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Comparison of Systemic and Local Immunity in Dogs with Canine Parvovirus Gastroenteritis

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To determine whether resistance to canine parvovirus (CPV) gastroenteritis is mediated by local or systemic immunity or both, an enzyme-linked immunospecific antibody assay (ELISA) was developed that quantitated different classes of antibody to CPV. Antibody levels in serum and feces of dogs with CPV-associated gastroenteritis were compared with their clinical signs and viral hemagglutination (HA) titers. Dogs with high levels of CPV coproantibody had a favorable clinical prognosis, high serum antibody levels (hemagglutination inhibition [HI] and ELISA), and low viral HA titers in feces. Conversely, dogs with little or no detectable CPV coproantibody had severe clinical signs and associated mortality rates and high viral HA titers in feces. Many of these dogs had high HI antibody titers. Statistical analysis revealed that only coproantibody level correlated (inversely) with HA titer; serum antibody, whether measured by HI or ELISA, did not. These data suggest that local intestinal immunity is more important than humoral immunity in developing immunological resistance to CPV gastroenteritis.

Canine parvovirus (CPV) infection in dogs has been associated with outbreaks of acute gastroenteritis characterized by bloody diarrhea, vomiting, depression, leukopenia, pyrexia, dehydration, and sometimes death (1). Within a year of the initial description, outbreaks were reported throughout the United States, in Europe, and in Australia (1).

The rapid spread of the disease has accentuated the demand for effective immunoprevention measures. Several vaccines are currently in use, including the closely related feline panleukopenia virus (FPLV) and attenuated CPV vaccines. However, an important and as yet unanswered question concerns the type of immunity required for protection from CPV gastroenteritis. Because there is a viremic phase and concomitant lymphopenia and neutropenia (1) accompanying intestinal infection, one or several types of antiviral immunity may be important. Humoral antibody alone could contain the disease by preventing viral spread to secondary sites such as the intestine. On the other hand, local intestinal antibody (coproantibody) or a combination of local and humoral immunity may be required for complete protection. Thus, humoral antibody may lessen the severity of disease by limiting viremia yet still permit CPV replication in the gut. Without coproantibody the dog could conceivably become an inapparent carrier of CPV.

To study local and systemic immunity to CPV, we have developed an enzyme-linked immunospecific antibody assay (ELISA) to quantitate viral antibody of the immunoglobulin G (IgG), IgA, and IgM classes. We examined sera and feces of dogs with a clinical diagnosis of CPV gastroenteritis and compared serum antibody and coproantibody levels with clinical signs, viral hemagglutination (HA) titers, serum hemagglutination inhibition (HI) titers, and outcome of disease to ascertain the relative importance of local versus systemic immunity in resistance to CPV gastroenteritis.

MATERIALS AND METHODS

Dogs. Dogs chosen were clinical patients at either The Ohio State University College of Veterinary Medicine Hospital or the Columbus Animal Control Center. Clinical signs of CPV enteritis included bloody diarrhea, vomiting, depression, leukopenia, pyrexia, and dehydration. Blood and fecal samples were taken whenever possible. Vaccinated dogs were part of another study (Jack Gordon and William Rogers, Ohio State University College of Veterinary Medicine). Dogs were vaccinated with feline viral rhinotracheitis, calicivirus, panleukopenia virus vaccine containing modified live FPLV. The vaccine was administered to the dogs according to the manufacturer's directions in July 1980. Dogs returned to the Ohio State University Veterinary Hospital for a booster in January 1981. At that time, blood and fecal samples were collected. Two weeks later, blood and feces were again collected.

