Pathological and Molecular Biological Studies on Canine Distemper

CHAPTER VI

Relationship Between Growth Behavior in Vero Cells and Molecular Characteristics of Recently Isolated Canine Distemper Viruses

Summary

Ten recent isolates of canine distemper virus (CDV) strains were classified according to the growth ability and development of a syncytium cytopathic effects (CPE) in Vero cells. Strains P94S, Ac96I, S124C, MD231, MS232, MAS5 and 095Cr classified as Type 1 hardly not only grew, but also developed a syncytium CPE in Vero cells. Strains 007Lm, 009L and 011C classified as Type 2 grew well but failed to develop a syncytium CPE in Vero cells. A comparison of the sequences and phylogenetic trees of H and P genes showed that all Type 1 strains belonged to Asia 1 group and all Type 2 strains belonged to Asia 2 group. These results showed that the growth behavior in Vero cells had the relationship with the molecular characteristics of recently isolated CDV. To understand this relationship would be useful and practical for classifying and identifying the growth properties in Vero cells and molecular characteristics of CDV strains.

Key words: CDV, classification, phylogenetic analysis, sequence and Vero cells.

Introduction

Canine distemper virus (CDV) is a pantropic, negative sense, single-stranded RNA morbillivirus, of the family Paramyxoviridae (Pringle, 1999). The CDV genome has 15,960 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). The P gene encodes C and V non-structural proteins (Barrett, 1999).

The hemagglutinin (H) and fusion (F) proteins of CDV are major target antigens for the host immune system. The highest antigenic variation is in the H protein (Blixenkrone-Moller et al., 1992). The most appropriate protein to be monitored for detection of genomic changes of CDV is the H protein (Orvell et al., 1990; Haas et al., 1997). The P gene is most conserved within clades of a given CDV lineage (Carpenter et al., 1998).

Previously, we showed strains MD77, Onderstepoort and KDK1 of CDV can grow, and a syncytium cytopathic effect (CPE) in Vero cells expressing canine signaling lymphocytes activation molecule (SLAM; CD150) with a tag (Vero.Dog SLAM tag; Vero-DST cells) but behave differently against Vero cells without SLAM (Lan et al., 2005a). Vero cells are useful to classify various CDV strains after propagation in Vero-DST cells. CDV strains MD77, Onderstepoort and KDK1 show different phenotypic behavior against Vero cells: 1) KDK1 hardly grows or form a syncytium, 2) MD77 grows well but forms no syncytium or indistinguishable rounding CPE and 3) Onderstepoort grows and shows a syncytium CPE (Lan et al., 2005a). However, in the previous study, the growth profiles of only three CDV strains were tested in Vero cells. The sequences of CDV strain MD77 and the inferential relationship between the growth behavior in Vero cells and molecular characteristics of recent CDV isolates had not been researched. Therefore, in this study, to clarify and complete the idea that Vero cells were useful to classify various CDV strains (Lan et al., 2005a), we investigated the sequences and growth properties in Vero cells of 10 CDV recently discovered isolates in Japan. Since then, the inferential relationship between growth and molecular characteristics of CDV was identified.

Materials and methods

Viruses: Table 9 lists 10 new isolates of CDV used in this study. Strains MD231 from a severe diseased dog lung that was moribund due to pneumonia, MS232 from the lung of a dog that died from CD, MAS5 from a swab of a dog anus with little clinical signs and 095Cr from a dog cerebrum were newly isolated by using the virus isolation methods as described previously (Lan et al., 2005b). Briefly, fresh samples were homogenized and were sonicated in 5 ml of DMEM. After the mixtures were clear, the suspensions were inoculated into monolayers of Vero-DST cells in 24-well plates and were incubated in 5% CO_2 at 37^0C. CPE was checked by using immunocytochemistry with CDV N-protein.

