RESEARCH COMMUNICATION

Alternative cell lines for the propagation of lumpy skin disease virus

Y.S. BINEPAL, F.A. ONGADI and J.C. CHEPKWONY

Kenya Agricultural Research Institute (K.A.R.I.), P.O. Box 57811, Nairobi, Kenya

ABSTRACT

BINEPAL, Y.S., ONGADI, F.A. & CHEPKWONY, J.C. 2001. Alternative cell lines for the propagation of lumpy skin disease virus. *Onderstepoort Journal of Veterinary Research,* 68:151–153

In our Institute lumpy skin disease virus is grown on primary lamb testis cells for isolation, identification and vaccine production. However, the availability of lambs in Kenya has been seriously reduced over the past few years. This has led to an increase in the cost of using primary lamb testis cells. This study was undertaken to investigate other primary cell lines, which are easily available and provide an equivalent or better yield of lumpy skin disease virus. Foetal bovine muscle (FBM) cells were found to be an adequate alternative for lamb testis cells.

Keywords: Capripox virus, foetal bovine muscle cells, Kenya, lumpy skin disease, tissue culture

INTRODUCTION

Lumpy skin disease (LSD), an infectious viral disease of cattle caused by a member of the *Capripoxvirus* genus, which also includes sheeppox virus and goatpox virus, is a member of the Poxviridae family. The causative agent of LSD was identified as the 'Neethling' type poxvirus, which reproduced the disease in susceptible cattle (Alexander, Plowright & Haig 1957). LSD virus is classified as a poxvirus on the bases of morphology, cytopathic effects in tissue culture and the presence of intracytoplasmic inclusion bodies typical of poxviruses (Plowright & Ferris 1959; Munz & Owen 1966; Weiss 1968). More recently molecular biological evidence has corroborated this and shown the capripoxvirus to be distinct from other members of the pox family (Black, Hammond & Kitching 1986; Gershon & Black 1987; 1988).

The initial isolation of the Neethling strain was on monolayers of primary calf and lamb kidney cells (De Lange 1959; Ramisse, Serres & Rakotondramary 1969a; b). Weiss in 1968 used primary lamb testis (LT) cells to grow the capripoxvirus. Davies in a review in 1986 notes that the Neethling LSD virus can be propagated in a number of mammalian cell cultures including tissues from lambs, calves, rabbits, monkeys and hamsters. Davies (personal communication) stated that LSD virus has also been grown successfully in foetal calf skin cells and foetal calf muscle cells (FBM) from foetuses of exotic (*Bos taurus*) and cross breed (*Bos taurus X Bos indicus*) cattle. All tissue culture work on capripoxviruses is presently done using LT cells from exotic sheep, mainly of the Merino breed. Tissues from exotic breeds are used for virus isolation and vaccine production, as virus yield is nearly three fold greater than from tissues obtained from local breeds.

However, the availability of Merino lambs has declined in Kenya, due to a decrease in the number of farmers rearing Merino sheep. This has led to an increase in the cost of obtaining LT cells. An investigation into other primary cell lines, which are more easily available and might provide an equivalent or better virus yield, was therefore carried out.

Accepted for publication 9 January 2001—Editor

MATERIALS AND METHODS

Cell cultures

Three bovine foetuses were collected from a slaughterhouse. Two were at 6 months of gestation and were of exotic breeds, while the third was a crossbreed and was at 3 months of gestation. Foetal bovine skin (FBS) cells, foetal bovine muscle (FBM) cells and foetal bovine kidney (FBK) cells were successfully grown. LT cells were grown from 3-day-old Dorper (Dorset X Persian cross) lambs. Bovine thyroid cells (BT) were grown from a newly born calf.

The cell cultures were prepared as described by Freshney (1990). The cells were then seeded in Glasgow minimum essential medium (GMEM) with 10 % foetal calf serum (FCS) and antibiotics at 10^6 cells/ml in 25 mm^3 tissue culture flasks. The medium in each flask was changed after 24 h.

