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The fusion protein of wild-type canine distemper virus is a major determinant of persistent infection

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Abstract

The wild-type A75/17 canine distemper virus (CDV) strain induces a persistent infection in the central nervous system but infects cell lines very inefficiently. In contrast, the genetically more distant Onderstepoort CDV vaccine strain (OP-CDV) induces extensive syncytia formation. Here, we investigated the roles of wild-type fusion (F_{WT}) and attachment (H_{WT}) proteins in Vero cells expressing, or not, the canine SLAM receptor by transfection experiments and by studying recombinants viruses expressing different combinations of wild-type and OP-CDV glycoproteins. We show that low fusogenicity is not due to a defect of the envelope proteins to reach the cell surface and that H_{WT} determines persistent infection in a receptor-dependent manner, emphasizing the role of SLAM as a potent enhancer of fusogenicity. However, importantly, F_{WT} reduced cell-to-cell fusion independently of the cell surface receptor, thus demonstrating that the fusion protein of the neurovirulent A75/17-CDV strain plays a key role in determining persistent infection.

Keywords: Canine distemper virus; Persistence; Fusogenicity; Envelope glycoproteins; SLAM

Introduction

Canine distemper virus (CDV) is a *Morbillivirus* closely related to measles virus and depending on the viral strain may cause encephalomyelitis in dogs. The wild-type virulent A75/17-CDV strain typically produces a chronic demyelinating disease associated with a persistent infection in the central nervous system (CNS) (Bollo et al., 1986; Higgins et al., 1989; Imagawa et al., 1980; Vandevelde et al., 1980). The antiviral immune response, which follows invasion of immune cells in the CNS, leads to viral clearance within the inflammatory lesions (Bollo et al., 1986). However, this viral clearance is restricted to certain lesions, and simultaneously, A75/17-CDV has been shown to further spread in astrocytes in other areas of the brain without eliciting an inflammatory response. Thus, A75/17-CDV persistent infection in the CNS was suggested to be in part determined by a defective immune response (Bollo et al., 1986; Johnson et al., 1988; Vandevelde et al., 1986). However, in primary dog brain cell culture, wild-type CDV persistence was also associated with selective spread and very little virus release when compared to the Onderstepoort (OP) CDV vaccine strain (Zurbriggen et al., 1995), suggesting an important role also for viral factors in the establishment of persistent infection.

A75/17-V is a Vero cell-adapted CDV strain producing a persistent infection characterized by a very limited cytopathic effect (CPE). The virus spreads in a cell-to-cell manner without obvious syncytium formation (Hamburger et al., 1991; Meertens et al., 2003; Plattet et al., 2004). In contrast, the Onderstepoort (OP) CDV vaccine strain, which

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has been extensively passaged in vitro, produces a pronounced CPE in many cell types accompanied by the formation of large syncitia (Appel and Gillspie, 1972; Haig, 1956; Rockborn, 1959). Therefore, the non-cytolytic A75/17-V strain represents a promising tool to study the genetic determinants underlying viral persistence.

Both cell culture-adapted A75/17-V and OP-CDV have very similar genome organization as compared to the A75/17 wild-type strain and consists of a single-stranded negative-sense RNA of 15690 nucleotides. While A75/17-V differs from the wild-type strain by only seven amino acids located in the P, V, M and L proteins (Plattet et al., 2004), OP-CDV is genetically more distant and presents about 9% amino acid differences.

The CDV envelope contains the attachment (H) and the fusion (F) glycoproteins. The H protein binds to the CDV receptor of the host cell membrane, and the F protein mediates the membrane fusion event, which allows the entry of the viral genome into the cytoplasm. The OP-CDV cytolytic strain was demonstrated to spread through cell cultures both by producing infectious extracellular particles and by lateral cell-to-cell fusion (Gassen et al., 2000; Schmid et al., 2000; von Messling et al., 2001; Zurbriggen et al., 1995). Interestingly, it was shown that the coexpression of the F and H glycoproteins was necessary and sufficient to induce cell fusion, and that the CDV H protein was the major factor determining cell tropism (Stern et al., 1995). In addition, reverse genetics technology based on the OP-CDV strain suggested that not only the CDV tropism, but also the cytopathogenicity, also termed fusogenicity, in Vero cells were mainly determined by the H protein (von Messling et al., 2001).

