ADAPTATION OF CAPRINISED RINDERPEST VIRUS TO GROW IN VERO CELLS *IN VITRO*

M.M. PARIDA, S.K. BANDYOPADHYAY*

Division of Virology, Indian Veterinary Research Institute, Mukteswar-Kumaon 263138, Nainital (UP), India

Received July 31, 1995; revised November 13, 1995

Summary. – Caprinised rinderpest virus GTV strain (GTV) was adapted to grow in Vero cells (vGTV) by polyethylene glycol-mediated fusion of infected goat spleenocytes with non-infected Vero cells. The usual methods of infection of cell culture, i.e. virus adsorption or co-cultivation, were not successful. vGTV-induced cytopathic changes in Vero cells were similar to those reported for rinderpest virus. Virus titers increased with the passage number but the virulence for goats decreased. Immunoblot analysis did not reveal any difference between vGTV and vRBOK, the tissue culture rinderpest virus RBOK strain adapted to Vero cells.

Key words: caprinised rinderpest virus; Vero cells; adaptation

Rinderpest is an acute febrile and highly contagious disease primarily of cattle but other ruminants, e.g., sheep, goat, pig, camel etc., are also affected. The disease is characterized by high fever followed by erosion and haemorrhage in gastrointestinal tract leading to diarrhoea, dehydration and death within 7 to 10 days (Plowright, 1968).

Serial propagation of cattle rinderpest virus in goats ("caprinised virus") resulted in attenuation of the virus for cattle but in appearance of virulence for goats (Edwards, 1930). This attenuated virus has been extensively used in the past for immunization of cattle of Indian and African origin (Libeau and Scott, 1960). However, clinical reactions including abortion and loss of milk yield have been observed among certain breeds of cattle as a result of vaccination with this virus (Krishnamurti, 1945; Datta, 1954). With the development of tissue culture rinderpest vaccine (Plowright and Ferris, 1962) and the advantages it offered (Plowright and Taylor, 1967; Bansal *et al.*, 1980), the practice of vaccination with the under-attenuated goat virus has been abandoned.

The aim of the present study was the adaptation of GTV to Vero cells *in vitro* and its characterization.

Vero cells (ATCC, USA) were grown in Eagle's Minimal Essential Medium (MEM, Sigma) supplemented with 5% foetal bovine serum (FBS) and employed in their 125th to 145th passage.

Viruses. GTV (Edwards, 1930) in its 614th passage in goats was used in the present study. The virus was passaged by subcutaneous (s.c.) inoculation of 1.0 ml of 1% suspension of spleenic tissue into one and half year-old hill goats. The goats were euthanised on the fifth day post infection (p.i.) when rectal temperature started to decline. Their spleens were collected aseptically for further manipulations. In some experiments also the tissue culture rinderpest virus strain RBOK (Plowright and Ferris, 1962) was used after 4 – 6 passages in Vero cells (vRBOK).

Methods of virus adaptation. Three methods were applied for *in vitro* adaptation of GTV to Vero cells.

Method 1. Confluent monolayers of Vero cells were inoculated with 0.5 ml of 10% suspension of GTV-infected spleenic tissue in PBS. After adsorption for 1 hr, the virus inoculum was discarded and fresh MEM was added into culture flasks. After 8 – 9 days of incubation at 37°C, the infected cells were subjected to three cycles of freezing and thawing. The resulting material was used for infection of fresh Vero cell monolayers.

*Corresponding author. Present address: National Biotechnology Centre, Indian Veterinary Research Institute, Izatnagar 243122, Bareilly (UP), India

Abbreviations: CPE = cytopathic effect; FBS = foetal bovine serum; GTV = caprinised rinderpest virus GTV strain; MEM = Eagle's Minimal Essential Medium; PBS = phosphate buffered saline; p.i. = post infection; s.c. = subcutaneous; vGTV = Vero cells-adapted GTV; vRBOK = Vero cells-adapted RBOK

Method 2. In this method (co-cultivation), GTV in the form of spleenic suspension was mixed with freshly trypsinized Vero cells before seeding the mixture into culture flasks. Subsequent passages were done by co-cultivation of infected cell suspension with non-infected Vero cells.

