



Original Article

A negative search of acute canine distemper virus infection in DogSLAM transgenic C57BL/6 mice

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Abstract

Canine distemper is a highly contagious and immunosuppressive viral disease caused by canine distemper virus (CDV), an enveloped RNA virus of the family *Paramyxoviridae*. The susceptible host spectrum of CDV is broad and includes all families of the order Carnivora. To accomplish the infection, CDV requires an expression of signaling lymphocyte activation molecule (SLAM) functioning as a cellular receptor which generally presents in a variety of different lymphoid cell subpopulations, including immature thymocytes, primary B cells, activated T cells, memory T cells, macrophages and mature dendritic cells. The distribution of SLAM-presenting cells is in accordance with the lymphotropism and immunosuppression following morbillivirus infection. In the present study, the C57BL/6 mice engrafted with dog-specific SLAM sequence (DogSLAM) were used. The weanling (3-week-old) transgenic offspring C57BL/6 mice were infected with CDV Snyder Hill (CDV-SH) strain via the intranasal (n=6), intracerebral (n=6) and intraperitoneal (n=5) routes. Clinical signs, hematology, histopathology, immunohistochemistry, virus isolation and RT-PCR were observed for two weeks post infection. Results showed that CDV-SH-inoculated transgenic mice displayed mild-to-moderate congestion of various organs (brain, lung, spleen, kidney, lymph node, and adrenal gland). By means of immunohistochemistry, virus isolation and RT-PCR, CDV could not be detected. The evidence of CDV infection in this study could not be demonstrated in acute phase. Even though the transgenic mouse is not a suitable animal model for CDV, or a longer incubation period is prerequisite, it needs to be clarified in a future study.

Keywords: C57BL/6 mice, Canine distemper virus, SLAM, Snyder Hill strain

1. Introduction

Canine distemper is a highly contagious and immunosuppressive disease caused by canine distemper virus (CDV), a morbillivirus of family *Paramyxoviridae*. The susceptible host spectrum is broad including all families of the order

Carnivora; the *Canidae* (dogs, foxes, dingos), *Felidae* (cats), *Mustelidae* (ferrets, minks, badgers, weasels) and *Proconidae* (raccoons) (Baumgärtner *et al.*, 2003; Wohlsein *et al.*, 2007; Beineke *et al.*, 2009). In addition, several cell lines and primary cells from different species and tissues have been used for CDV isolation and infection which mimic the *in vivo* situation (Appel and Jones, 1967; Baumgartner *et al.*, 1987; von Messling *et al.*, 2004; Puff *et al.*, 2009). The tropism of morbillivirus-infectable cells has been correlated to the presence of a well known cellular receptor the so-called signaling lymphocyte activation molecule (SLAM, CD150).

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SLAM, a membrane glycoprotein, is expressed in a variety of lymphoid cell subpopulations, which in accordance with the lymphotropism and immunosuppression following morbillivirus infection (Tatsuo *et al.*, 2001; Tatsuo and Yanagi, 2002; Wenzlow *et al.*, 2007; Schneider-Schaulies and Schneider-Schaulies, 2008).

Currently, transgenic mice are widely used in biomedical research on cancer and neurodegenerative disorders with increasing evidence that genetic host factors play a key role in susceptibility (Burgermeister *et al.*, 2000; Jucker *et al.*, 2000; Guk *et al.*, 2005; Gordon and Bosland, 2009). In humans, a devastating inflammatory demyelinating and neurodegenerative diseases of the central nervous system (CNS) is multiple sclerosis (MS), which commonly affects young adults (Franklin and French-Constant, 2008; Schreiner *et al.*, 2009). Although the pathogenesis of demyelination in MS has been intensively studied, the underlying mechanisms are still poorly understood. Therefore, naturally-occurring and experimentally-induced demyelinating animal models including canine distemper virus (CDV)-infected dogs and Theiler's murine encephalomyelitis virus (TMEV)-induced mice, respectively, are considered promising tools for investigating the pathogenesis of MS (Beineke *et al.*, 2009; Ulrich *et al.*, 2009).

