Effects of Vaccines on the Canine Immune System

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ABSTRACT

The effects of several commercially available polyvalent canine vaccines on the immune system of the dog were examined. The results demonstrated that the polyvalent vaccines used in this study significantly suppressed the absolute lymphocyte count and that most of the polyvalent vaccines significantly suppressed lymphocyte response to mitogen, but had no effect on natural effector cell activity, neutrophil chemiluminescence, nor antibody response to canine distemper virus. The individual vaccine components from the polyvalent vaccines when inoculated alone did not significantly suppress the lymphocyte response to mitogen. However, when canine distemper virus was combined with canine adenovirus type 1 or canine adenovirus type 2, significant suppression in lymphocyte responsiveness to mitogen occurred. The results indicate that interactions between canine distemper virus and canine adenovirus type 1 or canine adenovirus type 2 are responsible for the polyvalent vaccine induced suppression of lymphocyte responsiveness.

RÉSUMÉ

Cette étude portait sur plusieurs vaccins polyvalents, destinés aux chiens et disponibles sur le marché; elle visait à analyser les effets de ces vaccins sur leur système immunitaire. Les résultats démontrèrent que ces vaccins supprimèrent de façon significative le nombre absolu de lymphocytes et que la plupart inhibèrent de façon appréciable la réponse mitogène des lymphocytes, sans toutefois exercer d'influence sur l'activité cellulaire effective naturelle, la chimioluminescence des neutrophiles, ou sur la réponse immunitaire à l'endroit du virus de la maladie de Carré. L'inoculation séparée des divers composants des vaccins expérimentaux ne supprima pas significativement la réponse mitogène des lymphocytes, sauf quand on combina le virus de la maladie de Carré avec l'adénovirus du type #1 ou #2. De tels résultats indiquent que les interactions entre le virus de la maladie de Carré et l'adénovirus canin du type #1 et #2 sont responsables de la suppression de la réponse mitogène des lymphocytes, imputable au vaccin polyvalent.

INTRODUCTION

In 1908, von Pirquet first recognized viral induced immunosuppression by demonstrating that humans infected with measles virus had a suppressed dermal reaction to tuberculin (1). Since this initial observation on measles virus, other viruses have been shown to be immunosuppressive (2-15). Much of the early work centered on viral suppression of the cutaneous response to intradermally inoculated antigens. With the development of *in vitro* assays to evaluate the immune system, most of the recent research has concentrated on viral induced suppression of lymphocyte response to mitogen, natural effector (NE) cell activity, antibody dependent cell-mediated cytotoxicity activity (ADCC) and phagocytic cell functions. Immunosuppression is not restricted to virulent virus as vaccine strains of measles, mumps, rubella, polio, yellow fever, vaccinia and influenza also have immunosuppressive effects (16-21). It is common in human and veterinary medicine to vaccinate for several pathogens using polyvalent vaccines containing multiple viral and bacterial components. However, most studies of viral induced immunosuppression have investigated the effects of only monovalent vaccines. Thus, little is known about vaccine virus interactions and their potential immunosuppressive effects.

Dogs are routinely vaccinated with attenuated polyvalent vaccines. These vaccines are commercially available in a variety of combinations consisting of canine distemper virus (CDV), canine adenovirus type-1 (CAV-1), canine adenovirus type-2 (CAV-2), canine parainfluenza virus (CPI) and/or parvovirus (PV). The attenuated Rockborn strain of CDV does not cause immunosuppression (3). Virulent CDV, however, suppresses lymphocyte response to mitogen, increases kidney graft retention time, induces a hypogammaglobulinemic state, and causes a prolonged leukopenia (2,3,22,23). The immunosuppressive effects of virulent CDV may persist for a month or longer. Virulent and vaccine strains of canine parvovirus are reported to be immunosuppressive, although convincing experimental evidence to support these assertions is limited (25-29). Additionally, we have recently demonstrated

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that neither virulent nor vaccine strains of CPV are immunosuppressive (24). The effects of virulent and vaccine strains of CAV-1, CAV-2 and CPI on the immune system of the dog have not been extensively studied, nor have the potential immunosuppressive effects of polyvalent vaccines. The purpose of the present study was to determine the effects of commercially available polyvalent canine vaccines on the immune system of the dog and if found immunosuppressive, to determine the vaccine component or viral interactions which are responsible for the suppression.

