A comparison of the immune responses of dogs exposed to canine distemper virus (CDV) — Differences between vaccinated and wild-type virus exposed dogs

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Abstract

Canine distemper virus (CDV)-specific immune response was measured in different dog populations. Three groups of vaccinated or wild-type virus exposed dogs were tested: dogs with a known vaccination history, dogs without a known vaccination history (shelter dogs), and dogs with potential exposure to wild-type CDV. The use of a T-cell proliferation assay demonstrated a detectable CDV-specific T-cell response from both spleen and blood lymphocytes of dogs. Qualitatively, antibody assays [enzyme-linked immunosorbent assay (ELISA) and neutralization assay] predicted the presence of a T-cell response well, although quantitatively neither antibody assays nor the T-cell assay correlated well with each other. An interesting finding from our study was that half of the dogs in shelters were not vaccinated (potentially posing a public veterinary health problem) and that antibody levels in dogs living in an environment with endemic CDV were lower than in vaccinated animals.

Résumé

La réponse immunitaire spécifique au virus du distemper canin (CDV) a été mesurée dans différentes populations canines. Trois groupes de chiens vaccinés ou exposés à une souche sauvage du virus ont été testés : des chiens avec un historique connu de vaccination, des chiens sans un historique connu de vaccination (chiens de refuge), et chiens avec une exposition potentielle à une souche sauvage du CDV. L'utilisation d'une épreuve de prolifération des cellules T a démontré une réponse détectable des cellules T spécifiques au CDV par les lymphocytes sanguins et spléniques. De manière qualitative, les épreuves immuno-enzymatiques (ELISA) et de neutralisation ont bien prédit la présence d'une réponse des cellules T, bien que quantitativement il n'y avait pas une bonne corrélation entre les épreuves de détection des anticorps et l'épreuve des cellules T. Une trouvaille intéressante de l'étude était que la moitié des chiens dans les refuges n'étaient pas vaccinés (représentant un problème de santé publique vétérinaire) et que les titres d'anticorps chez les chiens vivant dans un environnement où le CDV est endémique étaient plus faibles que chez les chiens vaccinés.

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The immunogenicity of canine distemper vaccines and their protective capacity can be tested by experimentally infecting dogs with wild-type virus after vaccination (1,2). To address these questions in field studies, however, neutralizing antibodies are often used as a substitute marker for protection. Titers between 16 and 64 are considered to be (at least partially) protective (3,4). In some studies, seropositivity by other antibody detection methods, including enzyme-linked immunosorbent assay (ELISA), has been used to indicate successful vaccination (5). Although the induction of cytotoxic T-cells (6,7) and T helper cells (8) by vaccination has been demonstrated in individual dogs, no systematic analysis of CDV-specific T-cell response has been performed. It is also not clear how T-cell responses are linked to the production of antibodies and protection against disease.

In this study, samples were tested for a CDV-specific immune response by ELISA, neutralization assay, and a T-cell proliferation assay. The ELISA is highly sensitive for CDV-specific antibodies independent of the biological function and indicates contact with the virus. The amount of neutralizing antibodies, which recognize the hemagglutinin or the fusion protein, is related to protection and was correlated to CD4 T-cell proliferation because the role of T-cells in protection is not known. These parameters were tested in 3 different groups of dogs: 1 group of animals had a well-documented vaccination history; 1 group of shelter dogs had no vaccination history; and 1 group of free-roaming dogs which had not been vaccinated but had been exposed to high endemic levels of CDV wild-type virus infection.

The collection of blood and tissue from dogs was approved by IACUC at Ohio State University. Samples from dogs in the vaccinated group (n = 15 dogs) were derived from 2 populations. Blood samples were drawn from 3- to 6-year-old greyhounds which acted as blood donors to the OSU Veterinary Hospital. These dogs were vaccinated with Merial Recombitek C4 vaccine (Merial, Duluth, Georgia, USA). Spleen and serum samples were obtained from 1- to 2-year-old beagles vaccinated against CDV which were euthanized for reasons unrelated to the study described herein. All dogs had

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been re-vaccinated within the last 12 mo. Spleen and serum samples from shelter dogs were obtained from pit bulls and pit bull mixes ($n = 7$ dogs). Serum samples of dogs from a Native American reserve were derived from feral and free-ranging dogs that were collected during animal control activities. These dogs were of mixed age, sex, and breed ($n = 15$ dogs).

Canine distemper virus (CDV strain Onderstepoort) was grown and titrated on Vero cells, and purified by sucrose gradient according to standard procedures (9).