Various strains of laboratory mice, besides dogs and ferrets, have been employed as animal models for studying the pathogenesis of CDV including Swiss albino mice, ddY mice or SJL mice (Lyons *et al.*, 1980; Bernard *et al.*, 1983; Hirayama *et al.*, 1986). Recently, CD150 (SLAM) transgenic C57BL/6 mice have been generated and have displayed a high susceptibility to measles virus (MV), a closely-related morbillivirus to CDV (Welstead *et al.*, 2005; Sellin *et al.*, 2006). Studies using such transgenic models have significantly contributed to the understanding of the cellular and tissue tropism of measles virus infection that may reveal a new perspective on the implications of SLAM in the neuropathogenicity of other morbilliviruses. In this present study, we used offspring DogSLAM transgenic C57BL/6 mice generated by microinjection of the pCAGDogSLAM tag plasmid into the pronuclei of the founder's fertilized oocytes. To further elucidate the susceptibility to CDV, we investigated the pathological alterations in the following 2-weeks post infection.

2. Materials and Methods

2.1 Animals

Fifty 3-month-old transgenic C57BL/6 founder mice

(25 males and 25 females) were bought from the Kumamoto University, Japan. Animals were housed in a specific pathogen free (SPF) unit and used for in-house breeding (The protocol was authorized by DNA committee, Japan). Their offspring (F1) was tested for the DogSLAM gene using polymerase chain reaction (PCR) as previously described with modifications (Grunenwald, 2003). Briefly, the tail biopsy (1-2 cm, weight ~100-500 mg) from each F1 transgenic mice was used for genomic DNA extraction by using the phenol : chloroform extraction method followed by a RNase treatment (Pearson and Stirling, 2003). The 20 μ l-PCR reaction was carried out using a 2 μ l DNA, master mixed PCR solution (Qiagen, Germany) and a pair of specific DogSLAM primers (Table 1). pCAGDogSLAMTag plasmid was used as a positive control and the products were visualized on 1.2% agarose gel.

2.2 Cells and virus

Vero DogSLAMTag (Vero DST) cells were generated by the transfection of African monkey kidney (Vero) cells with pCAGDogSLAMTag plasmid as previously described (Seki *et al.*, 2003). Cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 7% heat inactivated fetal calf serum (FCS), 0.15% sodium carbonate and 0.4 mg/ml G418 until the culture reached 80% confluence. The culture was washed twice with FCS-free DMEM and incubated with 1 ml CDV Snyder Hill (CDV-SH) strain (dilution 1 : 100; kindly provided by Dr. Summers, the Cornell University, USA). Subsequently, the infected culture was maintained under standard condition (37°C, 4% CO₂, water-saturated atmosphere) for 60 min and followed by the addition of 9 ml 10% Tryptose Phosphate Broth (TPB) and FCS-free DMEM for a 24-hr incubation period. The culture was harvested for CDV-SH stock preparation when a 90% cytopathic effect (CPE) characterized by syncytial cell formation was observed. After centrifugation (3,000 rpm, 10 min), the supernatant was transferred into a new tube and sonicated for 7 cycles composed of 30 sec on and 1 min off per cycle (Biorupture®, Japan). The virus titration was done and calculated for the 50% log₁₀ tissue culture infectious dose (TCID₅₀). The virus stock was kept at -80°C until use.