MATERIALS AND METHODS

ANIMALS

The 92 mixed breed dogs used in this study ranged in age from 3 to 11 months. At the time these studies were initiated, the dogs were serologically negative to the various viral agents used in this study. All experiments on animals were performed in accordance with the guidelines as stated in the "Guide to the Care and Use of Experimental Animals", Canadian Council on Animal Care.

VACCINES

Seven commercially available canine modified live vaccines were obtained from various manufacturers. Vaccine #1 consisted of CDV, CAV-1, CPI and PV. Vaccine #2 contained CDV, CAV-1 and CPI. Vaccine #3 contained CDV and CAV-1. Vaccine #4 consisted of CDV and CAV-2. Vaccine #5 contained only CDV, while vaccine #6 consisted of measles virus (MV). Vaccine #7 contained CDV, CAV-1, CPI and PV and two serovars of leptospira. Additionally, the individual components of vaccine #1, CDV, PV, CAV-1, CPI and the CAV-2 component of vaccine #4 were obtained from the manufacturers as single viral components.

BLOOD COLLECTION AND HEMATOLOGY

Twenty milliliters of heparinized venous blood were collected. A differential leukocyte count was performed on Diff-Quick stained blood smears (100 cells counted per slide). Total white blood cell numbers were determined with a cell counter.

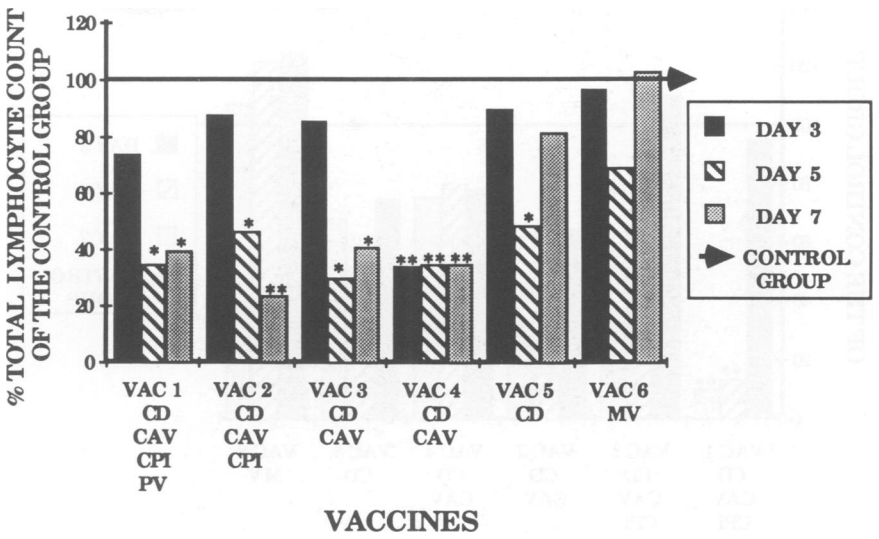

Fig. 1. Effects of vaccines on the absolute lymphocyte count. Data are represented as percent of the control group. The method of statistical analysis was a one way analysis of variance. On days when statistical differences were demonstrated by analysis of variance, individual *t* tests between the control group and each of the treatment groups were performed. *$p \leq 0.05$, **$p \leq 0.01$.

CELL SEPARATION

Boyum's method of peripheral blood separation was performed (30) as modified by Phillips *et al* (31).