TABLE 1. Summary of data collected from 15 dogs with high levels (>1.5 ng) of coproantibody to CPV[a]

Days after onset of clinical signs	Fecal analysis					Serum antibody			
	CPV HA titer	Occult blood	CPV antibody (ELISA) (ng)[b]			HI titer	CPV antibody (ELISA) (ng)[c]		
			IgA	IgG	IgM		IgG	IgM	
0–4	48 ± 5[d] (16)[e]	1.25 ± 0.31[f] (12)	0.92 ± 0.23[f] (15)	0.68 ± 0.16 (15)	0.66 ± 0.18 (15)	305 ± 50[d] (11)	0.64 ± 0.19[f] (11)	0.11 ± 0.03 (11)	
5–9	9 ± 3 (7)	1.86 ± 0.55 (7)	0.84 ± 0.23 (7)	0.82 ± 0.21 (7)	0.66 ± 0.27 (7)	768 ± 90 (5)	0.87 ± 0.28 (6)	0.17 ± 0.03 (6)	

[a] In 9 of 15 dogs, samples from two or more time periods were tested. Dogs (3 out of 15) were euthanatized early in the course of disease at the owner's request.
[b] Nanograms of antibody bound per 5 mg of lyophilized feces.
[c] Nanograms of antibody bound per 10 μl of serum.
[d] Geometric mean titer ± standard error of the mean.
[e] Numbers in parentheses are the numbers of samples tested.
[f] Arithmetic mean titer ± standard error of the mean.

Preparation of fecal extracts for HA and ELISA assays. The fecal extraction method of Haneberg and Tonder (7) was used. Feces were collected and frozen within 2 h of defecation. Samples were lyophilized, crushed, and weighed. Extracts were made by adding 10 ml of phosphate-buffered saline, pH 7.2, to each gram of dry feces. After gentle mixing for 30 min at room temperature, the mixtures were centrifuged at 20,000 × *g* for 30 min at 4°C. The supernatants, designated extracts, were decanted and stored at −70°C until use.

HA. CPV hemagglutinates porcine erythrocytes at 4°C (5). The method of Appel et al. (1) was used to quantitate CPV in fecal extracts. Extracts were tested in duplicate. The highest dilution of extract which caused formation of a uniform erythrocyte mat was regarded as the endpoint and its reciprocal as the HA titer. HA titers of <16 were considered negative.

HI. The method of Appel et al. (1) was used to quantitate serum HI antibody. The highest serum dilution which inhibited CPV-associated HA was regarded as the endpoint and its reciprocal as the HI titer.

ELISA for quantitation of CPV antibody of the IgG, IgA, and IgM classes. A modification of a previously published procedure (12) was used to quantitate CPV antibody. A 1:20 dilution of CPV tissue culture antigen was used to coat the wells. The antigen was prepared by infecting mink lung cells (CCL-64) with filtered (0.45 μm) fecal extract (808–10) of high (4,096) HA titer from a dog with hemorrhagic enteritis and, 5 days later, harvesting according to the method of Carmichael et al. (5). The HA titer of the resultant CPV antigen was 1,024. Rabbit anti-dog IgG, anti-dog IgA, and anti-dog IgM were purchased from Miles Laboratories, Inc., Elkhart, Ind. IgG was conjugated to horseradish peroxidase by the method of Nakane and Kawaoi (10). Duplicate samples (100 μl) of a 1:10 dilution of serum or a 1:2 dilution of fecal extract were tested. Standard curves for absorbency versus nanograms of anti-IgG (or anti-IgA or anti-IgM) IgG for each conjugate (20 μl of conjugate per well, four dilutions, each in quadruplicate) were constructed. Pooled CPV-negative, germfree canine serum and fecal extract were used as negative controls. A positive serum and fecal sample were used as positive controls. Absorbency values less than that of the lowest standard used but greater than the negative controls were recorded as trace and arbitrarily assigned a value of 0.2 ng. For anti-IgG, anti-IgA, and anti-IgM, the lowest standards used were 0.32, 0.60, and 0.65 ng of conjugated immunoglobulin, respectively.

Detection of fecal occult blood. Fecal extracts were tested with the hemoccult test (Smith Kline Diagnostics, Sunnyvale, Calif.), and based on color intensity, a score from 0 to 3+ was assigned.

