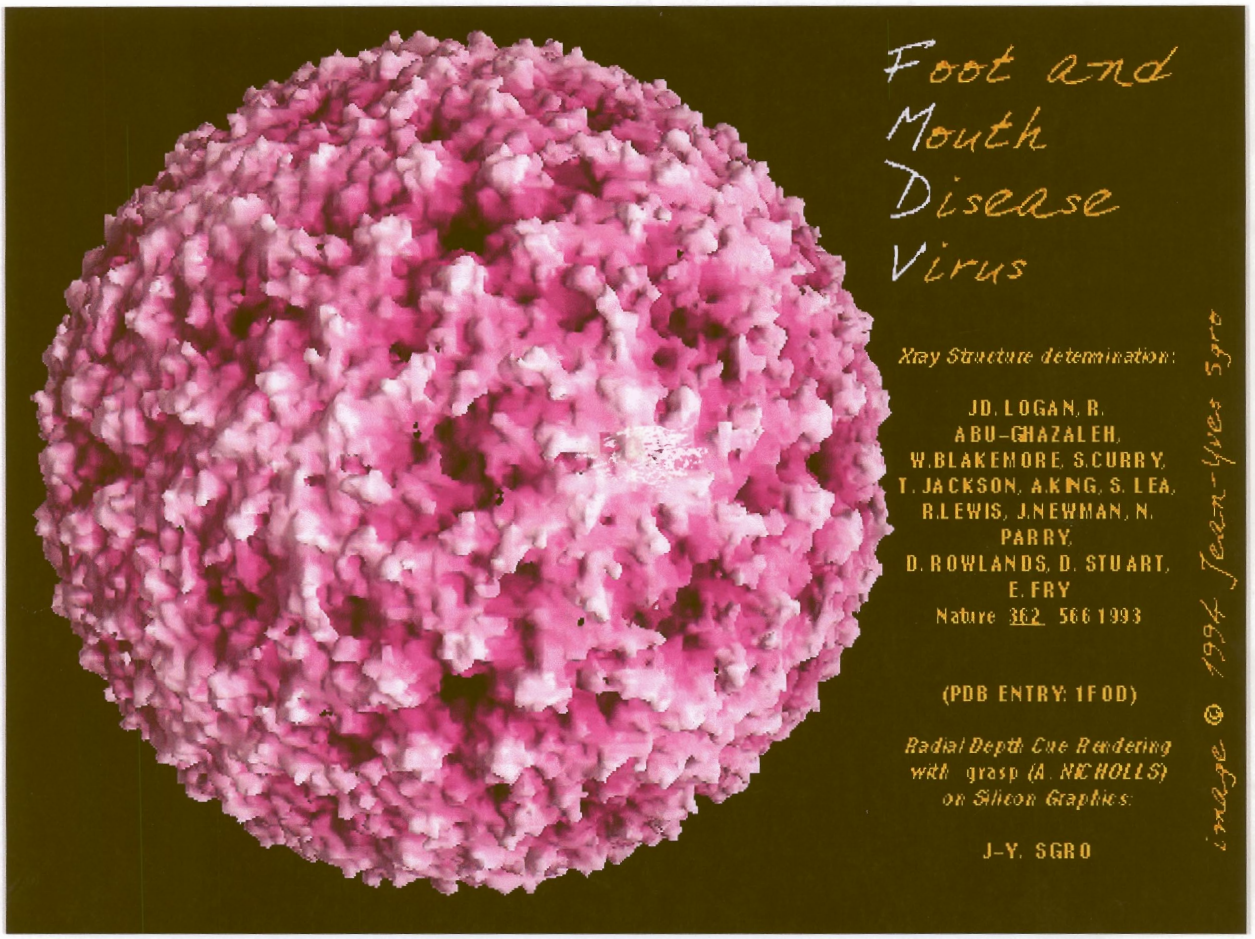

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"In the temple of science are many mansions ... and various indeed are they that dwell therein and the motives that led them there.

"Many take to science out of a joyful sense of superior intellectual power; science is there own special sport to which they look for vivid experience and the satisfaction of ambition;
many others are to be found in the temple who have offered the products of their brains on this altar for purely utilitarian purposes.