LYMPHOCYTE RESPONSE TO MITOGEN

This assay was performed as previously described (32). Briefly 2.5 x 10^5 mononuclear (MN) cells/well in RPMI 1640 with 10% autologous serum or 10% fetal bovine serum (FBS) were incubated for 48 h with 60 μg of phytohemagglutinin (PHA). The cells were labeled with 1 μCi ^{3}H thymidine/well. Eighteen hours later the plates were frozen and stored at -70°C until harvested. The samples were then counted in a scintillation counter and the counts per minute determined. The results of the lymphocyte response to mitogen assay

TABLE I. Effects of Vaccines on the Antibody Response to Canine Distemper Virus[a]

Group	Animal	Number of Days Postinoculation 3	5	7	17
Control	1	<2	<2	<2	<2
	2	<2	<2	<2	<2
	3	<2	<2	<2	<2
Vac #1	4	<2	<2	<2	64
	5	<2	<2	<2	1024
Vac #2	6	<2	<2	<2	128
	7	<2	<2	<2	128
	8	<2	<2	<2	64
Vac #3	9	<2	<2	<2	128
	10	<2	<2	<2	32
	11	<2	<2	<2	4096
Vac #4	12	<2	<2	<2	32
	13	<2	<2	<2	16
	14	<2	<2	<2	128
Vac #5	15	<2	<2	<2	2048
	16	<2	<2	<2	128
	17	<2	<2	8	4096
Vac #6	18	<2	<2	<2	<2
	19	<2	<2	<2	<2
	20	<2	<2	<2	<2

[a]No statistical differences were demonatrated between the serum neutralization antibody titers produced by vaccines #1-5

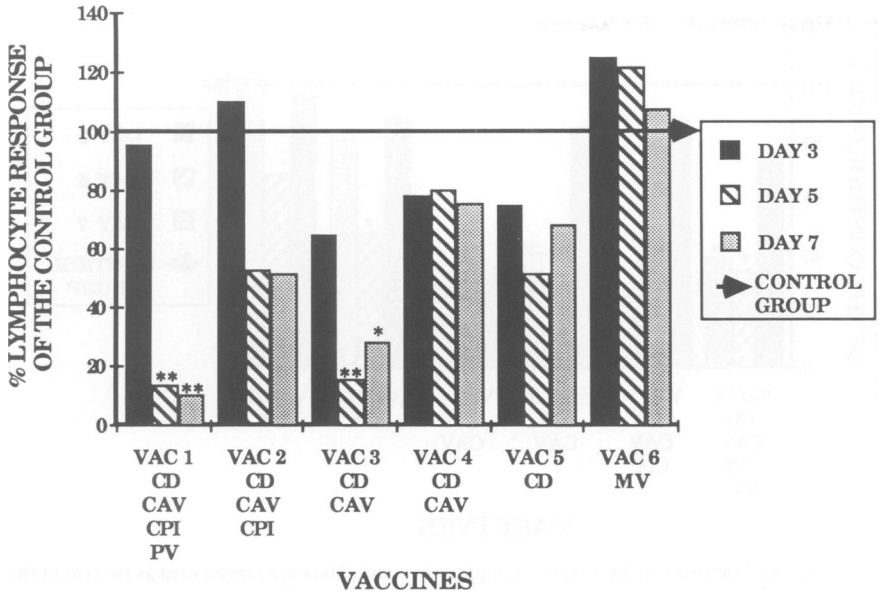

Fig. 2. Effects of vaccines on the phytohemagglutinin response of canine lymphocytes. Data are represented as percent of the control group. The method of statistical analysis was a one way analysis of variance. On days when statistical differences were demonstrated by analysis of variance, individual *t* tests between the control group and each of the treatment groups were performed. *p ≤ 0.05, ** p ≤ 0.01.

were expressed as percent response of the control (saline inoculated) group. The following formula was used to express the data: % response of control = (mean treatment value/ mean control value) x 100.

CHEMILUMINESCENCE ASSAY

The chemiluminescence response was performed as described by Allen (33) and modified by Phillips *et al* (31). Briefly, neutrophils at 39°C were stimulated to undergo an oxidative burst response by an opsonized zymosan luminol preparation. The peak chemiluminescence response was determined by a luminometer

NATURAL CELL-MEDIATED CYTOTOXICITY ASSAY

The natural cell-mediated cytotoxicity (NCMC) assay was performed as previously described by Phillips and Schultz (24). Briefly, the MN cells containing the natural effector cell population were incubated for 18 h with the ^{51}Cr labeled target cells, D17, a canine osteosarcoma cell line. The radioactivity released by the target cells was determined. The data are expressed as % killing of the control group.