RESULTS

Comparison of CPV coproantibody level with viral HA titer, serum CPV antibody (ELISA and HI), and clinical signs of CPV gastroenteritis. Viral HA titers, HI titers, serum CPV antibody (ELISA), CPV coproantibody, and clinical signs of dogs with diagnosed CPV gastroenteritis were

TABLE 2. Summary of data collected from 28 dogs with low levels (<1.5 ng) of coproantibody to CPV[a]

Dogs	Days after onset of clinical signs	Fecal analysis					Serum antibody		
		CPV HA titer	Occult blood	CPV antibody (ELISA) (ng)[b]			HI titer	CPV antibody (ELISA) (ng)[c]	
				IgA	IgG	IgM		IgG	IgM
Dogs that survived (n = 21)[d]	0–4	70 ± 6[e] (20)[f]	1.0 ± 0.48[g] (11)	0.08 ± 0.06[g] (18)	0.17 ± 0.06 (18)	0.06 ± 0.02 (18)	640 ± 71[e] (16)	0.20 ± 0.08[g] (14)	0.20 ± 0.13 (14)
	5–17	84 ± 4 (25)	0.19 ± 0.10 (16)	0.10 ± 0.02 (23)	0.13 ± 0.02 (23)	0.08 ± 0.03 (23)	1,629 ± 121 (11)	0.43 ± 0.12 (10)	0.31 ± 0.13 (10)
Dogs that died (n = 7)[h]	0–4	1,843 ± 114 (6)	1.0 ± 1 (2)	0.15 ± 0.03 (8)	0.25 ± 0.07 (8)	0.05 ± 0.03 (8)	498 ± 67 (9)	0.63 ± 0.24 (9)	0.23 ± 0.10 (9)
	≥5	—[i]	—	—	—	—	—	—	—

[a] In 12 of 28 dogs, samples from two or more time periods were tested.
[b] Nanograms of antibody bound per 5 mg of lyophilized feces.
[c] Nanograms of antibody bound per 10 μl of serum.
[d] Includes four dogs which were euthanized at the owner's request despite their stable clinical signs. They were euthanized at 4, 6, 8, and 15 days post-initial sign.
[e] Geometric mean titer ± standard error of the mean.
[f] Number in parentheses is number of samples tested.
[g] Arithmetic mean titer ± standard error of the mean.
[h] Includes two dogs which were euthanized in extremely depressed condition at 2 and 3 days post-initial sign. Dogs which died did so at 2, 3, 5, and 12 days post-initial sign.
[i] —, Not tested.

TABLE 3. Comparison of mean HA titers with levels of CPV coproantibody and serum CPV antibody and with HI titers[a]

Set	Group	Samples selected for:	n	Geometric mean HA titer	P[b]
1	a	High levels of coproantibody (>1.5 ng)[c]	14	24	<0.01
	b	Moderate levels of coproantibody (<1.5 ng > trace)	8	36	NS[d]
	c	Low levels of coproantibody (zero to trace)	45	107	
2	a	High levels of serum antibody (>1.0 ng)[e]	13	157	NS
	b	Moderate levels of serum antibody (<1.0 ng > trace)	11	38	NS
	c	Low levels of serum antibody (zero to trace)	18	89	
3	a	High HI titers (≥1,280)[f]	20	106	NS
	b	Moderate HI titers (160 to 640)	16	38	NS
	c	Low HI titers (<160)	9	170	

[a] Data are from Tables 1 and 2.

[b] Student's two-tailed *t* test was used to determine the significance between the mean HA titers of groups a and b with c.

[c] Geometric mean HA titer was determined on fecal samples which had total CPV coproantibody of >1.5 ng per 5 mg of lyophilized feces as determined by ELISA.

[d] NS, Not significant.

[e] Geometric mean HA titer was determined on fecal samples of dogs that on that day had total CPV serum antibody of <1.0 ng per 10 μl as determined by ELISA.