Were an angel of the Lord to come and drive all the people belonging to these two categories out of the temple, it would be noticeably emptier but there would still be some men of both present and past times left inside ... If the types we have just expelled were the only types there were, the temple would never have existed any more than one can have a wood consisting of nothing but creepers ...

... and those who have found favor with the angel ... are somewhat odd, uncommunicative, solitary fellows, really less like each other than the hosts of the rejected.

"What has brought them to the temple ... no single answer will cover ... escape from everyday life, with its painful crudity and hopeless dreariness, from the fetters of one's shifting desires. A finely tempered nature longs to escape from this noisy cramped surroundings into the silence of the high mountains where the eye ranges freely through the pure still air and fondly traces out the restful contours apparently built for eternity."

This is an excerpt from a speech given in 1918 by a young German scientist called Albert Einstein.

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ABBREVIATIONS

BEA – bromoethylamine hydrobromide

BGH – bovine growth hormone

BHK – baby hamster kidney

CMV – cytomegalovirus

CTE – C-terminal extention

DEAED – diethylaminoethyldextran

EIF – eukaryotic initiation factor

ELISA – enzyme linked immunosorbent assay

FLC – full-length clone

FMDV – foot-and-mouth disease virus

IB-RS-2 – Instituto Biologico Rim Suino

IRES – internal ribosome entry site

KNP – Kruger National Park

MEGA – molecular evolutionary genetics analysis

MOI – multiplicity of infection

NCR – non coding region

NTP – nucleotide phosphate

OIE – Office des Epizooties

ORF – open reading frame

PBS – phosphate buffered saline

PCR – polymerase chain reaction

PK – pig kidney

PTB – polypyrimidine tract-binding

RPMI – Roswell Park Memorial Institute

RT-PCR – reverse-transcribed polymerase chain reaction

SAT – South African Territories

TCID – tissue culture infectious dose

UTR – untranslated region

VNT – virus neutralization test

VP – viral protein

VPg – viral genome-linked protein

SUMMARY

The South African Territories (SAT) types of foot-and-mouth disease virus (FMDV) show marked genomic and antigenic variation throughout sub-Saharan Africa. This variation is to a large extent geographically linked and requires therefore the use of custom-made vaccines. Adaptation of field isolates as vaccine strains is cumbersome, time consuming and expensive. A possible means of circumventing the adaptation process is to construct recombinant or chimeric FMD viruses, followed by the production of conventional, inactivated vaccine utilizing these viruses. The advantage of such a strategy would be the ability to manipulate the antigenicity of these viruses by substituting the antigenic coding regions (i.e. structural proteins) of a full-length cDNA clone of a suitable strain.

Towards this objective the structural-protein-coding region (P1) of a SAT 2 vaccine strain, ZIM/7/83/2, was determined and compared with two other known SAT 2 P1 regions. Five hypervariable regions were identified of which four are situated in VP1. The cleavage sites for proteolytic processing and especially the regions adjacent to these sites, differ between types A and SAT 2. The genetic heterogeneity of two FMDV proteinases, the Leader and 3C, of representatives of six different serotypes, was subsequently investigated. The results revealed these genomic regions of the SAT viruses originating from southern Africa to be distinct from types A, O and C. Interestingly, it was also seen that the Leader and 3C proteinases of the SAT types are less variable than their European counterparts. These results were in contrast to that obtained for the structural proteins, which showed the SAT 2 P1 region to be at least 2-3 times more variable than that of types A, O and C. Despite the observed differences in the proteinases, a three-dimensional structural model for the Lb form of the ZIM/7/83/2 Leader proteinase predicted the three-dimensional fold of the enzyme to be conserved.

A chimeric cDNA clone between types A and SAT 2 was constructed by inserting the external capsid-coding region of ZIM/7/83/2 into the genetic backbone of the A_{12}

cDNA clone. The subsequent evaluation of the resulting recombinant FMD virus indicated the virus to be immunogenically identical to the wild type ZIM/7/83/2. However, the recombinant virus was found to be a slower antigen producer and less stable than the wild type SAT 2. These characteristics make the recombinant FMD virus constructed in this study unsuitable for conventional vaccine production. Alternative means, such as the use of a SAT 2 cDNA clone, should be investigated.