Experimental Parvovirus Infection in Dogs

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ABSTRACT

Five eight week old dogs were inoculated orally and intranasally with cell culture origin canine parvovirus. Three dogs became depressed and anorectic and developed a mild (one dog) to severe diarrhea five days postinfection. The remaining dogs had subclinical infections but developed a lymphopenia followed by a transient lymphocytosis. The ill dogs developed mild (one dog) to severe neutropenia and a moderate lymphopenia. One died nine days postinfection. Recovery was associated with cessation of viral excretion and with lymphocytosis and antibody production. Two of three dogs challenged intragastrically developed mild clinical signs and a moderate panleukopenia four to eight days postinfection.

The pathological changes of the experimental disease were very similar to that of spontaneous disease. Bone marrow changes included a severe granulocytic and mild erythroid depletion. The pathogenesis of canine parvovirus infection is discussed.

RÉSUMÉ

Cette expérience consistait à injecter un parvovirus, récolté sur cultures cellulaires, dans la bouche et dans la cavité nasale de cinq chiens âgés de huit semaines. Cinq jours après l'inoculation, trois de ces chiens manifestèrent de la dépression, de l'anorexie et une diarrhée d'intensité variable. Les autres chiens ne manifestèrent pas de signes cliniques, mais ils développèrent une lymphopénie à laquelle succéda une lymphocytose transitoire. Les chiens malades développèrent une neutropénie plus ou moins marquée et une lymphopénie modérée; l'un d'eux mourut, neuf jours après l'injection. La guérison s'accompagna d'un arrêt de l'excrétion du virus, d'une lymphocytose et de la production d'anticorps. Deux des trois chiens soumis à une infection stomacale de défi, manifestèrent des signes cliniques mitigés et une panleucopénie modérée, entre le quatrième et le huitième jour après l'infection.

Les lésions imputables à cette maladie expérimentale ressemblaient beaucoup à celles de la maladie spontanée. Celles de la moelle osseuse consistaient en une déplétion plus marquée des cellules de la lignée érythrocytaire que de celle des granulocytes. Les auteurs commentent aussi la pathogénèse de l'infection canine à parvovirus.

INTRODUCTION

An association between parvovirus infection and enteritis in dogs was first reported by Eugster and Nairn in 1977 (9). The association of parvovirus infection with enteritis and leukopenia has been confirmed by several investigators (1, 4, 6, 14, 16). Eugster and Nairn could not maintain the virus beyond two cell culture passages thus precluding definitive studies of the virus (9). However, several investigators recently reported the successful propagation of the virus in cell cultures (2, 3, 10, 12, 15). Canine parvovirus (CPV) is antigenically related to feline panleukopenia virus (2, 3, 10, 12, 15), and the disease in dogs is very similar to panleukopenia of cats (6, 14, 16). Myocarditis (11) and pneumonia (5) have also been associated with CPV infection.

Experimental production of the disease by oral challenge with the virus has not been reported, a problem which has slowed study of the disease and its control. It was the purpose of the study reported here to follow the course of CPV infection in dogs following oral and nasal challenge with a strain of CPV recently isolated from a dog with the disease. Since the disease sometimes occurs in dogs soon after routine vaccination against canine distemper (13, 22), some dogs included in this study were inoculated with canine distemper virus and canine adenovirus vaccine several days prior to challenge with CPV.

MATERIALS AND METHODS

CELL CULTURES

A continuous cat kidney cell line, CRFK (7), was used in this study. The cells were grown in Dulbecco's Modified Eagle's medium supplemented with ten percent fetal bovine serum.[1]

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VIRUS

A strain of CPV, isolated from a dog with acute hemorrhagic enteritis, serologically related to feline panleukopenia virus, and passaged five times in cell cultures, was used. The virus was grown by exposing a confluent monolayer of cell cultures to an inoculum for three hours at 37°C. The cells were then removed by trypsinization and seeded into new cell culture flasks. After 72 hours incubation, cells and cell culture fluids were collected and tested for virus. The latter was titrated by inoculating serial tenfold dilutions into 25 cm^2 cell culture flasks containing freshly seeded cells. After 48 hours incubation, the cells were examined for the presence of virus by the indirect fluorescent antibody test (IFAT) with a CPV antiserum. The latter was obtained from J.W. Black, C.E. Kord Animal Disease Laboratory, Nashville, Tennessee.