Freezing and revival of cells

The primary LT, FBM, FBS and FBK cell lines were stored and reconstituted as follows. The cells were trypsinised, washed with PBS and resuspended in GMEM with 10 % FCS. They were counted and diluted to 10^7 cells/mℓ, and were then cooled on ice and DMSO was added to a final dilution of 10 %. The cell suspensions were aliquoted in 1ml freeze drying vials and stored at –70 °C.

Cells were thawed at 37 °C, washed twice in PBS and seeded at 10^5 cells/mℓ in GMEM containing 10 % FCS and antibiotics.

Virus strains

The virus titration was carried out in 96-well flat-bottomed tissue culture plates. Cell suspensions containing 10^4 cells/mℓ, were seeded in volumes of 100 mℓ per well. They were allowed to form a monolayer for two days before infection with one of the viruses that were used (Table 1). The viruses were titrated in a tenfold dilution series up to 10^{-10}. Each virus was titrated in triplicate in each cell line.

Primary isolation from naturally infected cattle

Three cattle, numbers 199, 257 and 275 being used in a separate experiment showed typical clinical signs of LSD. Skin lesions from each of them were removed and prepared as described by Binepal (1996). Polymerase chain reaction (PCR) (Binepal 1996) and virus isolation in all cell lines was attempted with each of the specimens.

RESULTS

The plates were observed daily for the development of a cytopathic effect (CPE). The results of the titration of the different virus isolates in various cell cultures are shown in Table 2.

Primary Isolation

Three animals in a different experiment showed clinical signs of LSD. The lesions from the three cattle were all positive for LSD in the PCR. The results of the attempts to isolate the virus using three different cell lines are given in Table 3.

Virus titration following revival of frozen cells

Each of the cell lines was stored at –70 °C for at least a month before thawing and testing their viability and sensitivity to the viruses. No significant change in their viability or sensitivity was noticed after freezing.

TABLE 1 Viruses used in the experiment

Virus isolate	Species of origin	Virulence	Source
KS-1	Sheep	Vaccine	IAH, Pirbright
2490	Cattle	Virulent	Kenya
O40	Sheep	Virulent	Kenya
O181	Sheep	Virulent	Kenya
Neethling	Cattle	Virulent	South Africa
CPV/RPV[1]	Unknown	Vaccine	IAH, Pirbright
Isiolo[2]	Cattle	Virulent	Kenya
F1	Cattle	Virulent	Kenya
199	Cattle	Virulent	Kenya
275	Cattle	Virulent	Kenya
257	Cattle	Virulent	Kenya
G38	Goat	Virulent	Kenya
B158	Cattle	Virulent	Kenya
Kedong[2]	Sheep	Virulent	Kenya

[1] Capripox/Rinderpest recombinant vaccine (CPV/RPV)
[2] These isolates were given names from the area where the initial samples were collected

TABLE 2 Titration of viruses carried out in the various cell lines (average of three titrations)

	FBS	FBM	FBK	BT	LT
KS-1	10^{-4}	10^{-3}	10^{-1}	10^{-1}	10^{-5}
2490	$10^{-2.5}$	10^{-3}	$10^{-1.5}$	10^{-1}	10^{-3}
O40	10^{-2}	10^{-2}	Foci	$10^{-0.5}$	10^{-2}
O181	10^{-3}	10^{-4}	Foci	10^{-1}	10^{-3}
Neeth.	10^{-2}	10^{-4}	DNG	10^{-1}	10^{-6}
CPV/RPV	10^{-3}	10^{-4}	10^{-2}	10^{-1}	10^{-5}
Kedong	10^{-3}	$10^{-2.5}$	DNG	10^{-1}	10^{-4}
Isiolo	$10^{-2.5}$	$10^{-1.5}$	DNG	Foci	10^{-3}
F1	$10^{-2.5}$	10^{-2}	DNG	DNG	$10^{-2.5}$
199	10^{-2}	10^{-2}	DNG	DNG	10^{-2}
275	10^{-1}	10^{-1}	DNG	DNG	10^{-2}
257	10^{-2}	10^{-2}	Foci	DNG	$10^{-2.5}$
G38	10^{-2}	$10^{-1.5}$	Foci	DNG	10^{-2}
B158	10^{-1}	$10^{-1.5}$	DNG	DNG	10^{-2}

