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Monkey CV1 cell line expressing the sheep–goat SLAM protein: A highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens

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A B S T R A C T

Peste des petits ruminants (PPR) is an important economically transboundary disease of sheep and goats caused by a virus which belongs to the genus *Morbillivirus*. This genus, in the family *Paramyxoviridae*, also includes the measles virus (MV), canine distemper virus (CDV), rinderpest virus (RPV), and marine mammal viruses. One of the main features of these viruses is the severe transient lymphopaenia and immunosuppression they induce in their respective hosts, thereby favouring secondary bacterial and parasitic infections. This lymphopaenia is probably accounted for by the fact that lymphoid cells are the main targets of the morbilliviruses. In early 2000, it was demonstrated that a transmembrane glycoprotein of the immunoglobulin superfamily which is present on the surface of lymphoid cells, the signalling lymphocyte activation molecule (SLAM), is used as cellular receptor by MV, CDV and RPV. Wild-type strains of these viruses can be isolated and propagated efficiently in non-lymphoid cells expressing this protein. The present study has demonstrated that monkey CV1 cells expressing goat SLAM are also highly efficient for isolating PPRV from pathological samples. This finding suggests that SLAM, as is in the case for MV, CDV and RPV, is also a receptor for PPRV.

1. Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of sheep, goats and wild small ruminants (Abu Elzein et al., 2004; Furley et al., 1987; Lefèvre and Diallo, 1990). PPR is a transboundary animal disease causing significant economic losses due to its high morbidity and mortality, and has therefore been classified among diseases that must be notified to the World Organisation for Animal Health (OIE). It poses a serious threat to the development of small ruminant production in all areas where it occurs, namely Africa, the Middle East and Asia. As poor people in developing countries rely on small ruminants, particularly goats, for their livelihoods, it has been suggested that the control of PPR in endemic regions should be reflected in developing poverty alleviation policies (Perry et al., 2002). Clinically, PPR is characterized by pyrexia, necrotic stomatitis, catarrhal inflammation of the ocular and nasal mucosae, bronchopneumonia, diarrhoea and, in many cases, death. Apart from the bronchopneumonia, all these clinical signs resemble those of rinderpest, the cattle plague. The causal agents of both diseases are closely related viruses, peste des petits ruminants virus (PPRV) and rinderpest virus (RPV). They are classified in the genus *Morbillivirus* within the family *Paramyxoviridae* (Gibbs et al., 1979). Other members of this genus include measles virus (MV), a serious human pathogen, canine distemper virus (CDV) affecting animals of the family *Canidae*, phocine distemper virus (PDV) and cetacean morbilliviruses (CMV) which affect marine mammals. All these viruses induce in their respective hosts a transient but severe immunosuppression which favours secondary opportunistic bacterial and parasitic infections responsible for many of the disease symptoms and the severity of the infection (Obi et al., 1983; Seki et al., 2003; Ugochukwu and Agwu, 1991). It is likely that several mechanisms contribute to this morbillivirus-induced

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immunosuppression including leucopaenia, a major sign observed during infection (Heaney et al., 2002; Rajak et al., 2005; Schneider-Schaulies et al., 2001) since lymphoid cells are a major target of morbilliviruses (Takeda et al., 2007; Wohlsein et al., 1993, 1995). This tropism is linked to the presence of a protein receptor on the cell surface, the signalling lymphocyte activation molecule (SLAM) also known as CD150 which is used preferentially by wild-type morbilliviruses to bind to the host (Baron, 2005; Cocks et al., 1995; Kruse et al., 2001; McQuaid & Cosby, 2002; Ono et al., 2001; Sidorenko and Clark, 1993; Tatsuo et al., 2000, 2001; Yanagi et al., 2006). However, although lymphoid tissues are major sites of morbillivirus replication, it is also observed that they can infect and replicate in epithelial cells of other organs such as the alimentary track epithelial cells; lung and kidney cells by utilising other types of cell receptors that have yet to be clearly identified (Hashimoto et al., 2002; Leonard et al., 2008; Plowright, 1964; Tahara et al., 2008; Takeuchi et al., 2003; Wohlsein et al., 1993, 1995). The infection efficiency for those cells is up to 100–1000 times less than that of the lymphoid cells (Hashimoto et al., 2002; Takeda et al., 2007), but because they are easy to maintain in culture *in vitro*, they have been favoured preferentially for morbillivirus isolation. In the case of PPRV, primary cultures of bovine kidney, goat kidney, sheep kidney and lung cells have all been utilised for isolation and maintenance (Abu Elzein et al., 1990; El Hag Ali and Taylor, 1984; Lefèvre and Diallo, 1990; Taylor et al., 1990; Taylor and Abegunde, 1979). However not only is the availability of primary cells becoming more problematic their quality is not guaranteed and there is considerable variation from batch to batch. These drawbacks in the use of primary cell cultures have stimulated the use of cell lines for PPRV isolation, in particular the African green monkey kidney (Vero) cell line (Lefèvre and Diallo, 1990). Unfortunately, as with other morbilliviruses, PPRV isolation using these cells is inefficient: the likelihood of isolating the virus is very low and even if successful it often requires multiple, sequential blind passages and many weeks in culture before the development of any cytopathic effect (CPE) can be observed (Abu Elzein et al., 1990; Albayrak and Alkan, 2009; Gulyaz and Ozkul, 2005; Lefèvre and Diallo, 1990).