ANIMALS

Fourteen eight week old, mixed breed dogs were the test animals for this experiment. The dogs originated from a closed kennel and were free of antibodies to canine distemper virus (CDV), infectious canine hepatitis virus (ICHV) and CPV.

SEROLOGY

The IFAT as described earlier (17) was employed for detecting antibodies to CPV. The antigen was prepared by scraping infected cells off growth surfaces. The cells were suspended in PBS (17), centrifuged and resuspended in PBS to one tenth of the original volume. Drops of the cell suspension were placed on glass slides, allowed to dry and then fixed in acetone for ten minutes at room temperature.

SERA

Serum samples were obtained from the experiment dogs at various intervals after CPV inoculation.

HEMATOLOGY

Total leukocyte counts, differential cell counts, and bone marrow evaluation were done by conventional methods (20).

HISTOPATHOLOGY

Conventional methods were used for histopathology. Tissues from dead dogs were fixed in ten percent formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

EXPERIMENTAL DESIGN

Five pups were inoculated with four mL of CPV (10^4 tissue culture infectious doses — TCID/mL). Three mL were given orally and one mL was given intranasally. Five days prior to the CPV challenge, three of these pups were vaccinated with a commercial vaccine (DHL) containing modified live canine distemper virus and canine adenovirus type 1 grown in canine cell culture and *Leptospira canicola* and *Leptospira icterohemorrhagiae* bacterins. In addition, three DHL-vaccinated pups were challenged by intragastric inoculation, using a stomach tube, with 2×10^4 TCID CPV. A control group of three pups received DHL vaccine but was not challenged with CPV.

The dogs were housed separately in isolation. Body temperatures of the dogs were measured daily and blood samples were taken every three to four days from four to ten days before to 16 days after challenge with CPV. Leukocyte counts, antibody titrations, and virus isolation were done on the appropriate specimens.

RESULTS

Results of the experiments are summarized in Table I. The DHL-vaccinated dogs which were not challenged with CPV remained healthy. Their body temperatures and leukocyte counts remained normal and they did not develop antibodies to CPV.

Two unvaccinated dogs (880,881) challenged intranasally and orally with CPV developed a mild to moderate lymphopenia on the fourth day and a marked lymphocytosis from 11 to 14 days postinfection (Figs. 1a and 1b). Clinical

TABLE I. Summary of Sequelae in Dogs Challenged with CPV

Dog #	CPV Challenge	Vaccinated[a]	Anorexia[b]	Diarrhea[b]	Depression[b]	Lymphopenia[c]	Neutropenia[c]	Convalescent Leukocytosis[d]
884	No	Yes	0	0	0	No	No	No
886	No	Yes	0	0	0	No	No	No
891	No	Yes	0	0	0	No	No	No
926	IG[e]	Yes	0	0	0	92	85	119
927	IG	Yes	2+	1+	2+	46	27	122
928	IG	Yes	2+	1+	2+	34	26	120
880	PO-IN[f]	No	0	0	0	61	75	188
881	PO-IN	No	0	0	0	68	87	220
878	PO-IN	Yes	4+	4+	4+	53	2	N.D.[g]
879	PO-IN	Yes	4+	3+	4+	62	6	143
882	PO-IN	Yes	2+	2+	2+	52	53	147

[a]Vaccinated with DHL five days prior to challenge
[b]Rated subjectively in severity from 0 (normal) through 4+ (very severe)
[c]Lowest levels measured 1-11 days postchallenge expressed as a percentage of prechallenge levels
[d]Highest levels recorded 11-16 days postchallenge expressed as a percentage of prechallenge levels
[e]Intragastric
[f]Per os and intranasal
[g]Not done, dog died nine days postinfection

