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The V domain of dog PVRL4 (nectin-4) mediates canine distemper virus entry and virus cell-to-cell spread

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ABSTRACT

The entry of canine distemper virus (CDV) is a multistep process that involves the attachment of CDV hemagglutinin (H) to its cellular receptor, followed by fusion between virus and cell membranes. Our laboratory recently identified PVRL4 (nectin-4) to be the epithelial receptor for measles and canine distemper viruses. In this study, we demonstrate that the V domain of PVRL4 is critical for CDV entry and virus cell-to-cell spread. Furthermore, four key amino acid residues within the V domain of dog PVRL4 and two within the CDV hemagglutinin were shown to be essential for receptor-mediated virus entry.

Introduction

In the order *Mononegavirales*, measles virus (MeV) and canine distemper virus (CDV) belong to the genus *Morbillivirus* in the family *Paramyxoviridae* (Diallo, 1990; Rima and Duprex, 2006). Although MeV mainly causes a moderate disease in humans and certain non-human primates (de Vries et al., 2010), MeV-induced suppression of the immune system can result in an increased susceptibility to lethal secondary infections (Avota et al., 2010; Beckford et al., 1985). On the other hand, distemper viruses affect a wide range of canine and marine mammals causing mild discomfort to serious, neurological disease (Appel, 1970; Barrett, 1999; Deem et al., 2000; Harder and Osterhaus, 1997; Martella et al., 2008). Interestingly, CDV infections also occur in wild and captive feline populations (Daoust et al., 2009; Furtado et al., 2013; Nagao et al., 2012; Nava et al., 2008; Quigley et al., 2010; Terio and Craft, 2013).

Morbilliviruses infect a broad range of immune and epithelial cells (Sato et al., 2012). Specific interactions between cellular receptors and the viral hemagglutinin protein (H) facilitate virus entry and spread in host cells (Sato et al., 2012) by inducing virus–cell and cell–cell membrane fusion in cooperation with the fusion protein (F) (Baker et al., 1999; Colman and Lawrence, 2003; Hernandez et al., 1996; Takimoto et al., 2002). To date, two cellular receptors have been identified for CDV: (i) the morbillivirus receptor SLAM (signaling lymphocyte activation molecule), which is expressed on the surface of activated T- and B-lymphocytes, macrophages, and dendritic cells (Cocks et al., 1995; Hsu et al., 2001; Sidorenko and Clark, 1993; Tatsuo et al., 2000, 2001; von Messling et al., 2004, 2005) and (ii) PVRL4 (poliovirus-receptor-like-4; also known as nectin-4), the most recently identified receptor for morbilliviruses, which is found on epithelial cells (Birch et al., 2013; Muhlebach et al., 2011; Noyce et al., 2011; Noyce et al., 2013; Noyce and Richardson, 2012; Pratakpiriya et al., 2012). Interestingly, CDV has the intrinsic ability to use both human and dog PVRL4, without requiring adaptive mutations in H (Bieringer

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et al., 2013; Noyce et al., 2013; Sakai et al., 2013). CDV can also readily adapt to use the human SLAM receptor through a single mutation in its hemagglutinin gene (Bieringer et al., 2013; Sakai et al., 2013), suggesting that CDV has the potential to emerge as a novel human pathogen (Sakai et al., 2013).

PVRL4 is a member of the nectin family of adhesion molecules that belong to the immunoglobulin (Ig) superfamily, comprised of nectin-1, -2, -3 and -4, as well as the prototypic poliovirus receptor (PVR) (Kurita et al., 2011; Takai et al., 2008a, 2008b). Nectins are normally localized to the adherens junctions, and are components of the cell–cell adhesion system where they play a key role in limiting cell movement, facilitating intercellular communication, and regulating proliferation (Kurita et al., 2011; Takai et al., 2008a, 2008b). PVRL4 is a type I transmembrane glycoprotein with three Ig-like ectodomains (V, and two C2 domains), a transmembrane region, and a cytoplasmic tail (Fabre et al., 2002; Takai et al., 2008b). V domains are involved in homotypic and heterotypic interactions between the nectins, while C2 domains enhance the affinity of these interactions (Fabre et al., 2002; Satoh-Horikawa et al., 2000; Takai et al., 2008a).

Given that the V domain of human PVRL4 was previously shown to bind strongly to MeV and facilitate virus attachment and entry into cells (Muhlebach et al., 2011) and that the 3-D structure of V complexed to the H protein of MeV was reported (Zhang et al., 2013), we sought to functionally characterize key amino acid residues within the V domain of dog PVRL4 that are important for CDV entry. Molecular studies evaluating the interactions of the V domain during infection or membrane fusion experiments have not be been previously reported. We took advantage of the fact that Vero cells are not susceptible to CDV infection due to the absence of PVRL4 expression. However, there are low amounts of PVRL1 in Vero cells that cannot function as a receptor (Muhlebach et al., 2011; Noyce et al., 2011). Chimeric molecules were engineered in one polypeptide where the V domain of dog PVRL4 was replaced with the V domain of human PVRL1, and in another polypeptide where the V domain of human PVRL1 was replaced with the V domain of dog PVRL4. Key residues within the V domain of dog PVRL4 and the CDV hemagglutinin that dictate receptor-mediated virus entry were identified by mutational analysis.

