Complement-Mediated Neutralization of Canine Distemper Virus In Vitro: Cross-Reaction between Vaccine Onderstepoort and Field KDK-1 Strains with Different Hemagglutinin Gene Characteristics

Masami Mochizuki,1* Megumi Motoyoshi,2 Ken Maeda,2 and Kazunari Kai2

Laboratory of Clinical Microbiology, Kyoritsu Seiyaku Corporation, 1-12-4 Kudankita, Chiyoda-ku, Tokyo 102-0073,1 and Department of Veterinary Microbiology, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515,2 Japan

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The properties of neutralization of antigens of canine distemper virus Onderstepoort and a recent field isolate, KDK-1, were investigated with strain-specific dog sera. A conventional neutralization assay indicated antigenic dissimilarity between the strains; however, when guinea pig complement was included in the reaction mixture, the strains were neutralized with not only the homologous but also the heterologous antibodies.

Canine distemper virus (CDV) is a member of the Morbillivirus genus in the Paramyxoviridae family (22), and the disease caused by CDV is one of the important viral infections of puppies (1). In the 1950s CDVs were first cultivated in cells, and they were attenuated as vaccine viruses and adopted in modified live virus (MLV) vaccine products (1, 4). Most currently available MLV CDV vaccines, though not all, have been manufactured by using such strains as Onderstepoort (OSP), Rockborn, or Snyder Hill, which were isolated between the 1940s and 1960s (referred to here as “old CDVs”) (1, 4). As previously described by us as well as others, recent field CDVs (referred to here as “new CDV”) from various parts of the world possess different hemagglutinin (H) gene and protein characteristics (3, 11–14, 16, 17, 20). In addition, antigenic heterogeneities between new and old CDVs were also demonstrated previously by using either monoclonal (2, 15, 18, 21) or polyclonal (9, 14, 15) immune sera, and these observations make it clinically important to elucidate such antigenic variations.

Humoral immunity does not totally explain resistance to canine distemper (1, 4, 10), but it is not practical to monitor routinely the cell-mediated immune status of dogs (5). Thus, the level and the nature of antibodies to CDV have been utilized as valuable alternative indicators to assess vaccine efficacy and immune status of animals (8, 19, 23) and are correlated with protection against CDV (4, 8). However, the antigenic and genetic variations in H protein between different new CDV strains or between new and old strains may raise doubts about the efficacy of the present vaccine products (9, 18). The ideal approach to demonstrating vaccine efficacy is to perform challenge studies in vivo. However, it is well known that experimentally reproducible clinical canine distemper is hard to achieve, and to our knowledge, only one authorized challenge system has been adopted worldwide: an intracranial challenge exposure of the dog-passaged virulent Snyder Hill strain that belongs phylogenetically to the old CDVs (Fig. 1). In this context neutralizing antigenic relationships between OSP and new CDV strain KDK-1, belonging to the Asia/H1 genotype (16, 20), were investigated.

Both viruses were cultivated in Vero cells. For KDK-1, culture fluids from the 6th and 13th passages were used for inoculation of puppies and for a plaque reduction neutralization test (PRNT), respectively. The Vero cells were cultivated by a general cell culturing technique with Eagle’s minimal essential medium (MEM) supplemented with 7.5% fetal calf serum and antibiotics.