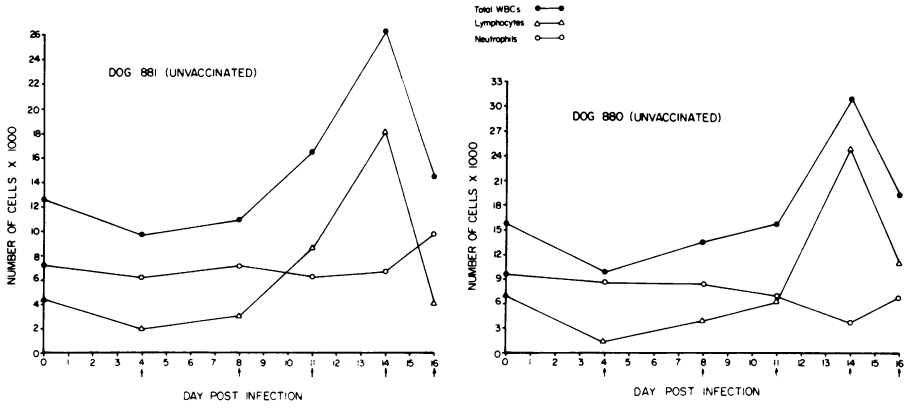

Fig. 1. Leukocyte responses in unvaccinated dogs 881 (1a) and 880 (1b) after oral and nasal inoculation of CPV.

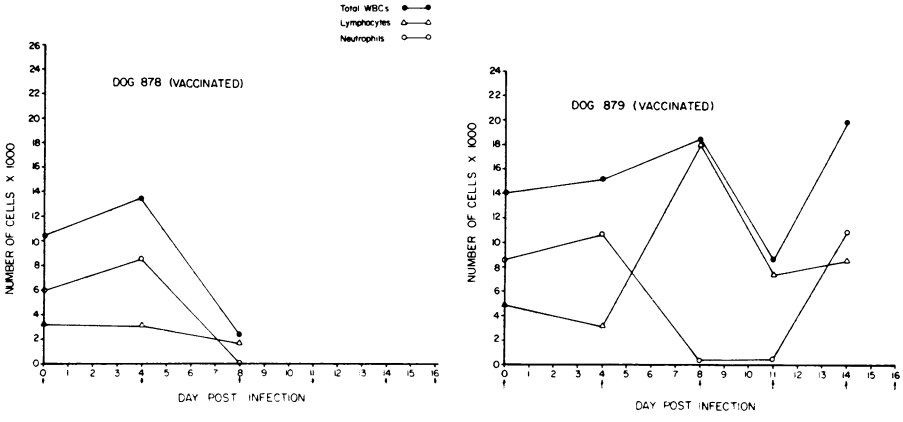

Fig. 2. Leukocyte responses in vaccinated dogs 878 (2a) and 879 (2b) after oral and nasal inoculation with CPV. Dog 878 died on the ninth day postinfection.

illness was not seen in these dogs.

Three DHL-vaccinated dogs (878,879,882) challenged intranasally and orally with CPV developed clinical illness on the fifth day postinfection. Marked anorexia and depression, and severe diarrhea were noted except for dog 882 in which these signs were moderate. Body temperatures were measured daily but elevated temperatures were not recorded. One dog died on the ninth day postinfection. The other two pups recovered by the tenth day. Of the three dogs with clinical signs, two (878, 879) had a severe neutropenia (Figs. 2a and 2b) and the third (882) a mild panleukopenia (Fig. 3). Both of the surviving pups had a moderate leukocytosis by the 14th day postinfection (Figs. 2 and 3). Two of three DHL-vaccinated dogs challenged intragastrically with CPV developed anorexia and a mild diarrhea four to six days postinfection. These two dogs also developed panleukopenia four to 11 days and a lymphocytosis 11 to 14 days after challenge.

Necropsy of the dog which died after challenge with CPV revealed emaciation and voluminous amounts of greenish mucoid fluid in the proximal two-thirds of the small intestine. Histological examination of tissues revealed crypt

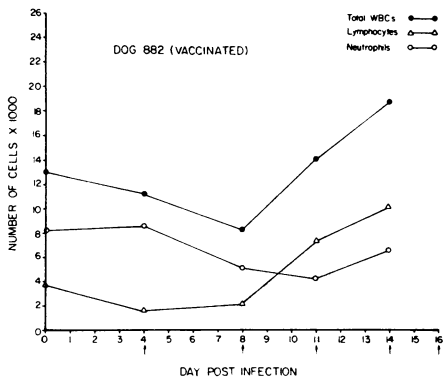

Fig. 3. Leukocyte responses in vaccinated dog (882) after oral and nasal inoculation with CPV.