[f] Geometric mean HA titer was determined on fecal samples of dogs that on that day had an HI titer of ≥1,280.

compared. We divided the dogs into two groups: those which, on at least 1 day, had total (IgG, IgA, and IgM) CPV coproantibody levels of greater than 1.5 ng, and those with little or no detectable coproantibody. Data from dogs with high levels of coproantibody are presented in Table 1. Most of these dogs had favorable prognostic signs. Of the 15 dogs in Table 1, 1 had recovered when tested, 9 were in early convalescence, and 3 were euthanatized early in the course of the disease. Only two of the dogs were still sick, but the latest samples available for coproantibody testing were 3 days after presentation. It is likely that these dogs would have recovered in a few days, as indeed the drop in their HA titers suggests. None of the dogs in Table 1 died from CPV gastroenteritis.

Serum HI titers of dogs in Table 1 were generally high (geometric mean = 416) but varied greatly (20 to 5,120). The mean titer increased approximately twofold in the latter half of the clinical course of disease. Serum CPV antibody, as determined by ELISA, was related to coproantibody level only in the IgG class. IgA antibody was the most abundant class in feces but was either absent or present in minute amounts in serum. IgM antibody was also more abundant in feces. Viral HA titers were generally low in dogs with coproantibody (geometric mean of 48 from 0 to 4 days after clinical onset) and decreased to 9 on days 5 to 9 after the onset of clinical signs. Presence of fecal occult blood was not related to coproantibody levels (Tables 1 and 2), indicating that coproantibody of all three classes was actively secreted into the gut and that it had not entered via intestinal hemorrhage.

Data from dogs whose total coproantibody on any day tested was less than 1.5 ng are presented in Table 2. The overall clinical picture of these dogs was different from that of dogs in Table 1. Dogs with severe clinical signs were selected from both tables. By the *t* test on two samples from binomial populations, the dog populations in Tables 1 and 2 are significantly different ($P < 0.005$). Data from dogs that survived the disease and those that did not are presented separately in Table 2. Mean serum HI titers at days 0 to 4 post-initial sign were approximately the same in the two groups and similar to HI titers of dogs in Table 1. The mean HI titer increased more than twofold by days 5 to 17 in the surviving dogs. Compared with values in Table 1, serum IgG antibody (ELISA) was slightly lower, whereas serum IgM antibody was slightly higher. Mean serum antibody (HI and ELISA) and coproantibody levels were similar in dogs that either survived or died. The mean viral HA titer, however, was much higher (1,843) in dogs that died than in those that did not (70). An interest-

ing comparison between dogs in Table 1 and Table 2 is the change in their HA titers in the latter stage of the disease (≥5 days after clinical onset). The mean HA titer of dogs in Table 1 decreased fivefold whereas there was no decrease and even a slight increase in mean HA titer of dogs in Table 2.

Inverse correlation of coproantibody level and HA titer. To determine whether the general associations presented in Tables 1 and 2 were statistically significant, samples were selected for high (>1.5 ng), moderate (< 1.5 ng > trace), and low (zero to trace) levels of coproantibody (set 1); for high (>1.0 ng), moderate (< 1.0 ng > trace), and low (zero to trace) levels of serum antibody (ELISA) (set 2); and for high (≥1,280), moderate (160 to 640), and low (<160) HI titers (set 3).

The geometric mean HA titers of the three groups in each set were compared by the Student's two-tailed *t* test, and the results are presented in Table 3. There was a significant difference ($P < 0.01$) in mean HA titer only between the group with high levels and the group with low levels of coproantibody. No statistically significant relationship was found between HA titer and serum antibody (ELISA) or between HA titer and HI titer.

The association of high CPV coproantibody levels with low HA titers was further analyzed by the method of least squares. Samples with the same HA titer were selected, and their coproantibody values were averaged. The amount of coproantibody was found to be negatively correlated with HA titer. The slope of the line was −0.13, the correlation coefficient was −0.65, and the probability that coproantibody level and HA titer were not related was <0.02 by the two-tailed *t* test.