2.3 Experiment design

Twenty 3-week-old DogSLAM-transgenic F1 mice were divided into four groups and inoculated with 1x10⁵ TCID₅₀ of CDV-SH through different routes of infection. **Group I** consisted of three mock-infected control mice inocu-

Table 1. List of used primers: sequences, annealing temperatures and the length of amplicons

Genes	Forward primers	Reverse primers	Ta (°C)	Amplicon (bp)
DogSLAM	5'-GGTACTGCTGCTCTGGGTTTC-3'	5'-CCTTCATTTCCCTCCTGCT-3'	50	427
CDV-NP	5'-ATGTTTATGATCACAGCGGT-3'	5'-ATTGGGTTGCACCACTTGTC-3'	50	429

lated with DMEM via intranasal (IN), intracerebral (IC) and intraperitoneal (IP) route, **Group II-IV** composed of five to six CDV-SH-infected mice inoculated via IN, IC and IP route (Table 2). Animals in each group were separately housed and supplied *ad libitum* with sterile water and food.

2.4 Clinical observation and hematology

Body weight and clinical signs of CDV-SH-infected mice including respiratory (oculonasal discharge, pneumonia), gastrointestinal (diarrhea) or nervous system (staggering gait, lack of coordination) were routinely monitored for 14 days post infection (dpi). Detection of CDV viral inclusion bodies was performed from the conjunctival swab in every three days by using Dip Quick[®] and Shorr's staining.

At 14 dpi, the infected mice were sacrificed. Heart blood was collected for hemogram evaluation which included a total red blood cell count (RBCs), hemoglobin (Hb), hematocrit (Hct), total white blood cell count (WBCs), WBC differential count and thrombocyte count. Macroscopic lesions of the nervous, respiratory and gastrointestinal systems were recorded. Tissues from these organs were collected for detection of CDV by viral isolation, histopathology, immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR).

2.5 CDV isolation

Single cell suspension was prepared from 0.5 g of the brain, lung and spleen of mock- and CDV-SH inoculated mice. Tissues were mechanically minced and sonicated in 10 ml DMEM. After centrifugation (5,000 rpm, 4°C, 5 min), the supernatant was collected and the virus was isolated using Vero DST cells. Subsequently, the cultures were maintained under standard conditions and daily observed for the CPE (see above).

2.6 Histopathology

Selected tissues (brain, spinal cord, gastrointestinal tract, lung, spleen, lymphoid tissue, thymus, kidney, urinary bladder and prepuce or vagina) were preserved in 10% neutral buffered formalin. Routine histological processes were done and the 4- μ m thick sections were stained with hematoxylin and eosin (H&E) and Luxol fast blue-cresyl echt violet stain. Samples were evaluated microscopically under light microscope.

2.7 Immunohistochemistry

For the detection of CDV antigen and DogSLAM receptor, the selected tissues (see *Histopathology*) were immunostained using a labeled streptavidin-biotin (LSAB) method. Briefly, the 4 μ m-thick sections were deparaffinized, autoclaved in phosphate buffer saline (PBS, 121°C, 5 min) for antigen retrieval and immersed in 0.3% hydrogen peroxide

(H₂O₂) in methanol (room temperature, 10 min) for non-specific endogenous peroxidase blocking. After 3 washing steps with PBS, the monoclonal anti-CDV (dilution 1:10, Monotope Virostat[®], USA) or anti-HA antibodies (dilution 1:200, Boehringer Mannheim, Germany) were applied for 60 min at 37°C and followed by the biotinylated anti-mouse IgG antibodies and envision polymer (Envision Polymer DAKO[®], Denmark) for 30 min at 37°C. The reaction was visualized with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) for 2 min and counterstained with Mayer's hematoxylin. The non-infected Vero DST cells and mock-infected mouse brain were used as negative controls whereas the infected Vero DST cells and clinically CDV-infected dog's brain were used as positive controls. The percentage of positive cells was quantitatively scored under light microscope as (-) negative, (+1) less than 10% of positive cells, (+2) 10-50% of positive cells, (+3) more than 50% of positive cells.