VIRUS NEUTRALIZATION TEST

The canine distemper virus neutralization test was performed as previously described (34).

STATISTICAL ANALYSIS

The method of statistical analysis for the study was the one-way analysis of variance after log transformation of the data. When significance was found in the analysis of variance, individual *t* tests between the control group and treatment groups were performed (35).

EXPERIMENTAL DESIGN

Experiment 1: A survey study was conducted to determine the effects of commercially available vaccines on selected parameters of the immune system. Twenty dogs were placed into seven groups (a control group, and six treatment groups). The dogs in the control group received a subcutaneous inoculation of sterile saline. The dogs in each of the six treatment groups were inoculated subcutaneously with one of the commercially available canine vaccines (vaccine #1 through vaccine #6). Each of the groups contained three dogs except the group that received vaccine #1 which consisted of two dogs. The lyophilized vaccines were rehydrated with sterile water. The dogs were bled on days 3, 5 and 7 postinoculation and the following assays performed: total white blood cell count, differential white blood cell count, lymphocyte response to mitogen and chemiluminescence. The animals were also bled on day 17 postinoculation. Canine distemper virus serum neutralization test was performed with the serum obtained on days 3, 5, 7 and 17 postinoculation.

Experiment 2: The duration of the decreased lymphocyte response to mitogen, the effects of vaccination on NCMC, and the effects of using leptospirosis bacterin (a common diluent for canine vaccine) as the vaccine diluent instead of sterile water were examined. Fifteen dogs were placed into one of three groups with five dogs in each group The lyophilized vaccines were rehydrated with leptospirosis bacterin (*Leptospira*

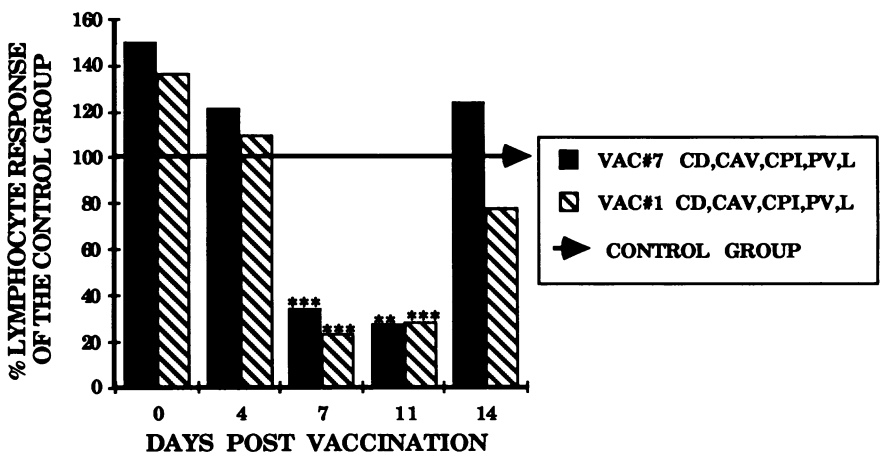

Fig. 3. Kinetics of vaccines effects on the phytohemagglutinin response of canine lymphocytes. Data are represented as percent of the control group. The method of statistical analysis was a one way analysis of variance. On days when statistical differences were demonstrated by analysis of variance, individual *t* tests between the control group and each of the treatment groups were performed. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