Method 3 consisted of the technique described by Ishii *et al.* (1986). Briefly, 2 x 10^7 GTV-infected spleenic lymphocytes were mixed thoroughly with 2 x 10^6 non-infected Vero cells in 10 ml of MEM. The cells were centrifuged at 500 x g for 10 mins, the pellet was broken by gentle tapping and 1 ml of prewarmed 50% (w/v) polyethylene glycol 1500 (Boehringer Mannheim) was added dropwise over a period of 1 min under constant shaking in 37°C water bath. Then 15 ml of prewarmed MEM were added. The cells were pelleted by light centrifugation and resuspended in 250 ml of MEM containing 10% FBS. The cell suspension was distributed into culture flasks and incubated at 37°C in CO_2 atmosphere.

Virus infectivity titration was done according to Rossiter and Jesset (1982).

Immunoblot analysis was described previously (Hemadri and Bandyopadhyay, 1994).

Both the virus adsorption to monolayers and co-cultivation techniques (Methods 1 and 2) failed to produce virus-induced cytopathic changes even after seven serial blind passages. Infected cover-slip cultures at the 3rd and 7th passages did not reveal any virus-specific changes or inclusion bodies in cells. In contrast, the cell-fusion method (Method 3) produced cytopathic changes once the fused cell monolayers were trypsinized after 4 days and reseeded. Small foci of infection appeared within 48 hrs of reseeding which resulted in complete detachment of monolayer within seven days. Subsequent passages were done by co-cultivation of infected culture fluid with trypsinized non-infected Vero cells. Clear rinderpest virus-specific cytopathic effect (CPE) was observed from the 3rd passage onwards which included formation of intracytoplasmic and intranuclear inclusion bodies, syncytia, and rounding and clustering of cells leading to detachment of the monolayer. The time required for development of total CPE decreased with the increase of passage number, and remained constant between the 5th and 15th passages, studied so far. Attempts to infect a preformed Vero cell monolayer with infected culture fluid was successful only after 12 passages by co-cultivation.

Table 1. Infectivity titers of GTV strain during its adaptation to Vero cells

Passage No. after fusion	Log $TCID_{50}$/ml
1	–
2	–
3	1.6
6	2.5
9	3.0
12	3.5
15	4.6

Titrations were done in microtiter plates by co-cultivation of freshly trypsinized Vero cells with serial dilutions of infected culture fluids. (–) = virus not detected.

Table 2. Virulence of unadapted GTV and Vero cells-adapted vGTV strains for goats

Virus	Rectal temperature (C°)	Clinical symptoms	Post mortem lesions[a]
GTV	41.5	Diarrhoea, eye discharge	Spleenomegaly, atrophy of lymph nodes
vGTV (passage No.)			
4	41.3	Diarrhoea	–
8	40.2	Undetectable	Spleenomegaly, enlargement of lymph nodes
14	39.0	Undetectable	Spleenomegaly

[a]Inoculated goats were euthanised 8 days p.i. In all the animals, rinderpest antigen was detected in lymph nodes and spleens by AGP and CIE tests.

The infectious virus titer of culture fluid following adaptation of GTV to Vero cells (vGTV) increased with the passage number (Table 1). However, the cell-free virus was not detected in first two passages. The growth pattern of vGTV was compared with that of Vero cells-adapted RBOK virus (vRBOK, Fig. 1). Maximum concentrations of both the cell-associated and cell-free virus were obtained 96 hrs p.i. of Vero cells. The growth pattern of vGTV was similar to that of vRBOK, albeit, with a lower titer.

In order to study the viral immunogenic polypetides, vGTV-infected Vero cells were subjected to immunoblot analysis using rabbit rinderpest hyperimmune serum. Three major immunogenic polypeptides were detected in both vGTV- and vRBOK-infected Vero cells (Fig. 2). These polypeptides, on the basis of their molecular mass and previously reported data (Hemadri and Bandyopadhyay, 1994), can be putatively designated as H, N, and F1. Molecular mass values of vGTV-induced polypeptides were similar to those induced by vRBOK in Vero cells, suggesting similar immunological properties.