2.8 RNA extraction and reverse transcriptase (RT)-PCR

The viral RNA was extracted from the brain, lung, spleen, and/or peripheral blood mononuclear cells (PBMCs) by the acid phenol guanidine isothiocyanate method. Briefly, 200-300 μ l supernatant of homogenized tissues was lysed with Trizol[®] (ratio 1:1, GibcoBRL[™], USA) and incubated at -80°C overnight. Sixty μ l of phenol:chloroform:isoamyl alcohol (ratio 25:24:1) was added and mixed well. After centrifugation (13,000 rpm, 15 min, 4°C) the supernatant was transferred into a new tube and followed by addition of 0.5 l glycogen (20 mg/ml) and 150 μ l isopropyl alcohol, respectively. After 15 min incubation and centrifugation (10,000 rpm, 15 min) RNA pellet was washed with 70% ethanol and dried at room temperature for 20-30 min. Finally, the pellet was dissolved in deionized water and kept at -20°C until use.

For RT-PCR, the CDV nucleoprotein (CDV-NP) gene was amplified employing 5 μ l RNA and a one step RT-PCR kit (Invitrogen, Japan) according to the manufacturer's instructions. The PCR conditions are displayed in Table 1. CDV-SH strain was used as a positive control and PCR products were visualized as mentioned above.

2.9 Statistical analysis

Data was analyzed using the Statistical Analysis System version 9.1 software (SAS Institute, Cary, NC). The clinical hematology data was expressed as mean and standard deviation (SD) and compared using a one-way analysis of variance (ANOVA). P-values of less than 0.05 were considered to indicate statistically significant differences.

3. Results

3.1 Expression of DogSLAM gene in F1 transgenic mice

The founder transgenic mice that harbored DogSLAM gene were bred in-house. To ensure the transmission of the

DogSLAM transgene to F1 progeny, all offspring were tested using gene-specific PCR analysis from tail biopsies (Figure 1). The results showed that they displayed the specific band in 427 base pairs (bp) when compared to the pCAGDogSLAMTag plasmid used as a positive control and indicated the incorporation of DogSLAM gene in their genome.

3.2 Weight, clinical observation, hematology following CDV-SH infection

The 3-week-old F1 transgenic mice were divided into four groups and inoculated with 1×10^5 TCID₅₀ of CDV-SH through different routes of infection including mock-infection (Table 2). Mice were clinically observed and daily weighed for two weeks. The infected mice displayed no clinical signs in any inoculated group throughout the observation period when compared to the controls. In addition, they did not show significant weight loss after infection suggesting a normal appetite (Table 2). Moreover, the CDV inclusion bodies from conjunctival swab were done every 3 days using Dip Quick® and Shorr's stain. No evidence of viral inclusion bodies was observed at any time point in any inoculated group (Figure 2).

Clinical hematology was performed at 14 dpi to evaluate the status of blood cell component including erythrocytes, leukocytes and thrombocytes (Table 3). The number of erythrocytes (RBC) from intraperitoneal inoculation group was significantly different from control group (**, $p < 0.05$) but remained in the normal range. The packed cell volume or hematocrits (Hct) in all infected groups were increased when compared to control group. A decrease in thrombocyte number (platelets) was found in all groups including the control.

3.3 Macroscopic, microscopic and immunohistochemical study following infection

Mice were humanely euthanized and post-mortem examined following 14 dpi. By macroscopic investigation,

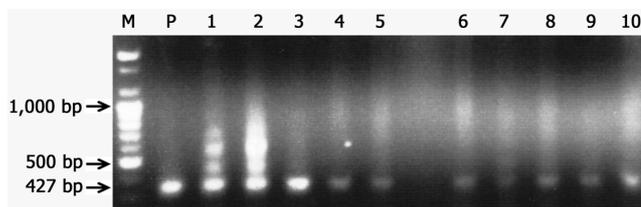


Figure 1. PCR analysis of DogSLAM gene expression from tail biopsies of F1 transgenic C57BL/6 mice indicating a specific band at 427 base pairs (bp). M: DNA ladder marker, P: positive control (pCAGDogSLAMTag plasmid), 1-10: DNA sample from tails of individual F1 transgenic mice.