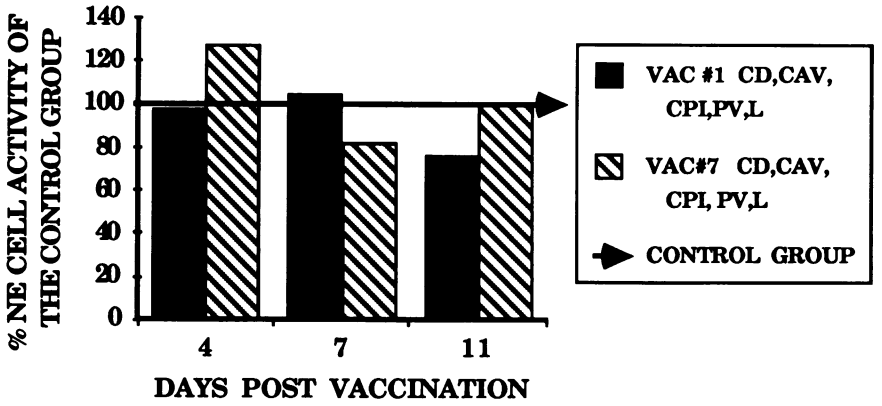

Fig. 4. Effects of vaccines on the natural effector cell activity. Data are represented as percent of the control group. The method of statistical analysis was a one way analysis of variance. No statistical differences were demonstrated.

ictohemorrhagica and *L. canicola* serovars). The dogs in the control group received an inoculation of saline. The dogs in one of the treatment groups were inoculated with vaccine #1 while the dogs in the second treatment group were inoculated with vaccine #7. The animals were bled on days 0, 4, 7, 11 and 14 postinoculation. The lymphocyte response to mitogen assay was performed on days 0, 4, 7, 11 and 14 while the NCMC assay was performed on days 4, 7 and 11 postinoculation.

Experiment 3: The effects on lymphocyte response to mitogen of individual vaccine components CDV, PV, CAV-1, CPI from vaccine #1, and CAV-2 from vaccine #4 were determined. Three dogs were inoculated with attenuated Rockborn strain of CDV. Eight dogs received the attenuated strain of PV. Three groups with five dogs per group received the attenuated strains of either CAV-1, CAV-2 or CPI. A saline inoculated control group was included in each trial. All of the individual vaccine components were inoculated at titers which were approximately equal to the titers in the polyvalent vaccines. The dogs were bled on days 0, 3, 5 and 7 postinoculalion and the lymphocyte response to mitogen was determined.

Experiment 4: The effects of inoculating dogs with CDV (from vaccine #1) in combination with CAV-1 (from vaccine #1) or CAV-2 (from vaccine #4) on the lymphocyte response to mitogen were determined. Fifteen dogs were placed into three groups with five dogs in each group. One group of dogs was inoculated with the CDV component of vaccine #1 and the CAV-1 component of vaccine #1. Another group of dogs was inoculated with the CDV component of vaccine #1 and the CAV-2 component of vaccine #4. The control group was inoculated with saline. All of the vaccine components were inoculated at titers which were equal to their respective titers in the polyvalent vaccines. The animals were bled on days 0, 3, 5, 7 and 10 postvaccination and lymphocyte response to mitogen assay was performed.

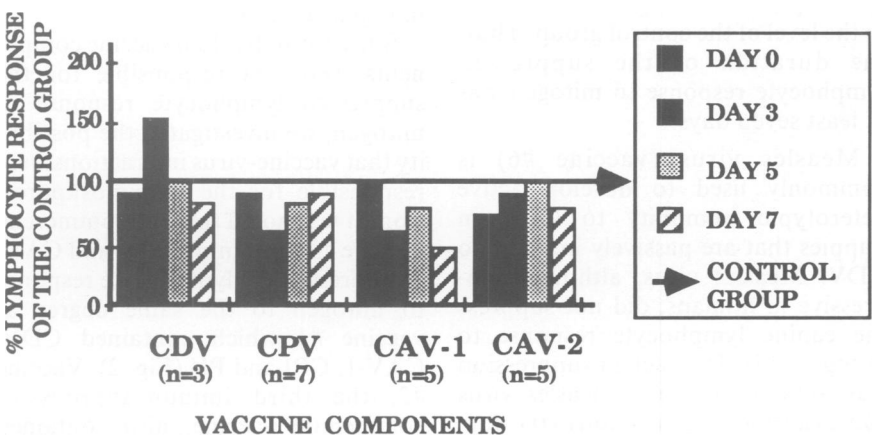

Fig. 5. Effects of individual vaccines components on the phytohemagglutinin response of canine lymphocytes. Data are represented as percent of the control group. The method of statistical analysis was a one way analysis of variance. No statistical differences were demonstrated.