Enzyme-linked immunosorbent assays were performed according to standard procedures. In brief, 10 μg/mL gradient purified, UV-inactivated CDV or Vero cell lysate was coated in 200 mM $NaCO_3$ buffer (pH 9.6) at 4°C overnight, blocked with PBS/10% FCS/0.05% Tween 20 and incubated with dilutions of dog serum at room temperature for 1 h. After washing, the plate was incubated for 1 h at room temperature with a horseradish-peroxidase coupled goat-serum specific for canine IgG (Bethyl Laboratories, Montgomery, Texas, USA) and was subsequently developed with 0.5 mg/mL ortho-phenyl-diamine in buffer (35 mM citrate, Na_2HPO_4 66 mM, pH: 5.2) and 0.01% H_2O_2. The comparison of positive and negative sera demonstrated that the threshold of seropositivity in this assay was a CDV-specific reaction of an optical density (OD) twice as high as that against control Vero cell antigen. This amount of antibody was arbitrarily assigned 1 ELISA unit (EU), and sera with 1 EU or higher were considered positive. A high titer dog serum of 10 EU from a vaccinated animal was used to obtain a standard curve by dilution and to calculate all canine sera in EU for easy comparison. All sera were tested at a 1:100 dilution.

For the neutralization assay, serum dilutions were incubated with 50 pfu CDV Onderstepoort strain for 1 h at 37°C and plated in duplicate onto 10^4 Vero cells per well of a 96-well plate. Titers were determined microscopically after 7 d. Based on results with negative sera, a titer of 1:20 or higher was considered CDV specific.

Spleen cells were isolated through a tea strainer and lymphocytes from spleen and whole blood were purified using a Ficoll-paque Plus gradient (1.077 g/mL density). Spleen cells were plated in triplicate at 1.25 to 2.5 $\times$ 10^5 cells/well in a 96-well-plate in serum free stem cell media (Stemline II, Sigma, St. Louis, Missouri, USA) for splenocytes, and in media containing human serum albumin (AIM-V, Invitrogen, Carlsbad, California, USA) for peripheral blood lymphocytes with 10 μg/mL CDV antigen, 40 μg/mL pokeweed mitogen or medium alone. After 4 d 0.5 μCi 3H-thymidine/well was added and 18 to 24 h later cells were harvested onto glass filters and counted with a Betaplate Counter (Wallac, Turku, Finland). The stimulation index (SI) was calculated as the mean of proliferation of CDV stimulated cells in counts per minute (cpm)/proliferation of cells in medium in cpm. To control for the specificity of the assay, spleen cells from virus immune animals were tested against Vero cell antigen and proliferation did not exceed a stimulation index of 2 indicating the threshold of the assay.

The immune response to either CDV vaccination or infection was monitored in 3 different canine groups. All data from T-cell assays were used in aggregate because preliminary data indicated that data from the T-cell proliferation assay with lymphocytes from spleen were comparable to those from blood (data not shown).

Table I. Test results for vaccinated dogs, dogs with unknown vaccination history, and for dogs exposed to wild-type CDV. Serum samples from vaccinated dogs (dogs 1 to 15), dogs with unknown vaccination history (dogs 16 to 22), and for dogs exposed to wild-type CDV (23–37) were tested by ELISA and neutralization assay. T-cell proliferation was performed with 4 spleen samples and peripheral blood lymphocyte samples. T-cell responses are displayed as stimulation index of lymphocytes stimulated with CDV antigen or grown in medium control. The ELISA results are shown as ELISA units (EU) which were derived from a high titer serum arbitrarily used as standard. The NT titers are shown as the reverse of the serum dilution which protected indicator cells against CDV infection

Dog number	T-cell proliferation (SI)	ELISA (EU)	Neutralization titer
1*	3.0	5.8	320
2	16.4	5.8	160
3*	3.5	1.1	120
4	2.7	5	120
5	5.6	1.6	80
6	2.8	6.4	80
7	3.9	2.1	60
8	20.1	2.0	40
9*	8.0	1.9	40
10	2.5	1.5	40
11	2.0	1.7	40
12	6.3	2.1	20
13	6.0	1.4	20
14	4.3	1.2	20
15*	< 2	< 1	< 20
16	2.1	4	120
17	4.0	2.2	60
18	< 2	< 1	< 20
19	< 2	< 1	< 20
20	< 2	< 1	< 20
21	< 2	4	60
22	3.9	1.2	< 20
23	n.d.	2.1	160
24	n.d.	4	60
25	n.d.	< 1	40
26	n.d.	< 1	20
27	n.d.	< 1	20
28	n.d.	< 1	20
29	n.d.	3.9	20
30	n.d.	19.5	< 20
31	n.d.	16.6	< 20
32	n.d.	4.5	< 20
33	n.d.	1.5	< 20
34	n.d.	1.1	< 20
35	n.d.	1	< 20
36	n.d.	< 1	< 20
37	n.d.	< 1	< 20

*Spleen samples.