The observation that Vero cell adaptation of wild-type CDV does not necessarily require amino acid changes in the H protein (Nielsen et al., 2003; Plattet et al., 2004) is inconsistent with the idea that host cell specificity is determined exclusively by the H protein. Thus, the question remains as to whether the phenotype of infection observed with A75/17-V is mainly determined by the H protein, as described for OP-CDV (von Messling et al., 2001).

Fusogenicity was previously suggested to be mediated both by viral factors and by cell surface receptors (Takeuchi et al., 2002). The CDV receptor in Vero cells has not yet been identified, although the CD9 molecule was shown to influence CDV-induced cell-to-cell fusion, but not virus–cell fusion (Loffler et al., 1997; Schmid et al., 2000). In contrast, the expression of signaling lymphocytic activation molecule (SLAM; also known as CD150) in non-susceptible cell lines was reported to allow binding and entry of both wild-type and vaccine CDV strains (Seki et al., 2003; Tatsuo et al., 2001). In humans, SLAM expression is restricted to activated T and B lymphocytes, immature thymocytes (Cocks et al., 1995; Sidorenko and Clark, 1993) and mature dendritic cells (Ohgimoto et al., 2001). The SLAM cDNA of the dog has recently been isolated (Tatsuo et al., 2001) but the tissue distribution of SLAM in this species has not yet been determined.

In this study, genetic determinants of the persistent infection phenotype of A75/17-V, which exhibits identical F and H proteins compared to the wild-type strain, were characterized. Towards this end, the F and H proteins from wild-type and OP-CDV were analyzed with respect to their cell-to-cell fusion-inducing ability using a transient-expression system and a newly established A75/17-V-based reverse genetics technology (Plattet et al., 2004). The role of SLAM in fusogenicity was also evaluated. Our results showed that both the wild-type F and H proteins as well as the canine SLAM receptor act in concert to determine the phenotype of infection.

Results

Differences in fusogenicity between A75/17-V-CDV and OP-CDV

Vero and Vero-dogSLAMtag cells were infected with the two different CDV strains at an MOI of 0.001 and their respective CPE-inducing ability was examined 24 h after infection by phase contrast microscopy and immunofluorescence. Although no CPE was observed in Vero cells infected with A75/17-V (Fig. 1A), immunofluorescence analysis showed single cell staining of A75/17-V throughout the cell monolayer (Fig. 1E). In these cells, OP-CDV produced the expected pronounced level of CPE (Fig. 1B), which was also confirmed by immunofluorescence analysis (Fig. 1F).

In the presence of the canine SLAM receptor, the level of CPE of OP-CDV was even more pronounced than in SLAM-negative Vero cells (Figs. 1D, H). In contrast, A75/17-V produced only few and small syncytia (Figs. 1C, G). These results demonstrate that viral proteins are the major determinants of cell-to-cell fusion but that SLAM also acts as a fusion-enhancing molecule. Since both morbillivirus envelope glycoproteins have been described as important fusion modulators, we wished to dissect the role of the A75/17-V F and H proteins in determining the low CPE-inducing phenotype of infection.

Both viral surface glycoproteins and SLAM modulate cell-to-cell fusion

As wild-type A75/17 and A75/17-V present identical F and H proteins (Plattet et al., 2004), they were named in this study wild-type F (F_{WT}) and wild-type H (H_{WT}). In order to investigate the role of the two surface glycoproteins of OP-CDV and A75/17-V CDV in producing a cytolytic versus persistent infection, we first carried out a comparative analysis of the predicted amino acid sequences between F_{WT} and F_{OP} as well as of H_{WT} and H_{OP}. The sequence of F_{WT} has already been published (Cherpillod et