Coproantibody and serum CPV antibody in dogs vaccinated with modified live FPLV. We had established that only CPV coproantibody and not serum antibody (ELISA or HI) was negatively correlated with HA titers and severity of clinical signs. It was, therefore, of interest to determine whether coproantibody production was induced by vaccination with a currently used modified live FPLV vaccine. Blood and fecal samples from 13 dogs were collected 6 months after vaccination and 2 weeks after a booster vaccination. All dogs were clinically healthy throughout the study.

CPV coproantibody levels, serum CPV antibody (ELISA and HI) levels, and viral HA titers are shown in Table 4. HA titers in all 13 dogs both pre- and postbooster were negative (<16), indicating that there was no active CPV infection during the period in which samples were obtained. The immune responses to vaccination were similar in all dogs. Vaccinated dogs did not develop significant CPV coproantibody. In contrast, all dogs developed high or very high serum IgG antibody (ELISA) (values were much higher than seen in infected dogs [Tables 1 and 2]). IgM antibody (ELISA) was much lower than IgG, and all dogs were negative for serum IgA antibody both before and after the second vaccination. Serum HI titers were moderate (geometric mean = 208) but ranged from negative (<20) to >2,560. After booster vaccination, HI titers increased in half of the dogs to a mean of 373. An almost parallel increase was seen in serum IgG (ELISA) levels, from a mean of 41.0 to 70.0 ng of antibody bound per 10 μl of serum. No increase was seen in serum IgM or in any class of coproantibody.

DISCUSSION

In the present study, an ELISA assay was developed and used to quantitate different classes of antibody to CPV. By examining antibody levels in the sera and feces of dogs admitted to the hospital or pound with a diagnosis of CPV gastroenteritis, in conjunction with quantitative comparisons of these antibodies to viral HA titers and severity of clinical signs, we found that only coproantibody was correlated with low HA titer and with favorable prognostic signs. Serum CPV antibody, measured by either ELISA or HI, did not appear to be related to either parameter.

Our results are in agreement with the observation that immunological resistance to mucosal infection can exist in the absence of detectable serum antibody (2). Tomasi and Bienenstock (13) measured IgA antibody to a variety of viruses, bacteria, and other agents and found that resistance correlated best with this local antibody rather than with circulating antibody. Other workers (16) have shown that parenteral inoculation of susceptible patients with inactivated poliovirus vaccine did not prevent subsequent intestinal infection despite the development of serum antibody. Local resistance was associated with development of intestinal secretory IgA activity against poliovirus. Recent studies (3, 4, 6, 11) with the Norwalk agent, a parvo-like enteric viral agent that causes acute diarrheal disease in humans, have also emphasized the lack of correlation of serum antibody (as well as local antibody [3, 4, 6]) with resistance to enteric disease. Serum antibody reflected only prior infection, not protection, and, paradoxically, even constituted a risk factor for those who possessed it (3).

IgA is the predominant class of antibody on mucosal surfaces. It is synthesized in local plasma cells, combines with secretory components in epithelial cells, and then is actively secreted

TABLE 4. Dogs vaccinated and boosted with modified live FPLV[a]