The virulence of Vero cells-adapted vGTV and unadapted GTV in goats was compared in terms of clinical reactions and post-mortem lesions (Table 2). The unadapted GTV produced severe clinical reactions in infected goats indicated by high fever (41.5°C), nasal and ocular discharge, and diarrhoea. Post-mortem lesions indicated enlargement of spleen and lymph nodes. Vero cells-adapted vGTV maintained the same virulence for goats even at the 4th passage. However, the severity of clinical reactions declined with increased passage number, e.g. between the 8th and 14th passages. This suggests further attenuation of GTV occuring simultaneously with its adaptation to Vero cells. The decrease in virus virulence for goats with the increase in passage number in Vero cells is in accordance also with the findings of Ishii *et al.* (1986) on lapinised rinderpest virus.

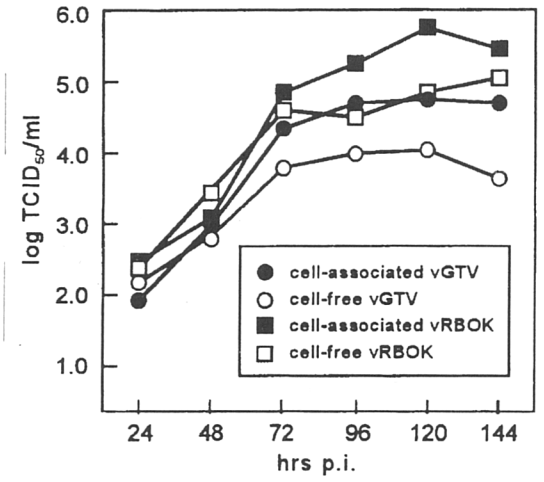

Fig. 1
Growth curves of Vero cells-adapted vGTV and vRBOK strains in Vero cells

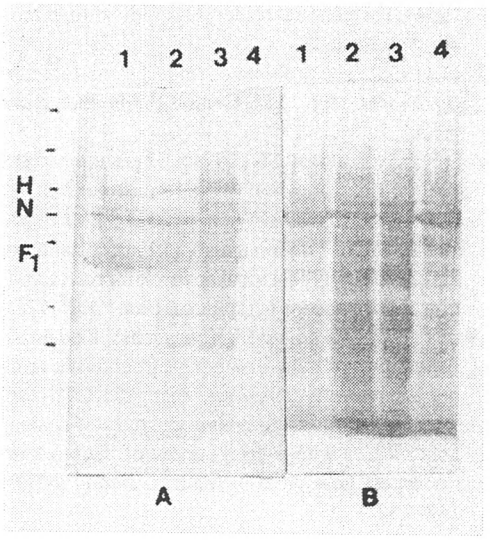

Fig. 2
Immunoblot analysis of Vero cells-adapted vGTV and vRBOK strains

Infected or non-infected Vero cells lysates were subjected to SDS-PAGE and immunoblot analysis (B). SDS-PAGE profiles after staining with Coomassie Brilliant Blue (A). vGTV, 100 hrs p.i. (lane 1); vGTV, 114 hrs p.i. (lane 2); vRBOK, 80 hrs p.i. (lane 3); mock-infected control (lane 4). H, N and F1 indicate positions of the three major viral immunogenic polypeptides. Positions of size marker proteins of 180 K, 116 K, 84 K, 58 K, 48.5 K, 36.5 K and 26.6 K are marked on the left side.

GTV has been extensively used in the past as a vaccine for cattle (Libeau and Scott, 1960). Attempts to grow this virus in Vero cells either by infecting a monolayer culture of or trypsinized Vero cells (co-cultivation) failed but the virus was successfully adapted to grow *in vitro* by polyethylene glycol-mediated fusion of infected spleenocytes with non-infected Vero cells. Although its exact mechanism was not studied, the failure to grow the virus by a normal method of infection suggests that GTV possibly could not adsorb onto or penetrate into cultured Vero cells. Since the polyethylene glycol-mediated fusion bypassed the above two steps in virus replication, it became possible to adapt GTV to grow in Vero cells by this technique. A similar approach for replication of a virus in non-permissive cells has been described with a retrovirus, lapinised rinderpest virus and transmissible gastro-enteritis virus (Rohde *et al.*, 1978; Ishii *et al.*, 1986, 1992). In our experiments, the Vero cells-adapted vGTV was able to infect preformed Vero cell monolayers only from the 13th passage onwards. Thus the intervening passages were carried out by co-cultivation of freshly trypsinized Vero cells with the infected culture fluid. Regards the above two features, the in vitro growth characteristic of vGTV was different from that of lapinised rinderpest virus as described by Ishii *et al.* (1986). The cytopathic changes induced by vGTV in Vero cells were similar to those reported for a rinderpest virus (Plowright, 1968).