Foci Cytopathic was seen in the first well but in isolated foci and did not spread
DNG Did not grow

TABLE 3 Primary isolation of LSD virus using three different cell lines (CPE at 80 %)

Sample no.	LT	FBS	FBM
199	1st passage—6 days	1st passage—8 days	2nd passage—5 days
257	1st passage—6 days	1st passage—6 days	1st passage—8 days
275	1st passage—5 days	1st passage—6 days	2nd passage—4 days

LT cells could be maintained to the 8th passage level. FBS and FBM cell lines could be maintained up to the 7th passage, while FBK cells went beyond the 10th passage. No loss of sensitivity was noticed with increasing passage levels.

DISCUSSION

FBK and BT did not adequately support virus growth and were therefore discounted.

FBM and FBS cells were very comparable in their ability to support viral growth, though they were less sensitive than LT cells even after freezing. However, the skin cells were fastidious, slow growing and prone to contamination. The availability of foetuses during the rainy season may be a problem, as there are fewer exotic animals being slaughtered. It is concluded that FBM cells are an effective alternative to LT cells for growing lumpy skin disease virus for diagnostic and vaccine production purposes.

ACKNOWLEDGEMENTS

This paper is published with the permission of the Director of Kenya Agricultural Research Institute. The authors acknowledge the funding for this study by DFID and thank Dr H.M. Wamwayi and Mr Alastair Grieve for their help, guidance and support in this study.

REFERENCES

ALEXANDER, R.A., PLOWRIGHT, W. & HAIG, D.A. 1957. Cytopathic agents associated with lumpy skin disease of cattle. *Bulletin of Epizootic Diseases of Africa*, 5:489–492.

BLACK, D.N., HAMMOND, J.M. & KITCHING, R.P. 1986. Genomic relationship between capripoxviruses. *Virus Research*, 5:277–292.

BINEPAL, Y.S. 1996. Genes involved in cell-virus interaction: possible roles in pathogenesis of lumpy skin disease. Ph.D. Thesis. University of Hertfordshire.

DAVIES F.G. 1981. Lumpy skin disease, in *Virus disease of food animals. Disease monographs*, 2, edited by E.P.J. Gibbs. London: Academic Press, 752–764.

DE LANGE, M. 1959. The histology of the cytopathic changes produced in monolayer of epithelial cultures by viruses associated with lumpy skin disease. *Onderstepoort Journal of Veterinary Research*, 28:245–255.

FRESHNEY, R.I. 1990. *Animal cell culture; A practical approach*, edited by D. Rickwood & B.D. Hames. London: Oxford University Press.

GERSHON, P.D. & BLACK, D.N. 1987. Physical characterization of the genome of a cattle isolate of capripoxvirus. *Virology*, 160: 473–476.

GERSHON, P.D. & BLACK, D.N. 1988. A comparison of the genomes of capripox isolates of sheep, goats and cattle. *Virology*, 164:341–349.

MUNZ, E.K. & OWEN, N.C. 1966. Electron microscope studies on lumpy skin disease virus type 'Neethling'. *Onderstepoort Journal of Veterinary Research*, 33:3–8.

PLOWRIGHT, W. & FERRIS, R.D. 1959. Ether sensitivity of some mammalian poxviruses. *Virology*, 7:397–407.

RAMISSE, J., SERRES, H. & RAKOTONDRAMARY, E. 1969a. Isolation of viruses associated with bovine lumpy skin disease in Madagascar. *Revue d'elevage et de Medecine Veterinaire des pays tropicaux*, 22:357–362.

RAMISSE, J., SERRES, H. & RAKOTONDRAMARY, E. 1969b. Adaptation to the rabbit renal cells of viruses associated with bovine lumpy skin disease. *Revue d'elevage et de Medecine Veterinaire des pays tropicaux*, 22:363–371.

WEISS, K.E. 1968. Lumpy skin disease virus, in *Virology monographs*, 3, edited by S. Gard. New York: Springer-Verlag, 111–131.