Macrophage Fc Receptors Control Infectivity and Neutralization of Canine Distemper Virus-Antibody Complexes

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Dogs that are persistently infected or that become moribund after exposure to canine distemper virus (CDV) have antibody that neutralized CDV when tested in dog lung macrophage cultures but failed to neutralize CDV when tested in epithelial, fibroblastic, or lymphatic cells. The antibody attached to protein A and was found in the immunoglobulin G fraction. The antibody bound complement and lysed CDV-infected target cells. The neutralizing activity in macrophages could be abolished (i) by pepsin digestion and removal of Fc portions from the antibody, (ii) by blocking the Fc receptors of macrophages with heat-treated normal dog serum, and (iii) by binding of protein A to Fc portions of the antibody. It was concluded that attachment of the CDV-antibody complex to Fc receptors of macrophages was essential for virus neutralization. If this attachment was hindered, the CDV-antibody complex became infectious for macrophages. In contrast, serum from recovering dogs neutralized CDV when tested in epithelial, fibroblastic, or lymphatic cells as well as in macrophages.

Antibody attachment to virus may render the virus noninfectious (9), enhance the infectivity of the virus (6, 12, 24, 25), or leave the infectivity of the virus unchanged (7, 14, 16, 22, 23, 27). Addition of complement (8) or species-specific antibody (19) to an infectious virus-antibody complex may reduce or abolish the infectivity.

The host cell system in which the residual virus infectivity is tested greatly influences the degree of neutralization of the virus (9, 15, 17). However, the manner in which a cell responds to a virus-antibody complex, which leads to either infection of the cell or neutralization of the virus, remains undefined.

We report here that some canine distemper virus (CDV)-antibody complexes are infectious for cells without Fc receptors and for lymphocytes, but are noninfectious for macrophages. Infectivity in macrophages of these complexes is restored when their binding to Fc receptors is blocked.

MATERIALS AND METHODS

Dogs. Specific-pathogen-free (SPF) beagle dogs from the James A. Baker Institute colony were kept in isolation units and fed a commercial ration and water.

Virus. Three strains of virulent CDV were used for in vivo and in vitro studies: Snyder-Hill (SH) and Cornell A75-17 were isolated in our institute (3) and Ohio R252 was kindly supplied by A. Koestner (Ohio State University, Columbus). Virulent virus was maintained by intravenous inoculation of susceptible dogs and by harvesting lymphatic tissues 5 days later, when virus titers were high. Infected tissues were stored at -70°C until used. The SH and A75-17 strains were adapted to Vero cells and used for neutralization tests in vitro. The Vero cell-adapted Onderstepoort strain (2) was used for immunization of dogs and in neutralization tests in vitro.

Tissue culture. Dog lung macrophage (DLM) cultures were prepared as described previously (4), with some modifications. Lungs from 3- to 6-week-old SPF beagle pups were minced in medium 199 (GIBCO Laboratories) with antibiotics and stirred for 1 h at room temperature. Free cells were

filtered through cheesecloth and were used for virus neutralization (VN) tests in microplates as described below. Approximately 70% of attached cells had esterase activity and the morphological features of macrophages when stained with hematoxylin.

Vero cells were obtained from the American Type Culture Collection, and secondary dog kidney cells were prepared from 3- to 6-week-old SPF dogs and propagated in Eagle minimum essential medium (MEM; GIBCO) with 10% fetal calf serum, antibiotics, and 29.2 mg of glutamine per 100 ml. Both cell types were stored in liquid nitrogen until used.

Lymphocytes from thymus and spleen tissue from 3- to 6-week-old SPF dogs were separated on Ficoll-Hypaque gradients and cultured in the presence of phytohemagglutinin in medium RPMI 1640 with 20% fetal calf serum for 4 days at 37°C in 5% CO₂-95% air.

Dog inoculations. Eight- to 14-week-old SPF dogs were inoculated intranasally with approximately 5×10^3 DLM 50% infectious doses of virulent CDV, strain SH, A75-17, or R252. Dogs were monitored for progression of disease or recovery as described elsewhere (5, 33). Six dogs were immunized with approximately 5×10^3 50% tissue culture infectious doses of the Onderstepoort strain of CDV.

Serum and CSF. Blood was collected at weekly, sometimes daily, intervals by venipuncture from dogs after exposure to CDV. Cerebrospinal fluid (CSF) samples were taken aseptically by puncture of the occipital foramen from anesthetized or euthanized dogs. Serum, CSF, and serum preparations were stored at -20°C until used.

