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Characterization of two recent Japanese field isolates of canine distemper virus and examination of the avirulent strain utility as an attenuated vaccine

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ABSTRACT

Recently, several new strains of canine distemper virus (CDV) have been isolated in Japan. To investigate their pathogenesis in dogs, the Yanaka and Bunkyo-K strains were investigated by infecting dogs and determining clinical signs, amount of virus, and antibody responses. The Yanaka strain is avirulent and induced an antibody response. The Bunkyo-K strain induced typical CDV clinical signs in infected dogs and virulence was enhanced by brain passage. Molecular and phylogenetic analyses of H genes demonstrated the Bunkyo-K strains were of a different lineage from Asia-1 group including the Yanaka strain and Asia-2 group that contain recent Japanese isolates, which were recently identified as major prevalent strains worldwide but distinct from old vaccine strains. Based on these data, we tested the ability of the Yanaka strain for vaccination. Inoculation with the Yanaka strain efficiently induced CDV neutralizing antibodies with no clinical signs, and the protection effects against challenge with either old virulent strain or Bunkyo-K strain were equal or greater when compared with vaccination by an original vaccine strain. Thus, the Yanaka strain is a potential vaccine candidate against recent prevalent CDV strains.

1. Introduction

Canine distemper virus (CDV) is an enveloped, single-stranded, negative sense RNA virus in the genus *Morbillivirus* and family *Paramyxoviridae*, which includes measles virus (MeV) and rinderpest virus. CDV is highly infectious and causes an often-fatal systemic disease in dogs and other carnivores. CDV in dogs is generally transmitted as an aerosol infection to the upper respiration tract. Primary virus replication occurs in the lymphoid tissues leading to severe immunosuppression, and the incubation period may range from 1 to 4 weeks or more (Appel, 1969; Krakowka, 1982; Krakowka et al., 1980). Transient fever reaches a peak 3–6 days after infection and is associated with an initial virus spread throughout the body. At about 10 days post-infection, CDV spreads by cell-associated viremia from sites of primary replication to various epithelial tissues and the central nervous system (CNS) (Appel et al., 1982; Winters et al., 1983). Subsequent to

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epithelial infection, respiratory, intestinal and dermatological signs occur. The most serious complication is infection of the CNS, leading to a variety of neurological syndromes, frequently with a bad prognosis (Tipold et al., 1996).

CDV is a monotypic virus and a single exposure normally confers long-lasting immunity, similar to MeV. In general, the extensive use of live attenuated CDV vaccines introduced in the 1950s has drastically reduced the incidence of canine distemper in dogs. However, cases of CDV in vaccinated dogs have been reported since the 1990s, and the prevalence of sporadic cases and large outbreaks of CDV are increasing worldwide (Blixenkrone-Moller et al., 1993; Calderon et al., 2007; Ek-Kommonen et al., 1997; Haas et al., 1997; Jozwik and Frymus, 2002; Simon-Martinez et al., 2008). Sequencing analyses have demonstrated that most cases are considered to be caused by infection with newly prevalent strains of CDV, but not by reversion to virulence of vaccine viruses.

Recently, the number of CDV outbreaks has also increased in Japan in both dogs and wild animals (Iwatsuki et al., 2000; Kai et al., 1993; Lan et al., 2006; Shin et al., 1995; Uema et al., 2005). In particular, our previous epidemiological investigations revealed that 44 of 62 dogs (71%) clinically diagnosed with canine distemper from 1985 to 1994 in the Tokyo area were previously vaccinated (Gemma et al., 1996). Furthermore, among six new Japanese CDV strains isolated between 1992 and 1997, an antigenic region in the H protein responsible for neutralization was altered compared with current vaccine strains (Iwatsuki et al., 2000). To examine this phenomenon further, we investigated the virulence of two recent Japanese field isolates, the Yanaka and Bunkyo-K strains. We also examined the potential use of the Yanaka strain as a novel live vaccine against recent prevalent CDV strains.

2. Materials and methods

2.1. Ethical statement

All animal experiments followed the Regulations for Animal Care and Use of the University of Tokyo and were approved by the Animal Experiment Committee at The University of Tokyo. All surgery was performed under anesthesia with a Dormicum and Domitor, and all efforts were made to minimize animal suffering. At the end of the experimental period, the dogs were euthanized by anesthesia with a ketamine-xylazine combination followed by exsanguination.

2.2. Experimental animals

Female beagle puppies with ages varying between 4 and 17 weeks old (details described below), and confirmed to be free from CDV infection by anti-CDV antibody enzyme-linked immunosorbent assay (ELISA), were purchased from the Narc Co. (Chiba, Japan). Dogs were group-housed in cages with ample space provided for exercise. Groups of dogs were kept in strict isolation to prevent viral cross-contamination during the course of all experiments.

2.3. Cells and virus

Vero cells and B95a cells were cultivated at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS) or RPMI 1640 containing 5% FCS, respectively (Kobune et al., 1990).

The Yanaka and Bunkyo-K strains were propagated in B95a cells as previously described (Iwatsuki et al., 1997). For challenge, the Snyder Hill strain, provided by Nippon Zenyaku Kogyo Co., Ltd (Fukushima, Japan), was intracerebrally inoculated into a 4-week old dog. After 7 days, homogenates of infected brain in phosphate-buffered saline (PBS) were used for testing as described below. The Onderstepoort strain, which was passaged in our laboratory (Yamanouchi et al., 1977), was grown in Vero cells and prepared as previously described (Gemma et al., 1995).

2.4. Clinical signs

Dogs were considered febrile if their body temperature increased more than 1 °C and leukopenic if white blood cells (WBC) decreased by more than 40% after challenge. Clinical signs specific for canine distemper (described below) were observed during virulence tests, counted daily and scored for diminished activity, depression, anorexia, lethargy, gastrointestinal signs (diarrhea, hematochezia), neurologic signs (convulsion, tremors, sialorrhea), and respiratory signs (sneezing, rhinitis, dyspnea).

2.5. Test for Yanaka strain virulence

To examine the virulence of the Yanaka strain, 500 μl of the virus solution diluted to 10^4 $TCID_{50}$/ml with maintenance medium were intracerebrally inoculated into six dogs with ages varying between 8 and 17 weeks old. Inoculated dogs were examined daily and rectal temperatures recorded.

The Yanaka strain (500 μl) was also inoculated intracerebrally into a weanling dog. After 7 days, a 20% (w/v) homogenate was produced from the brain and spleen in cold PBS. One dog was inoculated intracerebrally with 500 μl of brain homogenate and another intranasally with 500 μl and intravenously with 1 ml of spleen homogenate. Rectal temperature and clinical signs were monitored daily.

2.6. Test for Bunkyo-K strain virulence

Four weanling dogs (Nos.1-1 to 1-4) were inoculated intracerebrally with 500 μl of Bunkyo-K strain prepared in B95a cells at a titer of 10^4 $TCID_{50}$/ml, and examined daily for rectal temperature, leukocyte counts with a commercial kit (Unopette Test 58.56; Becton Dickinson), and clinical signs. Dogs were euthanized at 6 days post-infection (dpi) (1-2) or 7 dpi (1-4). At necropsy, the cerebrum, spleen, lung, and mesenteric lymph nodes were collected. Viral titers in 10% (w/v) homogenates of these tissues were determined in B95a cells and expressed as $TCID_{50}$/ml. Brain homogenates from infected dogs were inoculated intracerebrally into two additional dogs (Nos.