Virology

NOTE

Title:
Function of feline signaling lymphocyte activation molecule as a receptor of canine distemper virus

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Running head:
FELINE SLAM AS A RECEPTOR OF CDV

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ABSTRACT

Morbilliviruses use signaling lymphocyte activation molecule (SLAM) as a receptor for their entry to cells. In this study, a complete gene encoding SLAM of a domestic cat was identified. The identity of feline SLAM with canine one was 73%, and feline SLAM formed the same cluster with those of carnivores. Furthermore, feline cell expressing feline SLAM supported growth of canine distemper virus (CDV) as well as that expressing canine one. These results indicated that feline SLAM can function as a receptor for morbilliviruses, and our established feline cells that express feline SLAM might be useful for analysis of morbilliviruses originated from felids.

KEY WORDS: canine distemper virus, feline, signaling lymphocyte activation molecule
Canine distemper virus (CDV) infects dogs and various species of carnivores. It causes a fatal disease which involves the development of pyrexia, anorexia, nasal discharge, conjunctivitis, diarrhea, leukopenia and encephalitis [2]. Recently, CDV was recognized as a cause of morbidity and mortality in large felids, such as lions (Panthera leo) in Tanzania’s Serengeti National Park [18]. It has also been found in lions, tigers (Panthera tigris), leopards (Panthera pardus) and a jaguar (Panthera onca) in North American zoos in 1991-1992 [4], one Siberian tiger (Panthera tigris altaica) in Pokrovka, Russia, in 2004 [17] and tigers in a Japanese zoo in 2009 [14]. Although CDV causes severe disease in various species, including large felids, it is still unclear whether it can cause disease in domestic cats. In a serological survey of domestic cats, some cats possessed virus-neutralizing antibody to CDV [9]. Furthermore, a feline morbillivirus (FmoPV) was isolated from domestic cats in Hong Kong and might be associated with tubulointerstitial nephritis [21]. Therefore, this suggests that domestic cats can also be infected with morbilliviruses, including CDV.

Signaling lymphocyte activation molecule (SLAM) is expressed on activated T and B cells, immature thymocytes, memory T cells [5, 7, 19], activated monocytes [12] and mature dendritic cells [6, 11] and serves as a common receptor for morbillivirus [1, 20]. Canine SLAM expressed on cells of the immune system is also a major cellular
receptor for CDV infection [20]. However, a complete gene encoding SLAM of felids including domestic cats has not been identified, and it is unclear whether it functions as a cellular receptor for morbilliviruses, including CDV and FmoPV.

In this study, we identified the complete gene encoding feline SLAM and established feline SLAM-expressing cells to assess whether feline SLAM functions as a receptor for CDV.

To amplify the gene encoding feline SLAM, two primers, feline SLAM ATG-1F (5’-TTC TCT TTC TTC AGT GGC TG-3’) and feline SLAM TGA-R (5’-TCC CTT CGC TGA GTC TCT G-3’), were designed based on the portion of the cat genome homologous to the gene encoding canine SLAM. A feline thymus cDNA library of a domestic cat (Bastard) was prepared using a cDNA Cycle® Kit according to the manual (Invitrogen, Carlesbad, CA, U.S.A.). The gene encoding feline SLAM was amplified by Takara Ex-Taq kit (Takara, Otsu, Japan). The reaction was carried out with the following temperature profile: 94°C for 2 min, 40 cycles consisting of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min and final extension at 72°C for 10 min. The amplified products were purified by Minelute PCR Purification kit (QIAGEN, Hilden, Germany). Sequencing reactions were performed using six primers, feline SLAM ATG-1F, feline SLAM TGA-R, feline SLAM F
(5’-ATG AAC AGG TCT CCA CTC CG-3’), feline SLAM R (5’-CTG GAC TTG GGC ATA GAT CG-3’), feline SLAM F1 (5’-TCA TTG GTG TCA TCT TGA CG-3’), and feline SLAM R1 (5’-TGG GTC CAG TTC AAC ACC TT-3’) and a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, U.S.A.). The reaction was carried out with the following temperature profile: at 96°C for 1 min and 25 cycles consisting of 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min and nucleotide sequences were analyzed by the Center of Gene Research at Yamaguchi University. The nucleotide sequence was deposited in the DNA data bank of Japan (DDBJ) as accession number AB771742.

Feline SLAM consisted of 1,014 bp encoding 337 amino acids. There was 73% identity between the predicted amino acid sequences and canine SLAM. Feline SLAM differed from canine SLAM at five (66, 67, 72, 74 and 82) of the twenty-one amino acid residues at the SLAM interface which are involved in regulating the binding and specificity of morbillivirus [16]. There was 78% identity between feline SLAM and the pinniped SLAM of walrus (Odobenus rosmarus) and seals (Phoca larga). Phylogenetic analysis showed that feline SLAM was closely related to those of various carnivores (Fig. 1).