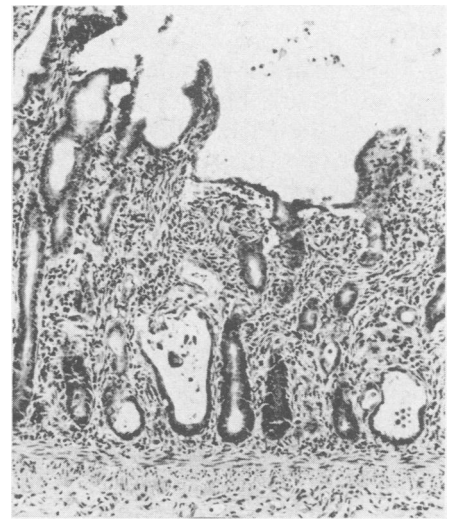

Fig. 4. Ileum of dog (878) with experimental CPV infection. Note dilated crypts, flattening of crypt epithelium, cellular debris in crypt lumens and villous atrophy. H & E. X93.

dilatation of the small and large intestine. Necrosis and flattening of the crypt epithelium of the small (Fig.4) and large (Fig. 5) intestine was observed. Marked lymphoid depletion was noted in the Peyer's patches (Fig. 6) and mesenteric lymph nodes. The myocardium and lungs were normal. A bone marrow smear from this dog indicated a severe granulocytic and mild erythroid depletion of the cells approaching maturity. A marked hyperplasia of neutrophilic granulocyte precursors with a definite left shift was seen (myelocytes and progranulocytes predominated). Few metamyelocytes and even fewer band cells were found. Mature segmented cells were rare. Eosinophils, lympho-

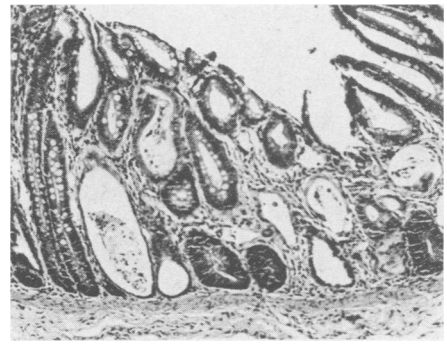

Fig. 5. Colon of dog (878) with experimental CPV infection. Note dilated crypts, cellular debris in crypt lumens and flattening of crypt epithelium. H & E. X73.

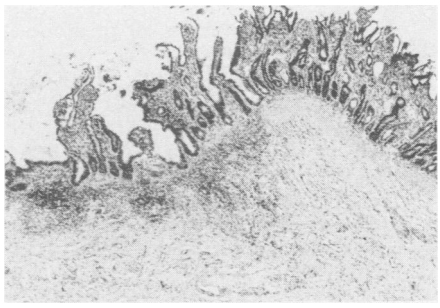

Fig. 6. Ileum of dog (878) with experimental CPV infection. Note depletion of lymphocytes in Peyer's patches and a few dilated crypts. The luminal part of the mucosa is autolysed. H & E. X50.

cytes, plasma cells, and macrophages were normal. Bone marrow cytology on dogs 880 and 881, which were never clinically ill, showed erythroid hyperplasia (both dogs) and granulocyte hyperplasia (dog 881) 14 days postinfection.

All dogs challenged with CPV developed antibodies by the eighth day postinfection (Table II). Maximal antibody titers were measured by the 11th day. Virus was recovered from the feces of all the dogs on the fifth and eighth days but not on the 11th and 14th days postinfection. All dogs challenged per os were killed 14 days after infection. Two dogs had a few localized areas of crypt epithelium necrosis and crypt dilatation at the ileocolonic junction. All had follicular hyperplasia of lymphoid tissues of the spleen, ileum and large intestine.

DISCUSSION

The pathogenesis of CPV-associated disease in dogs appears to be complex. Experimental inoculation of dogs with CPV does not result in disease (2, 15, 18) suggesting that other factors are involved. Moreover, evidence suggests that natural infections of dogs with CPV are often mild or subclinical (22). Recently, however, Robinson *et al* (19) reported experimental production of disease after intravenous virus challenge but were unable to reproduce the disease after oral challenge (W.F. Robinson, personal communication, 1980).

