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Stability of canine distemper virus (CDV) after 20 passages in Vero-DST cells expressing the receptor protein for CDV

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Abstract

Isolates 007Lm, S124C and Ac96I and a Vero cell-adapted Onderstepoort strain of canine distemper viruses (CDV) were examined for stability after passages in Vero cells expressing the canine signaling lymphocyte activation molecule (dogSLAM, the intrinsic receptor to CDV). These viruses passage once in Vero cells expressing dogSLAM (Vero-DST) cells (original) and after 20 passages (20p) were compared by using sequence analyses and growth characteristics. All four strains of 20p grew well and were slightly better than their originals. The 20p viruses developed a cytopathic effect slightly lower than the original strains. A few changes in amino acids in the H gene were between the 20p and the original viruses, but the sites of changes were not specific. Fragments of P, M and L genes of all strains showed no nucleotide changes after the passages. These results showed that: (1) passages of CDVs in Vero-DST cells induced amino acid changes only in the H gene, not in the P, M and L genes, unlike in a previous study with Vero cells; (2) passages did not markedly affect the growth characteristics of every viral strain. These results indicate that Vero cells expressing canine SLAM allow the isolation and passaging of CDV without major changes in viral genes.

Keywords: Adaptation; CDV; Growth; Passage; Sequence; Vero-DST cells

1. Introduction

Canine distemper virus (CDV) (genus *Morbillivirus*, family Paramyxoviridae) causes systemic disease in domestic and wild dogs, foxes and wolves. CDV infection has also caused deaths of large cats, including tigers in lions in zoos in the United States. CDV outbreaks have occurred among small carnivores, like minks, ferrets, raccoons and seals (Summers and Appel, 1994; de Swart et al., 1995; Harder and Osterhaus, 1997; Barrett, 1999). CDV may be related to some human diseases, like Paget's

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disease and multiple sclerosis (Mee and Sharpe, 1993; Fraser, 1997; Hodge and Wolfson, 1997). The CDV genome has 15,690 nucleotides and contains six non-overlapping genes encoding nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment (H) and large (L) proteins (Barrett, 1985, 1999; Bellini et al., 1986; Curran et al., 1991; Sidhu et al., 1993; McIlhatton et al., 1997). The P gene encodes the C and V non-structural proteins (Lamb and Kolakofsky, 1996; Barrett, 1999).

Adaptation of CDVs to Vero cells causes loss of pathogenicity (Hamburger et al., 1991) and changes in growth characteristics or cytopathic effect (CPE) (Plattet et al., 2005). Vero cell adaptation of wild-type CDV requires no amino acid changes in the H protein (Nielsen et al., 2003). A comparison of nucleotides of Vero-adapted A75/17-V strain passaged in Vero cells for 17 times with nucleotides of wild-type strain A75/17 showed only seven nucleotide changes: at nucleotide positions 2275, 2381 and 2399 in the P/V/C gene, at nucleotide positions 3610, 4422 and 4434 in the M gene and at nucleotide position 14,940 in the L gene (Plattet et al., 2004). Wild-type and Vero-adapted strains showed no nucleotide differences in the H gene.

Vero cells expressing canine signaling lymphocyte activation molecules (dogSLAM) have been developed to isolate CDVs efficiently from clinical samples (Seki et al., 2003). Previously, we showed that CDV strains grow in Vero cells expressing dog SLAM (Vero-DST) with an apparent CPE of cell-fusion and that they behave differently in normal Vero cells (Lan et al., 2005a). Vero-DST cells were used not only for isolation of CDV from clinical samples, but also for titration and research of biological properties of newly isolated CDVs. Field CDV strains isolated in Vero-DST cells do not change nucleotide sequences of P and H genes and keep virulence in dogs (Lan et al., 2005c) compared with samples of diseased animals. However, the effects of passages in Vero-DST cells on growth characteristics and genetic changes of CDVs isolated through Vero-DST cells were not tested. Thus, in this study, we investigated the growth characters in Vero-DST cells and genetic changes in H, P, M, and L genes of new isolates and a Vero cell-adapted Onderstepoort strain of canine distemper viruses after 1 and 20 passages in Vero-DST cells.

2. Materials and methods

2.1. Cell line

Vero-DST cells were used as previously described (Seki et al., 2003). Briefly, they were cultivated in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum, 100 unit/ml of penicillin and 100 μg/ml of streptomycin and 0.4 mg/ml of geneticin (G418) and were grown in a CO_2-incubator at 37 °C. H tag does not interfere with the binding of the H protein of CDV.

2.2. Viruses

The attenuated strain of Onderstepoort (Bussel and Karzon, 1965; Haig, 1948; Bolt et al., 1997) was provided by Dr. Summers (Cornell University, USA). This strain was isolated from a North American ranched fox diseased in an outbreak of canine distemper in the 1930s and has been passaged serially in ferrets 57 times, in chicken embryos 208 times, chicken embryo cell culture 62–66 times, ferret kidney cells 13–14 times, in Vero cells more than 100 times and in Vero-DST cells once. CDV strains 007Lm, Ac96I and S124C were used in this study as wild-type strains, which were isolated from the lymph node, large intestine and cerebellum of autopsied dogs. All strains passaged once in Vero-DST are referred to as original viruses. Viruses at a multiplicity of infection of 0.01 were used for every passage. After observing the CPE, viruses were harvested, were stored at −80 °C and were used for next passage. Viruses serially passaged at 20 times the passage of the original strains in Vero-DST are called 20p strains in this study.

2.3. Growth kinetics

A monolayer of Vero-DST cells in 48-well plates was infected with viruses at a multiplicity of infection of 0.001 and was incubated at 37 °C in 5% CO_2. The infected cells and supernatants were collected at 12, 24, 36, 48 and 72 h after inoculation, at which times, titers of released viruses into the supernatant and viruses inside the cells (cell-associated viruses) were made by using a 50% tissue culture infectious dose ($TCID_{50}$) assay and the growth profiles of viruses were recorded (Lan et al., 2005b).