ELISA — enzyme-linked immunosorbent assay.

n.d. — not done.

Among 15 dogs with a known vaccination history, 14 were positive for CDV-specific antibodies and the median NT titer was 80 (20–320). Overall, CDV neutralizing antibodies titers were comparable to those reported in previous studies (10,11). These seropositive dogs also had a T-cell response with a median stimulation index (SI) of 3.9 (2 to 20.1). Although hemagglutinin has been reported as a target for cytotoxic T-cells (7) and a T helper cell epitope has been defined on the fusion protein of CDV (8), no further studies have investigated the T-cell response and its correlation with the antibody response or protection against wild-type CDV infection. For this reason, a comparison of the T-cell data with other studies is not possible. However, in this study, the presence of a T-cell response correlated with the presence of neutralizing antibodies which are considered to be, at least partially, protective (3,4). One of the 15 dogs showed no detectable immune response using any of the 3 tests (Table I, animals 1 to 15). It is not clear whether this animal had never been vaccinated or lacked antibody due to vaccination failure.

Among 7 dogs with an unknown vaccination history, 3 showed no detectable immune response on any of the tests (Table I, animals 16 to 22), suggesting that these dogs had not been previously vaccinated or exposed to CDV. Due to the small number of animals in this group it is difficult to generalize our findings. Nonetheless, these data suggest that in certain canine groups, such as shelter dogs, a sizeable number of animals may not be immune to CDV. If CDV vaccination is used as a marker of immunization status of these dogs, they are most likely also not to be immunized against Rabies thus posing a potential risk/reservoir for zoonotic transmission. Another potential risk is the pool of unvaccinated dogs in shelters which are a susceptible reservoir to CDV infection and potentially a threat to other dogs and to wildlife. Two of 7 dogs had both an antibody and a T-cell response. Two dogs were of special interest because the T-cell and antibody responses did not correlate. Animal 21 had no detectable T-cell response but was antibody positive. Animal 22 had a T-cell response (SI of 3.9) and was seropositive by ELISA (1.2 EU) but no neutralizing antibodies were detected. It is possible that in these animals a preferential activation or suppression of either the B-cells or T-cells in response to vaccination has occurred. However, these findings will have to be confirmed in future studies.

From the 3rd group of dogs which lived in an environment where wild-type CDV is endemic, only serum samples for antibody experiments were obtained due to logistical difficulties. Two animals had no antibody response (Table I), 13 of 15 dogs were seropositive by ELISA but out of those 13, 6 individuals (numbers 30 through 35) had no detectable neutralizing antibodies. The other 7 dogs had NT titers ranging from NT 20 to 160, with a median NT of 50. This is consistent with the view that ELISA is the more sensitive antibody assay as it measures all CDV-specific antibodies (5). In contrast, the neutralization assay measures only functional antibodies that bind to CDV hemagglutinin or fusion protein and interfere with virus uptake. However, no clear relationship between the ELISA units of a serum and its neutralizing activity was found. This correlates with a published comparison of results by ELISA with neutralization assay (5). In this study, sera were assigned from 0 to 6 ELISA units and NT titers were associated with a wide range (up to 4 units) of ELISA data.

The difference in average titers between wild-type CDV-exposed and vaccinated dogs was not significant (Mann-Whitney test). However, the numbers of animals with neutralizing antibodies was higher in vaccinated versus wild-type virus exposed animals ($P > 0.03$, chi-square test). Interestingly enough, another study reported higher titers in vaccinated versus wild-type CDV infected dogs (2). One explanation for the low antibody levels in wild-type CDV-exposed animals might be a very recent exposure. This clearly was true for animal 28 which displayed signs of CDV infection when blood was taken. In contrast, other animals might have survived infection a long time ago with subsequent amnesic antibody titers.

In summary, this study demonstrates the usefulness of the approach to incorporate the measurement of both antibody and T-cell responses into the analysis of the immune response after CDV vaccination or wild-type virus infection. In future, the differences found in the different groups will have to be analyzed in more detail using these methods.

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