VN tests. VN tests were made in 96-well microtiter plates (Linbro Division of Flow Laboratories) as described before (2). Threefold dilutions of heat-inactivated serum or CSF in MEM were incubated with 30 to 50 PFU of CDV and incubated at room temperature for 1 h. Target cells were then added at concentrations of approximately 5×10^5 DLM cells or 10^4 Vero or dog kidney cells per well. Cultures were incubated at 37°C in 5% CO₂-95% air in a humidified incubator. Titration endpoints were determined by syncytia formation that was induced by free virus 4 days after inoculation with virulent CDV in DLM cultures, 3 days after inoculation in Vero cell cultures infected with the attenuated Onderstepoort strain of CDV, and 7 days in Vero cell

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cultures infected with Vero cell-adapted strain SH or A75-17. Results were calculated by the Kärber method.

Adsorption to protein A. Protein A (*Staphylococcus aureus*) covalently bound to Sepharose 4B-CL (Pharmacia Fine Chemicals, Inc.) was allowed to swell in 0.05 M Tris-buffered saline solution at pH 8.0. After several washes a column was prepared with 6 ml of gel in a 10-ml syringe. A 2-ml portion of serum or CSF was percolated through the column, and unbound material was eluted from the column with Tris-buffered saline solution at a flow rate of approximately 1 ml/min. After approximately 20 ml was collected, protein was no longer detectable in the eluate. Bound material was then eluted with 0.58% acetic acid in 0.15 M NaCl. Approximately 20 ml was collected. Preparations with bound and unbound material were concentrated to the original serum volume by PVP dialysis and then dialyzed extensively against phosphate-buffered saline. The presence of immunoglobulin G (IgG) and IgM in bound or unbound fractions was determined by agar gel immunodiffusion against commercial rabbit anti-canine IgG or IgM (Miles Laboratories, Inc.).

G200 gel filtration. A column (3 by 65 cm) was prepared with G200 Sephacryl Superfine (Pharmacia Fine Chemicals) in 0.1 M Tris-hydrochloride-1.0 M NaCl at pH 8.0. Samples of 4 ml of serum were passed through the column at a flow rate of 11 to 14 ml/h. Four-milliliter fractions were collected, assayed for protein content, and dialyzed against phosphate-buffered saline. Samples with VN activity were tested by agar gel immunodiffusion against commercial rabbit anti-canine IgG or IgM (Miles Laboratories).

Humoral cytotoxicity. Humoral cytotoxicity was tested as described previously (30). ^{51}Cr -labeled CDV-infected secondary dog kidney cells were used as target cells, and guinea pig serum was the source of complement. Threefold serum or CSF dilutions in MEM were made in 96-well tissue culture plates (Linbro Division of Flow Laboratories). Subsequently, 5×10^4 target cells suspended in 50 μl of a 1:4 dilution of guinea pig serum containing 4 U of complement were added. The serum, target cells, and complement were allowed to react for 2 h at 37°C in an atmosphere of 5% CO_2 in air. The percentage of specific cytotoxicity was computed as follows: specific cytotoxicity = $[(^{51}\text{Cr} \text{ released} - \text{spontaneous } ^{51}\text{Cr} \text{ released}) / (\text{total } ^{51}\text{Cr} \text{ added} - \text{spontaneous } ^{51}\text{Cr} \text{ released})] \times 100$.

Preparation of F(ab)_2 fragments by pepsin digestion. F(ab)_2 fragments were produced as reported before (28). Serum or CSF samples were diluted with equal volumes of acetate buffer (0.07 M acetate in 0.05 M NaCl, pH 4.0). Pepsin (2,617 U/mg; Millipore Corp.) was added at a concentration of 2 mg per ml of serum. The solutions were adjusted to pH 3.5 to 4.0 by addition of 1 M HCl and incubated at 37°C for 20 h on a shaker. These preparations were then centrifuged at $12,000 \times g$ for 15 min. Supernatants were decanted and adjusted to pH 7.5 to 8.0 by addition of solid Tris. These preparations were dialyzed against phosphate-buffered saline. Control serum samples were treated identically but without pepsin.

Final products were tested for absence of Fc activity by agar gel immunodiffusion and by their inability to bind complement in a humoral ^{51}Cr release cytotoxicity assay.

Blocking of Fc receptors. Heated (65°C for 60 min) SPF dog serum was used for blocking Fc receptors on macrophages. Macrophages (10^7) in 1 ml of medium were incubated on a shaker plate with an equal amount of heat-treated serum for 30 min at 37°C. Macrophages were then centrifuged, and the pellet was resuspended in medium before macrophages were added to CDV-antibody complexes in VN tests.