Results

Chimeric dog PVRL4 and PVRL1 protein receptors localize to the cell surface

Based upon the alignment of dog and human nectin sequences in the V domain showing low sequence homology (Fig. 1A), chimeric dog PVRL4 and human PVRL1 molecules were created by replacing the V domain of dog PVRL4 with the V domain from human PVRL1 (dog.PVRL4/V^{hPVRL1}) and the V domain of human PVRL1 with the V domain of dog PVRL4 (hPVRL1/$V^{dog.PVRL4}$) (Fig. 1B). Chimeric molecules were expressed in Vero cells. Flow cytometry analysis revealed that dog.PVRL4, dog.PVRL4/V^{hPVRL1} and hPVRL1/$V^{dog.PVRL4}$ proteins localized to the plasma membrane, although the PVRL4 staining was absent in the dog.PVRL4/V^{hPVRL1} cells, since the antibodies only recognize the V domain peptide (Fig. 2). Vero and Vero.dog.PVRL4 cell lines expressed comparable low levels of endogenous PVRL1. However, there were increased levels of PVRL1 cell surface fluorescence on the Vero.dog.PVRL4/V^{hPVRL1} and Vero.hPVRL1/$V^{dog.PVRL4}$ expressing cell lines. Together, these data suggest that the majority of the Vero.dog.PVRL4/V^{hPVRL1} and Vero.hPVRL1/$V^{dog.PVRL4}$ cells express the chimeric dog PVRL4 and chimeric human PVRL1 proteins at the cell surface membrane, respectively. In addition, the V domain of both PVRL1 and PVRL4 contain important epitopes that are recognized by their respective antibodies.

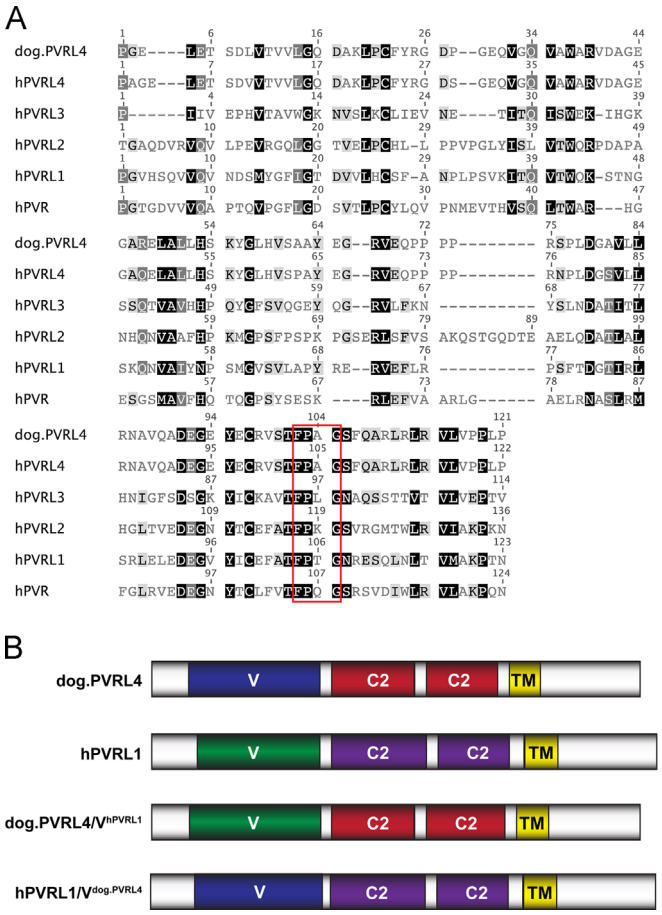

Fig. 1. Amino acid sequences of all human PVRL molecules and dog PVRL4 in the V domain. (A) Sequence alignment of the V domain from human PVRL1 to PVRL4, human PVR, and dog PVRL4. Residues having similarity are shaded (dark shading represents identical amino acid residues; dark gray shading represents residues with 80–100% identity, light gray shading represents residues with 60–80% identity, no shading represents residues with less than 60% identity). The consensus FPxG amino acid sequence is boxed in red. Pairwise protein alignments were performed using Geneious sequence alignment software (Drumond et al., 2010). (B) Schematic diagrams of the wild-type and chimeric dog PVRL4 and human PVRL1 proteins (dog.PVRL4, dog.PVRL4/V^{hPVRL1}, hPVRL1 and hPVRL1/$V^{dog.PVRL4}$). The V and C2 domains, along with the transmembrane domain (TM) are indicated.

The V domain of dog PVRL4 is essential for CDV 5804PeH infection

To address the contribution of the V domain from dog PVRL4 in CDV entry, the Vero stable cell lines that expressed the chimeric receptors were infected with CDV 5804PeH (von Messling et al., 2004) and monitored for syncytia formation and virus production (Fig. 3). Syncytia formation was observed in the Vero.dog.PVRL4 and Vero.hPVRL1/$V^{dog.PVRL4}$ cell lines but not in cells expressing dog.PVRL4/V^{hPVRL1} (Fig. 3A). Interestingly, smaller syncytia were detected in Vero.hPVRL1/$V^{dog.PVRL4}$ cells infected with CDV, suggesting that the V domain may not be solely responsible for efficient viral infection. At three days post-infection (dpi), CDV 5804PeH titers were higher in the Vero cells expressing dog.PVRL4 and hPVRL1/$V^{dog.PVRL4}$ proteins compared to the Vero and the Vero.dog.PVRL4/V^{hPVRL1} cell lines (Fig. 3B). We previously observed low levels of CDV production without syncytia formation in Vero cells lacking dog nectin-4 (Noyce et al., 2013). These background infections produced titers 2–3 logs below that of cells expressing SLAM or PVRL4. We postulate that the virus can enter these cells by an F protein-independent mechanism such as macropinocytosis and may or may not be relevant during in vivo infections. Taken together, the preceding data suggest that the V