All dog sera were diluted 10 times with Eagle’s MEM and heat inactivated at 56°C for 30 min, and antibody titers were examined by PRNT. The PRNT was performed by a general method briefly as follows. Reaction mixtures consisted of 50 μl of serum dilution, 50 μl of Eagle’s MEM or a guinea pig complement dilution, and 100 μl of virus fluid containing approximately 100 PFU in a 96-well plate (Ikawi Glass, Tokyo, Japan). The guinea pig complement was obtained from ICN Pharmaceuticals, Inc. (catalog no. 55854), and it was diluted to 20% (vol/vol) with Eagle’s MEM for use. The reaction mixture was incubated at 37°C for 1 h, and 100 μl of each mixture was inoculated onto Vero cell culture in a six-well plate (Ikawi Glass). After 1 h of incubation for virus adsorption at 37°C, the inoculum was aspirated and overlay medium was added to the cell sheet. The overlay medium was Eagle’s MEM containing 1% (wt/vol) Noble agar, 2% fetal calf serum, and antibiotics. For PRNT against OSP, the plate was incubated for 3 days and then the medium was additionally overlaid to add nutrients and incubated for a further 3 days before staining. Neutralization titer was defined as the reciprocal of the highest serum dilution showing 75% or more reduction of plaque numbers below those of virus control wells. The statistical analysis was performed with the Mann-Whitney test.
Four 8-week-old specific-pathogen-free (SPF) beagle puppies with no detectable antibody were divided into two groups, and mock-infected Vero cell culture fluid (puppies 1 and 2) or KDK-1 virus (puppies 3 and 4) were orally administered to each group. Total virus infectivity administered per puppy was $2.2 \times 10^5$ PFU. Puppies 3 and 4 passed the 28-day observation period without clinical abnormalities. However, they produced high titers of neutralizing antibodies to the inoculum (Fig. 2b). On the other hand, the titers against OSP were less than 1:40 in sera at all sampling points. When the samples were examined under conditions of supplementation of the reaction mixture with complement (Fig. 2a) and their titers were compared to the titers determined without complement, there was no obvious difference between the titers against KDK-1 and OSP. The control puppies, 1 and 2, remained healthy throughout the study. No neutralizing antibody responses against either OSP or KDK-1 were detected in their sera (Fig. 2b); however, when the reaction mixture was supplemented with complement, replication of both viruses at the lower serum dilutions such as 1:10 to 1:40 was inhibited (Fig. 2a).

Twelve 8-week-old SPF beagle puppies were immunized two times 3 weeks apart with a commercial canine vaccine product (Duramune Max 5; Fort Dodge Animal Health, Fort Dodge, Kans.). The vaccine is an MLV product and contains OSP together with canine parvovirus type 2, canine adenovirus type 2, and canine parainfluenza virus. As shown in Table 1, antibody titers against KDK-1 seemed to be comparable to those against homologous OSP; however, it was statistically shown that they were significantly different from each other ($P < 0.05$). When they were examined by the complement supplementation PRNT, the antibody titers increased not only against OSP but also against KDK-1. As a result, the titers against each strain were not significantly different from each other ($P < 0.01$).

Only one new CDV strain, KDK-1, was examined in comparison with vaccine strain OSP in the present study, for the following reasons (16, 20): (i) there are two H gene genotypes, Asia/H1 and Asia/H2, in Japan; (ii) CDVs including KDK-1 belonging to the former are the epidemiologically predominant type; and (iii) no cultivable strain is available for the Asia/H2 genotype. Thus, it will be worthwhile to confirm the present complement-mediated neutralization by using strains belonging to separate genotypes of foreign origin.

In general, several mechanisms by which antibody and/or the complement system may mediate viral neutralization have been identified elsewhere (6). Although the exact mechanisms of resistance to CDV have not been fully studied yet, complement-mediated viral lysis most probably accounts for this re-

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**FIG. 1.** Phylogenetic relations of the deduced amino acid sequences of the coding regions of CDV hemagglutinin for vaccine strain OSP and virulent strain Snyder Hill of the old type and recent field strain KDK-1 belonging to the Asia/H1 genotype of the new type. The tree is constructed on the basis of data in references 16 and 20.
response to CDV observed in vitro, as it does for responses to most and probably all enveloped viruses such as coronavirus, orthomyxovirus, arenavirus, retrovirus, paramyxovirus, and alphavirus (7).

In conclusion, we have demonstrated for the first time the antibody- and complement-mediated virus neutralization of CDV in dog serum. A neutralization assay without complement can distinguish two CDV strains antigenically, and in that regard, it is biologically a more valuable method than the assay with complement. On the other hand, we observed that differences in neutralization of antigens between these different CDV strains disappeared when complement was included in the neutralization reaction mixtures. This may be one of the immune mechanisms explaining why the present canine distemper vaccines using old CDV strains are still functional and sufficiently protect dogs against present, genetically varied field strains of CDV.

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REFERENCES