RESULTS

EXPERIMENT 1

The commercially available vaccines used in this study did not significantly affect the total white blood cell count, absolute polymorphonuclear neutrophil (PMN) count, or absolute monocyte count. However, the absolute lymphocyte counts in animals that received vaccines #1, 2, 3 and 4 were significantly decreased relative to the control group on days 5 and 7 postinoculation (Fig. 1). Animals that were inoculated with vaccine #4 also had a significantly decreased lymphocyte count on day 3 postvaccination. All the dogs, with the exception of those that received sterile saline or the measles vaccine, developed varying levels of neutralizing antibody to canine distemper virus which did not differ significantly among the vaccine groups (Table I). The vaccines did not significantly suppress the chemiluminescence response (data not shown). The polyvalent vaccines #1 and #3 significantly decreased the lymphocyte response to PHA on days 5 and 7 postinoculation (Fig. 2). Vaccine #2 caused a moderate suppression on days 5 and 7 postinoculation.

EXPERIMENT 2

On days 7 and 11 postinoculation, vaccine #1 and vaccine #7 caused a significant suppression of the lymphocyte response to mitogen assay (Fig. 3). By day 14 postvaccination, the lymphocyte response to mitogen in the animals receiving vaccine #1 and #7 had returned to the level of the control group. The suppression of lymphocyte response to mitogen induced by vaccine #1 occurred regardless of the

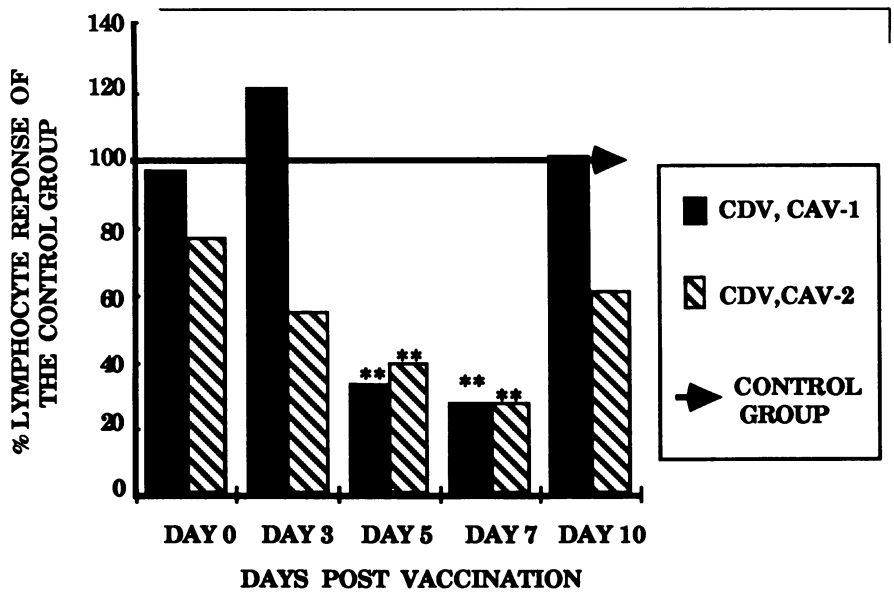

Fig. 6. Effects of vaccines components, CDV-CAV-1 or CDV-CAV-2, on the phytohemagglutinin response of canine lymphocytes. Data are represented as percent of the control group. The method of statistical analysis was a one-way analysis of variance. On days when statistical differences were demonstrated by analysis of variance, individual *t* tests between the control group and each of the treatment groups were performed. *$p \leq 0.05$, **$p \leq 0.01$.

diluent used (sterile water used in experiment 1 or leptospirosis bacterin used in experiment 2) (Figs. 2 and 3). Vaccines #1 and #7 did not significantly suppress the NEC activity (Fig. 4).

