IMPROVED METHODS FOR PRODUCTION AND CHARACTERISATION OF JEMBRANA DISEASE VIRUS PROTEINS

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DECLARATION

I declare that this is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution

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Abstract

Jembrana disease is an acute disease of Bali cattle (*Bos javanicus*) in Indonesia caused by *Jembrana disease virus* (JDV), a lentivirus most closely related to *Bovine immunodeficiency virus*. Control of the disease in Bali cattle, which are important to the economy of Indonesia, is dependent on the continued availability of protein antigens for immunosurveillance procedures that have been developed. Further investigation is also required to characterise of the proteins of JDV and to provide methods of producing commercial quantities of recombinant proteins for vaccine manufacture.

A problem with the large scale production of viral proteins using recombinant technology was that the proteins have been mainly produced as insoluble products within inclusion bodies in bacterial cells and in that insoluble format they were unsuitable for use as antigens. A method for solubilisation of the insoluble proteins was developed that involved solubilisation of the inclusion bodies with low concentrations of urea in an alkaline solution and the method could be performed easily and at low cost without any detectable loss of antigenicity. The solubilised protein was successfully renatured without the formation of aggregates by dilution of the urea in the presence of the reducing agent dithiothreitol. The method would be suitable for use during the large scale production of recombinant viral proteins for vaccine manufacture.

To provide an additional reagent for diagnosis of the disease, mice were immunised with the recombinant capsid (CA) protein of JDV and a hybridoma was produced that secreted monoclonal antibodies reactive with the CA protein. This monoclonal antibody was effectively used in an immunoperoxidase assay to demonstrate virus in tissues. As an alternative to this technology, recombinant antibody fragments, scFv, reactive with the CA protein of JDV, were also produced by phage display technology. These scFv were expressed as soluble products in the periplasmic space of transfected host bacterial cells. The scFv reacted specifically with the CA protein in western immunoblots and although further optimisation of the methods of production of this scFv are required, the reagents developed can be for expression of the antibody when required, without the need for maintaining liquid nitrogen storage facilities that are necessary for storage of hybridomas.

The size and nature of the glycosylation of the envelope proteins SU and TM of JDV harvested from infected cattle was determined. Two proteins of 75 and 60 kDa were initially identified in SDS-PAGE as the SU and TM, respectively. They were initially

identified using a specific glycoprotein stain and then matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometric (MS) analysis of the protein sequence. Further investigation on the unmatched mass value from MALDI-TOF/MS data suggested that post-translational glycosylation occurred on N-linked glycosylation sites of the JDV-SU and on O-linked glycosylation sites of the JDV-TM. This is the first report of the characteristics of the envelope proteins of JDV and their identification will facilitate further studies of the nature of the immune response to JDV infection using immunoblotting procedures.

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List of abbreviations

Viruses

BIV	:	*Bovine immunodeficiency virus*
BHV	:	*Bovine herpesvirus*
BPV	:	*Bovine papillomavirus*
CAEV	:	*Caprine arthritis-encephalitis virus*
EIAV	:	*Equine infectious anaemia virus*
FIV	:	*Feline immunodeficiency virus*
HIV	:	*Human immunodeficiency virus*
JDV	:	*Jembrana disease virus*
MVV	:	*Maedi-visna virus*
SIV	:	*Simian immunodeficiency virus*

Reagents

Amp	:	ampicillin
DAB	:	3,3' diaminobenzidine tetrahydrochloride
DMEM		Dulbecco's modified Eagle's medium
DMSO	:	dimethyl sulfoxide
DTT	:	dithiothreitol
EDTA	:	ethylenediamine tetra-acetic acid
FCS	:	foetal calf serum
HAT	:	hypoxanthine-aminopterin-thymidine
HT	:	hypoxanthine-thymidine
IPTG	:	isopropylthiogalactoside
LB	:	Luria-Bertani
PBS		phosphate buffered saline
PEG	:	polyethylene glycol
PMSF	:	phenylmethylsulfonyl fluoride
HRP	:	horseradish peroxidase
SDS		sodium dodecylsulfate
TBS	:	tris-buffered saline
TEMED	:	N,N,N',N'-tetramethylene-ethylenediamine
Tris	:	tris (hydroxymethyl) aminoethane

Other

aa	:	amino acid
Ag	:	antigen
bp	:	base pairs
CA	:	capsid
CDR		complementary determining region
cfu	:	colony forming units
DNA	:	deoxyribonucleic acid
dpi	:	day post inoculation
ELISA	:	enzyme-linked immunosorbent assay
Fab	:	fragment antibody
GST	:	glutathione transferase

HRP	:	horseradish peroxidase
IN	:	integrase
ISH		*in situ* hybridisation
LTR		long terminal repeat
MA	:	matrix
MAb		monoclonal antibody
MALDI-TOF	:	matrix-assisted laser desorption/ionisation – time of flight
MS	:	mass spectrometry
NC	:	nucleocapsid
PAGE		polyacrylamide gel electrophoresis
PCR		polymerase chain reactions
PR	:	protease
RNA		ribonucleic acid
RT	:	reverse transcriptase
RT-PCR	:	reverse transcription-PCR
scFv	:	single chain variable fragment
SDS	:	sodium dodecyl sulfate
SU		surface unit
TM		transmembrane glycoprotein
V_H		heavy chain variable domain
V_L	:	light chain variable domain