The gene encoding feline SLAM without the signal peptide and transmembrane
region was amplified from feline thymus cDNA by PCR with the primers, pDis-Bgl-feline SLAM F (5’-TCA GAT CTG AGA GCT TGA TGA CTT GTC AA-3’)

and pDis-Sal-feline SLAM R (5’-TCG TCG ACG GGT CTT AGT TCT GGA GGA T-3’). In order to amplify the corresponding canine gene, the gene encoding canine SLAM without the signal peptide and transmembrane region was amplified from pDisplay/cSLAM [15] with the primers, canineSLAM-95F (5’-CCA GAT CTG AGA GCT TGA TGA ATT GCC C-3’) and CDV724R (5’-TCG TCG ACT CTT GGC ACC GAA GAC TCT G-3’). PCR products were digested with restriction endonucleases BglII and SalI and cloned into BglII and SalI sites of the expression plasmid pDisplay (Invitrogen), which has genes for a signal peptide of mouse immunoglobulin kappa-chain V-J2-C, a hemagglutinin A (HA) epitope tag upstream of multiple cloning sites and neomycin-resistance. These expression plasmids were named pDisplay/fSLAM(PDGFR) and pDisplay/cSLAM(PDGFR), respectively.

CRFK cells originating from feline kidney (ATCC Number: CCL-94) were transfected with pDisplay/fSLAM(PDGFR) using Lipofectamine™ LTX and Plus™ Reagent (Invitrogen) and selected by the addition of 800 μg/ml of G418. The expression plasmid pDisplay/cSLAM(PDGFR) and an empty plasmid pDisplay were also transfected into CRFK cells. After selection with G418, SLAM expression was
confirmed by flow cytometric analysis using an anti-HA tagged monoclonal antibody (clone HA-7; SIGMA-ALDRICH, Tokyo, Japan) or normal mouse IgG1 (R&D systems, Minneapolis, MN, U.S.A.) as a negative control. Anti-normal mouse IgG labeled with ALEXA Fluor 488 (Invitrogen) was used as a secondary antibody. The results showed that feline and canine SLAM were expressed equally on the surface of CRFK cells (47.23 and 47.16%, respectively) (Fig. 2A, B). These cells were named CRFK/cSLAM(PDGFR), CRFK/fSLAM(PDGFR) and CRFK/pDisplay. For further experiments, these cells were passaged less than three times.

Three CDV strains were used in this study. KDK-1 (genotype Asia-1) was isolated from a diseased dog in Japan in 1991 using Vero cells [13] and propagated in A72/cSLAM cells [15] after six passages in Vero cells. KochiO1A (genotype Asia-1) was isolated from an oral swab of a dead masked palm civet in Kochi Prefecture in 2008 (manuscript in preparation) and was only propagated in A72/cSLAM cells. These field isolates were not plaque-purified and were propagated less than six times in A72/cSLAM cells. The Onderstepoort vaccine strain (genotype America-1) was only propagated in Vero cells. Viral titers were determined by plaque assay using CRFK/cSLAM [15] for KDK-1 and KochiO1A, and Vero cells (JCRB Number; JCRB9013) for Onderstepoort [10].
To analyze the function of these expressed SLAMs as receptors for CDV, CRFK/fSLAM(PDGFR), CRFK/cSLAM(PDGFR) cells and CRFK/pDisplay were infected with KDK-1, KochiO1A or Onderstepoort at a multiplicity of infection (MOI) of 0.002, and cytopathic effect was observed. KDK-1 and KochiO1A produced large syncytia in both CRFK/fSLAM(PDGFR) cells (Fig.2 D, G) and CRFK/cSLAM(PDGFR) cells (Fig.2 E, H), but not in CRFK/pDisplay cells (Fig.2 F, I). Onderstepoort produced syncytia in all cells (Fig.2 J-K).

CDV growth was compared in cells which were seeded on 6 well plates (5.0×10^5 cells per well) and infected with KDK-1, KochiO1A and Onderstepoort at an MOI of 0.002. After 1 hour adsorption, the inoculum was removed, the cells were washed twice with DMEM without FCS, and then 5 ml of DMEM containing 10% FCS was added. The supernatants were collected every 24 hr, and virus titers were determined by plaque assay. The growth kinetics showed that KDK-1 and KochiO1A efficiently replicated in both CRFK/cSLAM(PDGFR) and CRFK/fSLAM(PDGFR) cells, but not in CRFK/pDisplay cells (Fig.3). The highest viral titers of KDK-1 were 8.8×10^5 in CRFK/fSLAM(PDGFR), 9.3×10^5 in CRFK/cSLAM(PDGFR) and 1.9×10^4 PFU/well in CRFK/pDisplay cells at 96 hr postinfection. The highest titers of KochiO1A were 6.8×10^3 in CRFK/fSLAM(PDGFR) cells at 96 hr postinfection,
1.9×10⁴ in CRFK/cSLAM(PDGFR) cells at 96 hr postinfection and 2.5×10¹ PFU/well in CRFK/pDisplay cells at 72 hr postinfection. Compared to KochiO1A, KDK-1 replicated well in CRFK/pDisplay cells. Since KDK-1 initially isolated using Vero cells, the strain may also utilize alternative cellular receptor in addition to the SLAM. On the other hand, Onderstepoort replicated more efficiently in CRFK/pDisplay cells than in CRFK/fSLAM(PDGFR) or CRFK/cSLAM(PDGFR) cells. The highest viral titers of Onderstepoort were 9.8×10⁴ in CRFK/fSLAM(PDGFR) cells at 72 hr postinfection, 2.0×10⁵ in CRFK/cSLAM(PDGFR) at 72 hr postinfection and 1.8×10⁶ PFU/well, in CRFK/pDisplay cells at 96 hr postinfection. Similar tendency in intracellular virus growth was observed (data not shown).