Following the identification of the SLAM as the main receptor used by MV, CDV and RPV wild type strains (Baron, 2005; Hsu et al., 2001; Ono et al., 2001; Tatsuo et al., 2000, 2001), Vero or CHO cells expressing human, dog or bovine SLAM proteins have been used as a highly efficient means of isolation and propagation of these morbilliviruses (Ono et al., 2001; Seki et al., 2003; Tatsuo et al., 2001). This paper reports the development of a CV1 cell line stably expressing the goat SLAM protein. This new cell line, designated CHS-20, is highly efficient for isolating wild type PPRV from pathological specimens.

2. Materials and methods

2.1. Cell lines and culture

Flp-In-CV-1 cells, referred to as CV1, were purchased from Invitrogen (Eugene, Oregon, USA). They were grown in the Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% irradiated foetal calf serum (FCS) and 1% mixed antibiotic–antimycotic solution (Invitrogen, Eugene, Oregon, USA), 2 mM L-glutamine, 1 mM sodium pyruvate, and 100 μg/ml zeocin. The African green monkey kidney cells (Vero) were grown in DMEM medium containing 10% FCS and 1% mixed antibiotic–antimycotic solution (Invitrogen, Eugene, Oregon, USA).

2.2. Goat SLAM cDNA cloning

A cDNA sequence corresponding to open reading frame of the goat SLAM that was available in Genbank (Accession No. DQ228869) was synthesized and cloned into pUC57 vector by GenScript (Piscataway, NJ, USA). The cDNA was removed from the plasmid and sub-cloned into pcDNA5/FRT plasmid (Invitrogen, Eugene, Oregon, USA). The new plasmid, pFRT-CHS-20, was amplified in *Escherichia coli* DH5alpha bacteria, purified with the Endo-Free Plasmid Maxi Kit (Qiagen, Hilden, Germany) and used to transfect the cells.

2.3. Cells stably expressing goat SLAM

The pFRT-CHS-20 plasmid was used to transfect the CV1cells of the Flp-In system according to the manufacturer's instructions (Invitrogen, Eugene, Oregon, USA). Cells which had incorporated the recombinant plasmid were selected in a medium composed of the DMEM (Invitrogen, Eugene, Oregon, USA) supplemented with 10% FCS and the antibiotic hygromycin at a final concentration of 600 μg/ml. The cell clones which were resistant to this antibiotic were picked up and expanded in the selecting medium. The cloned cells were each cultured individually in a 25 cm^2 flask and after 10 passages, total RNA was extracted for each clone using the RNAeasy mini kit (Qiagen, Hilden, Germany). From this total RNA, mRNAs were purified using the Oligotex mRNA kit (Qiagen, Hilden, Germany) and submitted to RT-PCR amplification using the following goat SLAM primers: Slam-BCO273F (5′aagagcaggaaggaggatgaagg3′) and Slam-BCO273R (5′gccaagagtgagatacaagaggtg3′). Briefly, 8 μl of each RNA was reverse transcribed (RT) into cDNA using the First Strand cDNA Synthesis kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions and using the random primers pdN (6). Five μl of the cDNA was obtained were used for the detection of goat SLAM mRNA by PCR with the goat SLAM primers indicated above. As a control for the quality of the extracted mRNA, a further five μl of the same cDNA was submitted to PCR to amplify mRNA corresponding to the β-actin gene by using the specific primers Bact1 (5′accaactgggacgacatggaga3′) and Bact2 (5′agccatctcctgctcgaagtc3′).

2.4. Viruses and clinical specimens

In this study, the live attenuated PPRV Nigeria 75/1 vaccine strain was used. This virus was isolated originally from a sick goat on primary lamb kidney cell culture in Nigeria and attenuated by serial passages on Vero cells (Diallo et al., 1989; Taylor and Abegunde, 1979). In addition, clinical specimens from sick sheep and goats with suspected PPR were collected in 2008 and 2009 from different locations in Nigeria and Côte d'Ivoire respectively. The details of the samples are summarized in Table 1.

2.5. PPRV identification by RT-PCR and quantitative RT-PCR (RT-qPCR)

Before attempting isolation of the PPRV from the suspected infected pathological specimens used in this study, they were first screened by the reverse transcriptase polymerase chain reaction (RT-PCR) assay for the detection of viral nucleic acid. An RNeasy mini kit (Qiagen, Hilden, Germany) was used to extract total RNA from these specimens. RNAs were also extracted from cell culture medium to detect the presence of virus by RT-PCR.

These extracted RNAs were then submitted to the amplification of the PPRV RNA by the classical RT-PCR as described previously by Couacy-Hymann et al. (2002). The amplified cDNA samples were analysed by electrophoresis in 1.5% agarose gel stained with ethidium bromide at a concentration of 1 μg/ml.

The RNA samples that were extracted from the pathological samples were analysed by the one-step reverse transcription quantitative real-time PCR (RT-qPCR) using the iScript One-Step