The reason for successful oral-nasal and gastric challenge in the present study is unclear. It is possible that virus strains may vary in pathogenicity. In the study reported here, five of eight dogs became ill four to five days after virus challenge and a significant leukopenia developed in seven of eight dogs. The most severe disease developed in DHL-vaccinated dogs after oral-nasal challenge. Intragastric challenge resulted in mild clinical signs only which suggests that nasopharyngeal replication of the virus may be a significant step in the pathogenesis of the disease. The animals most severely affected also had the most severe neutropenia (2%-6% of normal levels) whereas lymphocyte levels only fell to approximately 50% of normal counts. Significant neutropenia and clinical illness were recorded only in dogs vaccinated with DHL prior to CPV challenge whereas the two unvaccinated dogs remained healthy. It is tempting to speculate that prior exposure of DHL may have influenced the course of subsequent CPV infection. Several mechanisms are theoretically possible but speculation at this point is premature since the number of dogs used was small and additional experiments are necessary to exclude the possibility of coincidence.

Nevertheless this report appears to be the first on successful reproduction of the disease after oral virus challenge and the study revealed several interesting aspects of the disease. The pathological changes of experimental CPV disease were similar to that of spontaneous disease. Infection with CPV resulted in characteristic enteritis, lymphopenia, neutropenia, or panleukopenia. Infected dogs which remained healthy developed a lymphopenia only. Recovery from infection was associated with a transient lymphocytosis, a rapid antibody response and a cessation of virus excretion. The incubation period was four to five days and the course of the disease was one to four days.

Some differences between experimental disease and spontaneous disease were noted. The enteritis observed was not hemorrhagic as is often seen in natural-occurring cases (4, 14, 16), nor was fever a feature in contrast to that recorded for spontaneous disease (1). Colitis was observed in three experimentally infected dogs but it has not been observed frequently in natural occurring disease (3, 16). However, colitis, usually localized, often occurs in feline panleukopenia (21). Knowledge of the incubation period is of importance but little information on this aspect of the disease has been published. Our data suggests it may be approximately five days, which is similar to that reported for feline panleukopenia (8). A slightly shorter incubation period, three days, has been observed after intravenous virus challenge (19).

A transient leukocytosis, primarily due to a lymphocytosis, was observed during a period which seemed to coincide with convalescence, rapid antibody production and cessation of virus excretion in the feces. These observations argue against the proposed concept of an autoimmune mechanism of disease with CPV (18). The leukocytosis reached levels as high as

TABLE II. Antibody[a] Response of Dogs Experimentally Infected Intranasally and Orally with CPV

	Days Postinfection				
Dog No.	0	4	8	11	14
878	<10	<10	40	ND[c]	ND
879	<10	<10	80	1280	2560
880[b]	<10	<10	160	2560	2560
881[b]	<10	<10	80	2560	2560
882	<10	<10	80	1280	2560

[a] Reciprocal of highest dilution in which antibody was detected by IFAT
[b] Did not receive DHL vaccine
[c] Not done; dog died

33,000 per μm^3, a phenomenon that could cause confusion in diagnosis especially with neoplastic disease such as leukemia. The important feature of the lymphocytosis with CPV is that it is transient. It is of interest that transient leukocytosis has also been observed in experimental feline panleukopenia (8) and has been observed in natural cases of CPV disease examined at the Veterinary Teaching Hospital, University of Tennessee.

Canine parvovirus infection resulted in a marked depletion of granulocytes in the bone marrow suggesting that neutrophil precursors constituted one of the targets of the virus. Erythroid elements were slightly affected but there was no effect on the other cellular elements of the bone marrow. During convalescence there was a hyperplasia of the granulocytic and erythroid elements of the bone marrow in an apparent effort to replenish cells lost during the acute phase of the disease. Anemia was not observed in any of the dogs. Circulating levels of erythrocytes apparently are not affected since mature cells have a long half life (20) relative to the short period the virus suppresses production in the marrow. The neutropenia can be profound in CPV infection. Severe to moderate neutropenia has been recorded in spontaneous disease (1) and was observed in five of eight dogs in this study. The dramatic drop in circulating neutrophils seems to be the result of the interrupted production in the bone marrow which, although transient, is important since the lifespan of neutrophils is very short (20). Extensive tissue injury creates a great demand for neutrophils (20). The damaged intestinal epithelium in CPV disease probably demands a tremendous neutrophil response which, since it occurs at a time when the supply from the bone marrow is interrupted, may be a major contributing factor to the neutrophil depletion.

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