In this study, the complete gene encoding feline SLAM was identified and found to have higher homology with SLAM of other carnivores which are susceptible to CDV infection rather than with SLAM of other animals susceptible to infection with other morbilliviruses such as measles virus, rinderpest virus and peste-des-petits ruminant virus. In addition, our established cell line, CRFK/fSLAM, supported replication of KDK-1 and KochiO1A as well as CRFK/cSLAM, indicating that feline SLAM, as well as canine SLAM, can serve as a receptor for CDV field strains. Furthermore, Appel et al. [3] reported CDV replication in lymphatic tissues and
macrophages of experimentally infected cats without any clinical signs. These results indicate that, in addition to canine lymphocytes, feline lymphocytes expressing SLAM might also become infected with CDV.

Fatal infection of large felids with CDV has recently been reported worldwide. In a Japanese safari-style zoo, 12 tigers developed respiratory and gastrointestinal disease, and one tiger died of a neurological disorder following CDV infection [14]. One Siberian tiger presented symptoms of neurological signs and anorexia and ultimately died in Russia [17]. Although SLAMs of large felids such as lions and tigers have not been identified, SLAM from domestic cats belonging to the same family could function as a receptor for CDV.

On the other hand, specific pathogen-free domestic cats inoculated with homogenized tissues from a Chinese leopard (Panthera pardus japonensis) that had died following natural infection with CDV did not show any clinical signs except for a transient leukopenia [8], indicating that CDV was less pathogenic in domestic cats. Our results show that CDV can use feline SLAM as a receptor. This is supported by the fact that the CDV experimentally infected cats showed a tentative viremia associated with CDV-infected PBMC [8]. Furthermore, in comparison with field strains, Onderstepoort grew well in CRFK/pDisplay cells, but not in feline or canine SLAM-expressing cells.
This result is consistent with our previous observation [15], but the reason why Onderstepoort cannot propagate well in SLAM-expressing cells remains unclear. Further experiments will be required to clarify the mechanism behind this inhibition.

In 2012, FmoPV was isolated from CRFK cells [21] following subculture over a prolonged period. On day 14 after the 8th passage, the cells displayed typical cytopathic effects of rounding, followed by detachment from the monolayer, and on day 10 after the 16th passage, syncytia were observed [21]. Although SLAM is a common receptor for morbilliviruses, including CDV, measles virus, rinderpest virus and peste-des-petits ruminant virus, it is unclear whether FmoPV uses SLAM as a receptor. However, this CRFK/fSLAM will be useful for isolation of morbilliviruses, including CDV, from domestic cats.

In conclusion, feline SLAM was shown to serve as a receptor for CDV infection.

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**Figure captions**

**Fig. 1.** Phylogenetic relationship based on the amino acid sequence of SLAM. Feline SLAM is shown in bold. Phylogenetic analysis was conducted using MEGA5.

**Fig. 2.** Expression of SLAM on the cell surface and development of syncytia after CDV infection. (A-C) Flow cytometric analysis of SLAM expression on CRFK cells. CRFK cells were transfected with expression plasmids and selected with G418. The resulting cell lines were named CRFK/fSLAM(PDGFR) (A), CRFK/cSLAM(PDGFR) (B) and CRFK/pDisplay (C). Cells were first incubated with anti-HA tagged monoclonal antibody (clone HA-7) and then incubated with anti-normal mouse IgG labeled with ALEXA Fluor 488. (D-K) CPE induced by CDV infection in CRFK/fSLAM(PDGFR) (D, G, J), CRFK/cSLAM(PDGFR) (E, H, K) and CRFK/pDisplay cells (F, I, L). Cells were infected with KDK-1 (D-F), KochiO1A (G-I) or Onderstepoort (J-K) strains of CDV for 72 hr and then observed using microscopy.

**Fig. 3.** Growth kinetics of CDV in SLAM-expressing CRFK cells. KDK-1 (A), KochiO1A (B) and Onderstepoort (C) infected into CRFK/fSLAM, CRFK/cSLAM and CRFK/pDisplay cells at an MOI of 0.002. Mean values from two independent experiments are shown.
Figure 1
Figure 3

A

B

C

Virus titer (PFU)

Hours post infection

CRFK/fSLAM(PDGFR)

CRFK/cSLAM(PDGFR)

CRFK/pDisplay

0 24 48 72 96 120 144

Virus titer (PFU)

Hours post infection

0 24 48 72 96 120 144

Virus titer (PFU)

Hours post infection

0 24 48 72 96 120 144

Virus titer (PFU)

Hours post infection

CRFK/fSLAM(PDGFR)

CRFK/cSLAM(PDGFR)

CRFK/pDisplay