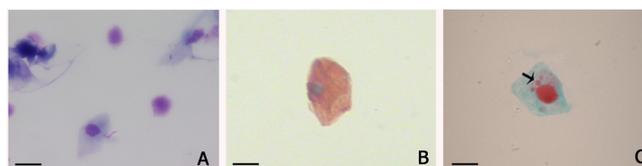


Figure 2. Detection of canine distemper virus (CDV) inclusion bodies on conjunctival epithelium every 3 days post infection. They showed no evidence of inclusion bodies throughout the observation period (A; DipQuick®, B: Shorr's staining) when compared to a positive eosinophilic intracytoplasmic inclusion bodies (arrow) derived from CDV-infected dog (C: Shorr's staining). Scale bar ~10mm.

Table 2. Body weights of the 3-week-old F1 transgenic mice in control and canine distemper virus Snyder Hill strain infected groups at 14 days post infection

Group	Weight (grams; Mean \pm Standard deviation)
I. Control	13.65 \pm 2.60 (n = 3)
II. Intranasal inoculation (IN)	14.52 \pm 3.07 (n = 6)
III. Intracerebral inoculation (IC)	15.32 \pm 3.67 (n = 6)
IV. Intraperitoneal inoculation (IP)	12.45 \pm 2.43 (n = 5)
Normal value*	12.3-13.6 \pm 1.6

*Normal value of body weight in C57BL/6 mice
(available from <http://jaxmice.jax.org/support/weight/000664.html>)

Table 3. Clinical hematology of F1 transgenic mice in control and canine distemper virus Snyder Hill (CDV-SH) strain infected groups at 14 days post infection

	Control (n = 3)	Intranasal inoculation (n = 6)	Intracerebral inoculation (n = 6)	Intraperitoneal inoculation (n = 5)	Normal value*
RBC ($10^6/\mu\text{L}$)	6.80±1.93**	8.24±0.63	7.31±1.26	8.47±0.74**	8.3 (6.5-10.1)
Hb (g/dL)	12.05±1.71	13.15±0.57	12.28±1.80	12.50±1.30	13.1 (1.1-16.1)
Hct (%)	37.70±11.30	45.01±1.86	40.25±6.68	45.70±2.81	40.4 (32.8-48.0)
MCV (fl)	55.15±1.79	54.73±2.50	55.13±1.95	54.04±1.94	49.1 (42.3-55.9)
MCH (pg)	18.85±6.43	15.96±0.70	17.2±4.18	14.74±0.62	15.9 (13.7-18.1)
MCHC (g/dL)	34.55±13.12	29.21±0.59	31.08±6.74	27.28±1.42	32.3 (29.5-35.1)
Platelet ($10^4/\mu\text{L}$)	0.02±0.01	0.14±0.31	0.04±0.05	0.15±0.09	1.16 (0.78-1.54)
WBC ($10^3/\mu\text{L}$)	4.35±1.26	4.58±1.95	3.50±1.59	3.80±1.01	6.33 (2.61-10.05)
Neutrophil (%)	0.25±0.10	0.31±0.52	0.14±0.13	0.31±0.07	1.20 (0.4-2.0)
Lymphocyte (%)	3.92±1.29	4.19±1.96	3.22±1.51	3.33±1.03	4.86 (1.27-8.44)
Monocyte (%)	0.17±0.11	0.06±0.05	0.12±0.07	0.14±0.02	0.14 (0-0.29)
Eosinophil (%)	0±0	0.008±0.02	0±0	0±0	0.08 (0-0.17)
Basophil (%)	0±0	0±0	0±0	0±0	0 (0-0.02)

Abbreviations:

red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC)

* Normal value (Pilny, 2008)