Results

Growth properties of CDV in Vero cells: According to the classification of CDV phenotypes in Vero cells by Lan et al. (2005a), the type names of viruses were: Type 1, the virus hardly grows and show no syncytium formation; Type 2, the virus grows well but shows no syncytium formation or indistinguishable rounding CPE; Type 3, the virus grows and shows a syncytium CPE. CDV strains 007Lm, 009L and 011C no syncytium CPE, but many CDV antigens were in single cells infected with those strains (Table 10; Fig. 15). The number of CDV positive single cells increased gradually from 24 to 72 hpi. These results indicated that strains 007Lm, 009L and 011C grew well in Vero cells and were classified as Type 2. Cells infected with CDV strains P94S, Ac96I, S124C, MD231, MS232, MAS5 and 095Cr developed no syncytia during the observation (Table 10). At 24 hpi, very small numbers of or no viral antigens were in the single infected cells infected with these strains. From 36 to 96 hpi, CDV-positive cells disappeared in all seven strains infecting Vero cells. This implied that strains P94S, Ac96I, S124C, MD231,

MS232, MAS5 and 095Cr failed to grow efficiently and develop syncytium CPE in Vero cells and are classified as Type 1. No strains of Type 3 were among these strains. In contrast, all CDV isolates grew and developed syncytium CPE in Vero-DST cells.

Sequence and phylogenetic analyses: Phylogenetic trees for sequence and phylogenetic analyses of P and H genes of 10 new isolates of CDV were constructed from the nucleotide sequence of a 390 bp fragment of the P gene (Fig.16) and a sequence of 607 amino acids of the H gene (Fig. 17) of new isolates. Ten recent CDV isolates in Japan were divided into two groups: Asia 1 and Asia 2. Strains P94S, Ac96I, S124C, MD231, MS232, MAS5 and 095Cr classified as Type 1 joined to the clade of Asia 1 group. Strains 007Lm, 009L and 011C classified as Type 2 belonged to Asia 2 group. Sequence analysis of H genes of Types 1 and 2 of new isolates showed that one N-glycosylation site at amino acid 584-586 was absent in the H gene of Type 2 (Fig.18). The change in charged amino acids of H genes of Asia 1 and Asia 2 groups, and strains MD77 and Onderstepoort were compared (Table11). At amino acid positions 29, 145, 186, 276, 330, 343, 401, strain Onderstepoort had different amino acid charges compared with the other strains. Asia 1 group included charged amino acids at positions 241, 313 and 415 different from the other strains.

The relationship between growth and molecular characteristics: All Type 1 CDV strains, which hardly grew and showed no syncytium CPE in Vero cells, belonged to Asia 1 group, while the CDV strains, which belonged to Asia 2 group in phylogenetic trees, could grow well but showed no syncytium formation or indistinguishable rounding CPE in Vero cells. These indicated that there was an inferential relationship between the growth properties in Vero cells and molecular characteristics of CDV strains. If the growth character of a CDV isolate is examined in Vero cells, the group in which CDV isolate belongs in phylogenetic trees basing on the sequence analysis was inferred; on the

contrary, the growth properties in Vero cells of a CDV isolate can be pointed from it's position in the phylogenetic trees.

Discussion

The present study showed that in Vero cells, CDV strains 007Lm, 009L and 011C could grow well but CDV strains P94S, Ac96I, S124C, MD231, MS232, MAS5 and 095Cr hardly grew. It is vague to understand that CDV binds to CD46, SLAM or other receptors to enter the Vero cells. The membrane cofactor protein CD46 is expressed on Vero cells as a cellular receptor for measles virus and some morbilliviruses (Naniche et al., 1992, 1993; Dorig et al., 1993; Manchester et al., 1994). CD46, a transmembrane glycoprotein of approximately 57- 67 kDa, is a member of the regulators of complement activation (RCA) superfamily of complement-binding proteins that protect host cells from autologous complementation by binding activated complement components and preventing their deposition on the host cell surfaces (Seya et al., 1986; Hourcade et al., 1989; Liszewski et al., 1991, 1992;). CD46 expression allows binding, entry and replication of laboratory strains of measles virus or some morbilliviruses (Dorig et al., 1993). The extra cellular domain of CD46 has four conserved modules called short consensus repeats (SCRs) that are typically found in RCA proteins (Liszewski et al., 1991, 1992). Mutant CD46 proteins with deletions in SCR 1 or SCR 2 cannot bind to measles virus or allow measles virus entry (Adams et al., 1991; Manchester et al., 1995). CD46 is not a receptor for all strains of measles virus or *Morbillivirus* (Yanagi et al., 1994; Lecouturier et al., 1996). No evidence for a role of CD46 as a receptor for CDV or Rinderpest virus has been found (Lamb, 1993; Stern et al., 1995; Galbraith et al., 1998). Some studies showed that envelope proteins of wild-type strains of *Morbillivirus* do not interact with CD46, but they interact with SLAM (Tatsuo et al., 2001).