Dog	Vaccination	Coproantibody[b]			Occult blood	Serum antibody[c]			
		IgG	IgA	IgM		HI	IgG	IgA	IgM
29	First	0	0	0	0	640	33.0	0	0.65
	Second	0	0	0	0	640	62.4	0	Trace
30	First	0	0	0	0	40	11.4	0	Trace
	Second	0	0	0	0	80	20.4	0	Trace
31	First	0	0	0	0	40	53.2	0	1.19
	Second	0	0	0	0	80	55.6	0	1.19
32	First	0	Trace	0	0	320	58.8	0	1.04
	Second	0	0	0	0	640	149.8	0	0.77
33	First	0	Trace	0	0	80	15.0	0	Trace
	Second	0	0	0	0	320	58.2	0	Trace
34	First	Trace	0	0	0	640	230.0	0	Trace
	Second	Trace	0	0	0	640	300.0	0	Trace
35	First	0	0	0	0	>2,560	19.2	0	Trace
	Second	Trace	0	0	0	>2,560	32.0	0	Trace
36	First	0	0	0	0	640	0.40	0	Trace
	Second	0	0	0	0	1,280	1.14	0	Trace
37	First	Trace	0	0	0	320	34.6	0	1.20
	Second	Trace	0	0	0	320	38.0	0	1.65
38	First	0	0	0	0	1,280	3.2	0	Trace
	Second	Trace	0	0	0	1,280	15.4	0	Trace
39	First	0	0	0	0	<20	1.92	0	0.65
	Second	0	0	0	±	40	2.6	0	0.70
40	First	0	0	0	0	320	3.2	0	0
	Second	0	0	0	0	ND[d]	ND	ND	ND
41	First	0	0	0	1+	<20	30.8	0	1.1
	Second	0	0	0	1+	80	104.2	0	1.3

[a] Dogs were vaccinated with feline viral rhinotracheitis, calicivirus, panleukopenia virus according to the manufacturer's directions. Six months later, blood and fecal samples were taken, and the dogs were boosted. Two weeks later, blood and fecal samples were again collected. HA titers in all 13 dogs were negative (<16) both pre- and postbooster.

[b] Anti-CPV coproantibody was quantitated by the ELISA assay. Values are nanograms of antibody bound per 5 mg of lyophilized feces.

[c] Anti-CPV serum antibody was quantitated by HI and ELISA assays. For HI, titers are the reciprocals of the highest dilutions which inhibited HA. For ELISA, values are nanograms of antibody bound per 10 μl of serum.

[d] ND, Not done.

onto mucosal surfaces (15). However, IgG and IgM are also produced locally in the intestinal lamina propria (9, 14, 15). These classes appear to be transported into the gut by a nonspecific process shared by other proteins entering the intestinal lumen (15). Our finding of IgG and IgM coproantibodies and their lack of association with fecal occult blood supports the above findings and indicates that all three classes of antibody are normally synthesized and transported into the intestines of dogs suffering from CPV gastroenteritis. A point to make is that actual quantities of antibody in the intestines are undoubtedly larger than the coproantibody values reported here due to dilution with fecal material as well as to the fact that secretory antibodies adhere very strongly to the mucous coat (15).

The relationship of high levels of coproantibody to recovery from disease is apparently not an all-or-none phenomenon. Of the 22 noneuthanatized dogs with little or no detectable coproantibody (Table 2), 8 were improving clinically. Thus, factors in addition to coproantibody may influence recovery from, and perhaps

resistance to, CPV gastroenteritis. Alternatively, intestinal coproantibody could have been present but not detected in the feces. For example, it could have been bound in complexes with CPV. Quantitation of CPV in feces before and after dissociation of possible immune complexes would help to settle this question. Interestingly, these clinically improving dogs which had little or no detectable coproantibody had HA titers which were not decreasing by 2 weeks after the onset of CPV gastroenteritis. This suggests that without sufficient coproantibody, CPV replication may persist for variable periods, resulting in a carrier state. Such a situation is described for rubella where lack of mucosal antibodies may result in a carrier state even in the presence of systemic immunity (14).

Finally, feline parvovirus origin vaccine products are known to provide only limited and short-term (less than 6 months) immunity to CPV infection (8). The data collected from the vaccination protocol described indicate that use of this product did not result in local CPV-specific coproantibody. This feature may explain the apparent lack of long-term success noted with this type of product.

In conclusion, we have examined dogs clinically affected with CPV-related gastroenteritis for evidence of determinants of resistance and immunity in this disease. Our data indicate that recovery, as measured by both resolution of clinical signs of disease and decreased shedding of viral antigen in feces, correlated only with the presence of CPV-specific coproantibody. Thus, it may be that effective immunoprophylactic measures for CPV must be centered around methods that are designed to induce local CPV antibody in the gut of CPV-susceptible dogs.

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