** Significant differences between control and CDV-SH-infected mice via intraperitoneal inoculation ($p < 0.05$)

there were no remarkable lesions in any infected group when compared to the control (data not shown). On the contrary, the main lesions found at the histopathological level of all groups were pulmonary hemorrhage, congestion and atelectasis (Table 4, Figure 3). The intracerebral inoculation group showed lesions both in the respiratory and lymphatic system characterized by mild to moderate pulmonary hemorrhage, congestion and mild interstitial pneumonia (Figure 3C) as well as lymph node congestion. Moreover, congestion at the corticomedullary junction of the adrenal gland and congestion of the brain were noted. In the intraperitoneal inoculation group, lesions were mainly found in the respiratory system and included pulmonary atelectasis, mild to moderate lung hemorrhage and congestion, focal vasculitis

in the lung and increased pulmonary alveolar macrophages (PAMs) (Figure 3D). Congestion and extramedullary hematopoiesis in the spleen and congestion of the kidney were also noted.

Detection of CDV antigen and SLAM receptor was done using monoclonal mouse anti-CDV antibodies and anti-HA antibodies, respectively, by the immunohistochemistry method. A panel of selected tissues was stained including the brain, spinal cord, lung, heart, spleen, lymph node, adrenal gland, liver, pancreas, stomach, small intestine, large intestine, kidney, urinary bladder and vagina/prepuce. Neither the CDV antigen nor the SLAM receptor was positive in any group when compared to the positive controls (data not shown).

Table 4. Histopathological lesions of various organs following CDV-SH infection in F1 transgenic mice

Organs/Lesions	Control (n = 3)	Intranasal inoculation (n = 6)	Intracerebral inoculation (n = 6)	Intraperitoneal inoculation (n = 5)
Brain				
· Congestion	+1 (1/3)	-	+1 (1/6)	-
Spinal cord	-	-	-	-
Lung				
· Edema	-	-	+1 (1/6)	-
· Congestion	-	-	+1 (3/6)	+1 (1/5)
· Hemorrhage	+2 (1/3)	+1 (1/6), +2 (1/6)	+2 (1/6)	+1 (1/5); +2 (1/5); +3 (1/5)
· Atelectasis	+1 (1/3)	+1 (1/6)	+1 (1/6)	+1 (2/5)
· Emphysema	-	-	-	+1 (1/5)
· Focal vasculitis	-	-	-	+1 (1/5)
· Increased PAM	-	-	-	+1 (1/5)
· Interstitial pneumonia	-	-	-	-
Heart	-	-	-	-
Spleen				
· Hemosiderosis	-	-	+1 (1/6)	+1 (2/5)
· Congestion	-	-	-	+2 (1/5)
Lymph node				
· Congestion	-	-	+2 (1/6)	-
Adrenal gland				
· Congestion at CM junction	-	-	+2 (1/6)	-
Liver	-	-	-	-
Pancreas	-	-	-	-
Stomach	-	-	-	-
Small intestine	-	-	-	-
Large intestine	-	-	-	-
Kidney				
· Congestion	-	-	+1 (1/6)	+1 (1/5)
Urinary bladder	-	-	-	-
Vagina/prepuce	-	-	-	-

Abbreviations: pulmonary alveolar macrophage (PAM), corticomedullary (CM)

Lesion score: (-) no remarkable lesion, (+1) mild degree, (+2) moderate degree, (+3) severe degree

3.4 CDV isolation and RT-PCR study following infection

Viral isolation was done using single cell suspension from the brain, lung and spleen and inoculated in Vero DST cells. Following infection, the cultures were monitored daily for CPE characterized by syncytial cell formation. All isolated tissues from the CDV-SH-infected groups displayed no CPE following infection. The cultures remained as a single cellular layer without CPE (Figure 4B). To confirm the presence of the CDV nucleoprotein (CDV-NP) gene at the molecular level, the selected tissues (brain, lung, spleen, PBMCs) from

infected F1 transgenic mice were monitored by RT-PCR. All samples were negative when compared to the CDV-SH strain used as a positive control, which represented a specific band at 429 bp (Figure 4C).