EXPERIMENT 3

The individual vaccine components (CDV, CAV-1 and PV of vaccine #1 and the CAV-2 component of vaccine #4) when given alone did not significantly affect lymphocyte response to mitogen (Fig. 5) nor did the CPI component of vaccine #1.

EXPERIMENT 4

On days 5 and 7 postinoculation, a significant suppression in lymphocyte response to mitogen occurred in dogs that were inoculated with a CDV-CAV-1 combination or a CDV-CAV-2 combination (Fig. 6).

DISCUSSION

Vaccine induced immunosuppression has been reported in humans with many different vaccines (16-21). This, however, is the first report of polyvalent commercially available canine vaccines causing significant suppression of lymphocyte response to mitogen. Three of the five polyvalent commercially available vaccines used in this study (vaccine #1, vaccine #3 and vaccine #7, see Figs. 2 and 3) caused a significant suppression of the lymphocyte response to mitogen. The suppression was first detected at five days postinoculation and subsequently seen on days 7 and 11 postinoculation. The level of lymphocyte suppression was pronounced. Vaccine #1 on day 7 postinoculation caused a 94% suppression in lymphocyte responsiveness relative to the saline inoculated control group. By day 14 postinoculation the lymphocyte response to mitogen had returned to the level of the control group. Thus, the duration of the suppressed lymphocyte response to mitogen was at least seven days.

Measles virus (vaccine #6) is commonly used to develop active heterotypic immunity to CDV in puppies that are passively immune to CDV. Measles virus, although suppressive in humans, did not suppress the canine lymphocyte response to mitogen (17). This lack of suppression was anticipated, since measles virus causes an abortive infection in the dog. Thus, measles virus, in effect, served as an additional control. Vaccine #5 which contained only CDV also had no significant effects on the lymphocyte response to mitogen. These results are similar to a previous study showing no suppression by the Rockborn strain of CDV (3).

No significant differences in the antibody levels to CDV were detected between vaccine groups irrespective of vaccine (immunosuppressive or non-immunosuppressive) received. The high variability in antibody response combined with the small number of animals may explain why a significant difference in the antibody responses to CDV was not demonstrated. Alternately, the vaccine induced suppression of lymphocyte response to mitogen did not occur until five days postinoculation. This may be enough time for the CDV vaccine to stimulate an antibody response prior to the onset of lymphocyte suppression. However, if an animal was exposed to a pathogen during the time of vaccine induced immunosuppression (days 5 through 11 postvaccination), the immune response to the pathogen may be suppressed. The results of this present study may explain the observations of Potgieter *et al* (27) where dogs vaccinated with a CDV, CAV-1 vaccine five days prior to a challenge with virulent canine parvovirus developed clinical signs and lesions of canine parvovirus disease whereas the unvaccinated dogs that were challenged with the same virulent canine parvovirus remained healthy.

The individual vaccine components CDV, CAV-1, PV and CPI from vaccine#1 and CAV-2, a component of vaccine #4, did not cause a significant suppression in lymphocyte response to mitogen (Fig. 5).

Since the individual vaccine components were not responsible for the suppressed lymphocyte response to mitogen, we investigated the possibility that vaccine-virus interactions were responsible for the immunosuppression. It was noted that the commercial vaccine #3 containing CDV and CAV-1 suppressed the lymphocyte response to mitogen to the same degree as vaccine #1 which contained CDV, CAV-1, CPI and PV (Fig. 2). Vaccine #7, the third immunosuppressive commercial vaccine, also contained CDV and CAV-1. When the CDV component of vaccine #1 was simultaneously inoculated with the CAV-1

component of vaccine #1 or the CAV-2 component of vaccine #4, a significant decrease in lymphocyte response to mitogen was demonstrated (Fig. 6). The onset and degree of this suppression was similar to that seen with the commercially available polyvalent vaccines (vaccines #1, #3 and #7). Thus, an interaction between the CDV strain of vaccine #1 and the attenuated strains of CAV-1 (from vaccine #1) or CAV-2 (from vaccine #4) was responsible for the decrease in lymphocyte responsiveness. Interestingly, vaccine #4, which contained attenuated CDV and CAV-2 did not cause a significant suppression in lymphocyte response to mitogen. This suggests that the ability of the CDV to interact with CAV-2 and cause suppression of lymphocyte responsiveness may be related to the specific vaccine strain of CDV.