The caprinised rinderpest virus has never been employed as a model for the study of rinderpest virus pathogenesis or for understanding the phenomenon of its attenuation vis-a-vis the host reactivity to it. The molecular make-up of and phylogenetic relationships among vaccine strains of rinderpest virus (e.g. lapinised strain, RBOK strain) and virulent virus isolates became known recently (Chamberlain *et al.*, 1993; Baron *et al.*, 1994). GTV, in spite of being the first attenuated rinderpest virus, has not been studied in detail yet. We hope that the successful growth of GTV *in vitro* will promote its further studies.

Acknowledgements. The authors are thankful to the Director of the Indian Veterinary Research Institute, Mukteswar-Kumaon for providing necessary facilities, and to Mr. B.S. Nayal for technical assistance.

References

Bansal RP, Joshi RC, Kumar S (1980): Studies on immunogenicity of tissue culture rinderpest vaccine. *Bull. Off. Int. Epizoot.* **92**, 57–70.

Baron MD, Goatley L, Barrett T (1994): Cloning and sequence analysis of the matrix (M) protein gene of rinderpest virus and evidence for another morbillivirus. *Virology* **200**, 121–129.

Chamberlain RW, Wamway HM, Hockley E, Shaila MS, Goatley L, Knowles NJ, Barrett T (1993): Evidence for different lineages of rinderpest virus reflecting their geographic isolation. *J. Gen. Virol.* **74**, 2775–2790.

Datta S (1954): The national rinderpest eradication plan. *Indian J. Vet. Sci.* **24**, 1–9.

Edwards JT (1930): The problem of rinderpest in India. *Bull. Imp. Agric. Res.* **199**, 1–16. Central Publication Branch, Calcutta.

Hemadri D, Bandyopadhyay SK (1994): Effect of ammonium chloride on multiplication of rinderpest virus in Vero cells. *Acta Virol.* **38**, 163–167.

Ishii H, Yoshikawa Y, Yamanouchi K (1986): Adaptation of the lapinised rinderpest virus to in vitro growth and attenuation of its virulence in rabbits. *J. Gen. Virol.* **122**, 275–280.

Ishii H, Watanabe J, Mukamoto M, Kobayashi Y, Kodama Y (1992): Adaptation of transmissible gastroenteritis virus in non-permissive vero cells. *Arch. Virol.* **122**, 201–206.

Krishnamurty R (1945): A comparative study of rinderpest bull goat viruses with a brief survey of the work done with rinderpest goat virus in Madras. *Indian J. Vet. Sci.* **15**, 247–252.

Libeau J, Scott GR (1960): Rinderpest in Eastern Africa today. *Bull. Epizoot. Dis. Afr.* **8**, 23–26.

Plowright W (1968): *Rinderpest Virus*. Virology Monograph, No. 3. Springer Verlag, New York, pp. 25–110.

Plowright W, Ferris RD (1962): Studies with rinderpest virus in tissue culture. I. The use of attenuated culture virus as vaccine for cattle. *Res. Vet. Sci.* **3**, 172–182.

Plowright W, Taylor WP (1967): Long term studies of the immunity in East African cattle following vaccination with rinderpest culture vaccine. *Res. Vet. Sci.* **8**, 118–128.

Rohde W, Pauli G, Herring J, Fris RR (1978): Polyethylene glycol mediated infection with avian sarcoma viruses. *Arch. Virol.* **58**, 55–59.

Rossiter PB, Jesset DM (1982): Microtitre techniques for the assay of rinderpest virus and neutralizing antibody. *Res. Vet. Sci.* **32**, 252–256.