4. Discussion

The close interaction between the infectivity of morbilliviruses and their specific cellular receptor, the signaling lymphocyte activation molecule (SLAM), of target cells is well recognized. The expression profile of SLAM distributes

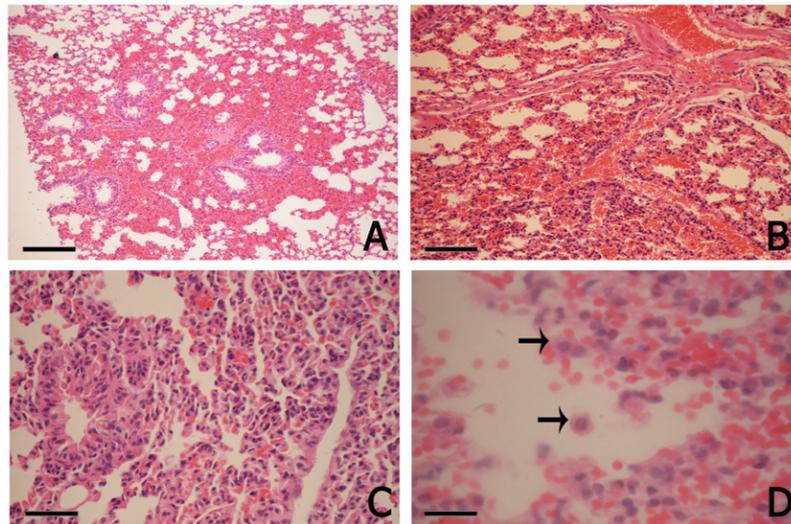


Figure 3. Histopathological findings of lungs in mock- (A) and canine distemper Snyder Hill (CDV-SH)-infected groups inoculated in different routes (B: intranasal, C: intracerebral, D: intraperitoneal route). A and B displayed moderate pulmonary hemorrhage (scale bar $\sim 50 \mu\text{m}$). C showed mild interstitial pneumonia (scale bar $\sim 25 \mu\text{m}$) while D elucidated mild degree of increased pulmonary alveolar macrophages (PAMs; arrows; scale bar $\sim 10 \mu\text{m}$).

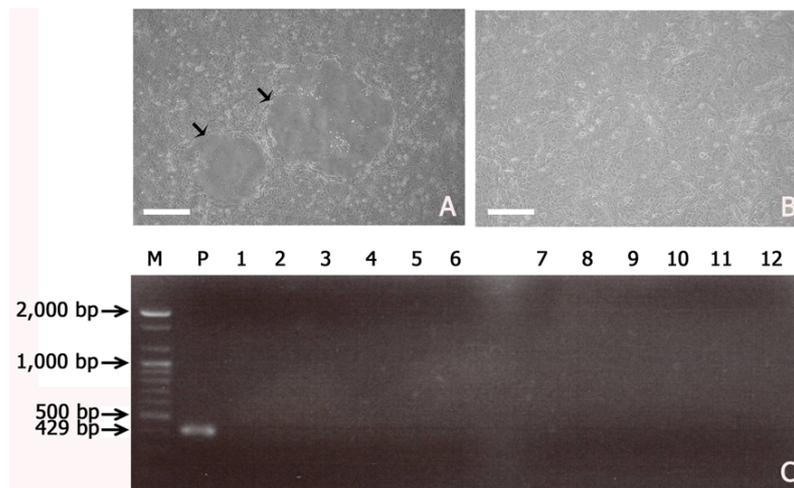


Figure 4. Detection of canine distemper virus (CDV) from infected F1 transgenic C57BL/6 mice using viral isolation on Vero DST cells (A-B) and RT-PCR (C). Tissue suspension from the brain, lung and spleen displayed no cytopathic effect (CPE, B) when compared to the CPE of a positive control characterized by syncytial cell formation of Vero DST (A, arrows, scale bar $\sim 100 \mu\text{m}$). RT-PCR analysis of CDV nucleoprotein gene expression from the brain, lung, spleen and peripheral blood mononuclear cells (PBMCs) of infected F1 transgenic mice showed all negative results. M: DNA ladder marker, P: positive control (CDV Snyder Hill strain), 1-12: RNA sample from F1 transgenic mice.