The Rockborn strain of CDV is used in the polyvalent vaccines #1, #2, #3 and #7. Vaccines #1, #3 and #7 caused significant suppression of the lymphocyte response to mitogen. Although vaccine #2 did not cause significant suppression of the lymphocyte response to mitogen, it caused a moderate suppression with kinetics similar to those observed with vaccine #1 and vaccine #3. The strain of CDV used in vaccine #4, which did not interact with CAV-2 to suppress lymphocyte response to mitogen, was an attenuated Snyder Hill strain. Thus, it would appear that the Rockborn strain of CDV, although not suppressive alone, interacted with CAV-1 or CAV-2 to suppress lymphocyte response to mitogen while the attenuated Snyder Hill strain of CDV did not.

Vaccines #1 through #4 caused a significant decrease in the number of peripheral blood lymphocytes on days 5 and 7 postinoculation (Fig. 1). The vaccine-induced suppression of lymphocyte responsiveness to mitogen and the decrease in absolute lymphocyte counts are independent events, since vaccine #4 decreased the lymphocyte counts as much or more than any of the other vaccines (Fig. 1) but did not suppress the lymphocyte response to mitogen (Fig. 2). Similarly, vaccine #1 caused the greatest suppression in the lymphocyte response to mitogen (Fig. 2) but caused no greater decrease in total lymphocyte counts than vaccine #2, #3 or #4 (Fig. 1). Thus, lymphopenia and functional suppression of lymphocytes are not directly related.

Although we have demonstrated that vaccine interactions between CDV and CAV-1 or CAV-2 are responsible for the decreased lymphocyte responsiveness, the exact mechanism is unknown. It is possible that CDV combined with CAV-1 or CAV-2 causes an alteration in lymphocyte circulation or trafficking (36), which removes the lymphocytes that are responsive to PHA from peripheral blood. Alternate explanations would be that a CDV-CAV combination has a lytic action on the population of lymphocytes responding to PHA or that CDV-CAV interactions prevent the lymphocytes capable of responding to PHA from undergoing cell division. Further investigations are needed to determine the mechanism of decreased lymphocyte response to mitogen.

The practical significance of the decreased lymphocyte responsiveness as it relates to the animal is currently unknown. Polyvalent canine vaccines have been demonstrated to be efficacious (37). Additionally, severe disease does not generally occur following the administration of these vaccines. However, the results of this study demonstrate that the majority of polyvalent canine vaccines significantly suppress lymphocyte responsiveness to PHA. We believe that the reason clinical disease is not widely associated with the use of these polyvalent vaccines is that the duration of the lymphocyte suppression is relatively short (approximately one week). Generally, for immunosuppression to cause clinically apparent disease, it must be present for a long period of time (weeks to months). However, in certain circumstances, even this relatively short duration of suppression could become clinically significant especially if the animal was in a partially immunosuppressed condition (e.g. nutritional deficiency). It is possible that vaccine induced immunosuppression may potentiate the severity of a concurrent disease or allow inapparent infection to become clinically apparent (3,27).

The results of this study do not suggest that dogs should not receive polyvalent vaccines. Polyvalent vaccines must be demonstrated safe and effective to be licensed. However, vaccination should not be viewed as an innocuous procedure and should be performed in accordance with manufacturer's recommendation (only healthy, clinically normal dogs should be vaccinated).

Polyvalent vaccines are frequently used in both human and veterinary medicine. This is the first report of individual vaccine components which are not immunosuppressive by themselves causing an immunosuppression when inoculated in combination. Thus, the results of this study may have implications for other species which receive polyvalent vaccines.

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