Syncytium formation of CDV and measles virus requires combined activities of H and F glycoproteins (Wild et al., 1991; Cattaneo et al., 1993; Stern et al., 1995). Receptor binding of the H protein induces conformational changes of the F protein needed for the fusiogenicity. Therefore, syncytium formation may be a sign of receptor binding, and formation of syncytia in Vero cells infected with CDV isolates depends on interaction between viral H proteins and receptors on the surface of Vero cells. In this study, all recent CDV isolates did not develop a syncytium CPE in Vero cells, but they all developed syncytia in Vero-DST cells. These strains may use dog SLAM, not CD46, to bind the H protein of CDV to cellular receptors. Type 1 CDV isolates of Asia 1 group in Vero cells did not grow but the Type 2 isolates of Asia 2 group grew well in Vero cells. CDV strains that provide a ligand domain in their envelope H protein(s) applicable to the CDV receptor in Vero cells that is different from SLAM can infect Vero cells but strains without the ligand domain can not infect Vero cells (Lan et al., 2005a).

Measles virus with wild type H protein have entry into Vero cells independent of both CD46 and CDw150 (Hashimoto et al., 2002; Takeuchi et al., 2002; Andres et al., 2003). Strains MD77 and Onderstepoort bind to another receptor of CD46 (Nielsen et al., 2003), but the property of their ligand domain may slightly differ between them. Thus, Type 2 strains might bind to receptors other than SLAM and CD46. Sequence analysis of the H genes of Types 1 and 2 of recent isolates and of reference strains MD77 and Onderstepoort showed that one N-glycosylation site at amino acids 584-586 was absent in H gene of Type 2, MD77 and Ondestepoort. Only Asia 1 group did not grow in Vero cells. These results might imply that the N-glycosylation site at amino acids 584-586 is related to the growth ability of CDV strains. The absence of this N-glycosylation site may alter receptor usage and allows virus growth in Vero cells. The Onderstepoort strain had no N-glycosylation sites at amino acids 391-393, 456-458 and 603-605 in contrast

with strain MD77 and the new isolates. However, in Vero cells, only strain Onderstepoort developed a syncytium CPE (Lan et al., 2005a). N-glycosylation sites at amino acids 391-393, 456-458 and 603-605 might be related to syncytium formation. Changes in charged amino acids showed that strain Onderstepoort had a different amino acid charge from the other strains at amino acid positions 29, 145, 186, 276, 330, 343, 401 that might be related to syncytial formation. Asia 1 group contained charged amino acids at positions 241, 313 and 415, which might be related to the growth ability of CDV strains, different from the other strains. However, to understand well these points, recombinant virus should be used in future studies.

In a previous study (Lan et al., 2005a), strain MD77 grew well, but did not develop a syncytium CPE. In this study, the sequence and phylogenetic analyses of strain MD77 and new isolates showed strain MD77 joined to different lineages from the Asia 2 group but it had apparently similar behavior in Vero cells to the Asia 2 group. This might be explained by different CPE formation of strain MD77 and strains belonging to Asia 2 group. Strain MD77 might produce rounding CPE and Asia 2 strains might not produce any CPE or in contrast, strain MD77 probably produced no CPE and Asia 2 strains formed rounding CPE. Another possibility is that strain MD77 and Asia 2 group had the same character of CPE formation, but after many passages in Vero cells, the Asia 2 group would change the genome and belong to the same lineage as strain MD77. To explain this clearly, electron microscopic analysis and molecular analysis of CDV strains after many passages in Vero cells should be done.

In conclusion, in this study, an inferential relationship was identified between the biological characteristics in Vero cells and molecular analysis of Asia 1 and Asia 2 group of CDV isolates.