to a variety of lymphoid cell subpopulations including immature thymocytes, primary B cells, activated T cells, memory T cells, macrophages and mature dendritic cells, which is in agreement with lymphopenia and immunosuppressive status following measles virus (MV) or CDV infection (Kruse *et al.*, 2001; Ostrakhovitch and Li, 2006). However, the mechanism of morbillivirus-induced demyelination is still fragmentary due to the lack of SLAM-positive

cells in the nervous system (McQuaid and Cosby, 2002). In addition, a significant number of oligodendrocytes, myelin-forming cells of the CNS, were not decreased, even in the chronic and completely demyelinated distemper lesions, suggesting the demyelination precedes oligodendrocyte loss (Schobesberger *et al.*, 1999; Schobesberger *et al.*, 2002).

The small animal model including mice for morbillivirus investigation has been broadly used. Interestingly, the

results of using the human SLAM transgenic C57BL/6 mice infected with MV, the closely related morbillivirus to CDV, have been revealed a high susceptibility to MV infection via intranasal route. These transgenic mice have developed the neurological signs prior to establish the systemic infection by using CD11c-positive dendritic cells (Shingai *et al.*, 2005; Welstead *et al.*, 2005; Sellin *et al.*, 2006). These findings open new perspectives for the analysis of the role of SLAM in the neuropathogenicity of other morbilliviruses, like CDV, which also utilize this molecule as a viral receptor. In addition, these transgenic mice may serve as a useful model for the investigation of the pathomechanism of CDV-induced demyelination.

In this present study, the weanling (3-week-old) offspring DogSLAM transgenic C57BL/6 mice were generated and infected with the CDV Snyder Hill (CDV-SH) strain via intranasal, intracerebral and intraperitoneal routes. The inoculated transgenic mice showed no evidence of CDV-SH infection regardless of the route of virus administration which were evaluated from clinical signs, hematology, histopathological alteration as well as antigen detection by immunohistochemistry, virus isolation and RT-PCR. Contrary to previous studies, our observations revealed the insensibility of C57BL/6 mice to the CDV-SH strain even though SLAM has been integrated into the genome of mice. This implies two possibilities of negative results. Firstly, CDV infection in rodents might use other molecules as viral receptors due to the fact that, so far, there is no entry receptor for CDV that has been identified in mice. Secondly, there is more than one molecular receptor that plays a role in the initial process of CDV entry including CD46 or CD9. The CD46 molecule clarifies as a membrane cofactor protein (MCP) or a complement regulatory molecule, which represents a primate-specific receptor for the laboratory-adapted Edmonston strain of MV and is expressed on all nucleated cells (Dörig *et al.*, 1993; Dörig *et al.*, 1994). The CD9, a tetraspan transmembrane protein, is considered an essential factor for CDV uptake by target cells, cell-cell (but not virus-cell) syncytial cell formation and the production of progeny virus (Löffler *et al.*, 1997; Schmid *et al.*, 2000). In addition, it has recently been shown that p75 neurotrophin receptor (p75^{NTR}) might play a role in CDV infection in mixed canine brain and glial cell cultures (Orlando *et al.*, 2008; Techangamsuwan *et al.*, 2009).

Taken together, the evidence of CDV infection in this study could not be demonstrated in acute phase. Future investigations have to generate other single or double transgenic mice that constitutively express both receptors and to investigate the susceptibility to CDV infection. Even though the transgenic mouse is not a suitable animal model for CDV, or a longer incubation period is prerequisite, it needs to be clarified.

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