Addition of protein A to virus-antibody complex. Various amounts of protein A (*S. aureus*; Pharmacia Fine Chemicals) ranging from 5 to 200 $\mu\text{g/ml}$ in MEM were incubated in 96-well microplates, 25 μl per well, with virus-antibody complexes (100 μl per well) for 1 h at 20°C before target cells in MEM (50 μl per well) were added in VN tests. After the lowest concentration of protein A with optimal activity had been determined, 40 μg of protein A per ml was used in additional serum neutralization tests when applicable.

Effect of weak bases on inactivation of virus-antibody complexes. Treatment of cells with weak bases blocks lysosomal acidification and prevents lysosomal fusions (13, 20,

FIG. 1. CDV (Vero cell-adapted SH strain) neutralizing antibody titers in sera of dogs after exposure to different strains of CDV. Symbols: (○) mean values of VN tests performed in DLM cultures; (□) mean values of VN tests performed in Vero cells. The range of VN titers represent (a) 10 dogs that became persistently infected with CDV strain Cornell A75-17; (b) 10 dogs that recovered from CDV strain Cornell A75-17; (c) 10 dogs that recovered from CDV strain SH; and (d) 6 dogs that were immunized with the Vero cell-adapted Onderstepoort strain of CDV.

TABLE 1. Virus strain comparison in VN tests

Serum from dog no.:	Days post CDV exposure ^a	VN titer (log ₁₀) tested in:					
		DLM			Vero cells		
		CDV-SH virulent	CDV-SH Vero cell passage 5	CDV-A75-17 Vero cell passage 7	CDV- Onderstepoort attenuated	CDV-SH Vero cell passage 5	CDV-A75-17 Vero cell passage 7
1	14	3.4	3.6	3.2	<0.8	<0.8	<0.8
2	14	1.9	2.4	2.2	<0.8	<0.8	<0.8
3	14	2.2	2.9	2.9	<0.8	<0.8	<0.8
4	14	2.2	2.2	2.6	<0.8	<0.8	<0.8
9	24	3.4	3.8	2.9	3.4	3.9	3.2

^a Cornell A75-17 strain.

26) which might be involved in inactivation of virus-antibody complexes after phagocytosis. Macrophages were incubated in medium containing 60 mM ammonium chloride or 0.03 mM chloroquine for 30 min at 37°C. They were then added to virus-antibody complexes which reduced the final concentration of NH₄Cl in the medium to 20 mM, as reported by Helenius et al. (13). The final concentration of chloroquine was 0.01 mM; higher concentrations were found to be toxic to DLM. Results were evaluated after a 4-day incubation period.

RESULTS

When VN antibody titers were determined in sera and CSF from dogs after exposure to different strains of CDV, considerable differences were detected with different indicator cells. At 14 and 21 days postinfection with virulent CDV strain A75-17 or R252, most infected dogs had VN antibody when their serum was tested in DLM cultures, but when tests were made in Vero cells, VN was only detected in sera from recovering dogs (Fig. 1b) and not in dogs that later became moribund or persistently infected (Fig. 1a) (3). In contrast, dogs vaccinated with attenuated CDV produced antibody within 7 days that neutralized CDV when tested in both DLM cultures and Vero cells (Fig. 1d). An intermediate effect was observed in dogs exposed to virulent CDV strain SH (Fig. 1c).

VN antibody in the central nervous system that was effective in DLM cultures but not, or to a lesser degree, in Vero cells was found in dogs persistently infected with CDV

strain A75-17 or R252. Recovered or vaccinated dogs had no VN antibody in the CSF.

VN antibody effects were tested in additional indicator cells: secondary dog kidney cells which are predominately of epithelial origin, secondary dog testicular cells which are of fibroblast origin, and spleen- and thymus-derived phytohemagglutinin-stimulated lymphocytes. With minimal deviation, results were identical to results obtained in Vero cells (data not presented).

To ascertain that antigenic differences between CDV strains would not account for the divergence in neutralization effects, we adapted strains A75-17 and SH to Vero cells and tested VN in different indicator cells. Regardless of the CDV strain used in VN tests, with minor variations, the difference in indicator cells remained unchanged (Table 1).

The following tests were performed to determine the nature and properties of the serum factor that inhibited virus replication in DLM cultures but not in epithelial or fibroblast cells.

Adsorption to protein A. When serum or CSF was percolated through a protein A-Sepharose 4B-CL column, the factor with VN activity remained attached and could be eluted with 0.58% acetic acid (Table 2). The eluted fraction contained IgG as well as IgM when tested by agar gel immunodiffusion.

G200 gel filtration. When serum was fractionated in G200 Sephacryl gel, three protein peaks were obtained (Fig. 2). By agar gel immunodiffusion tests, protein of the first peak reacted with anti-canine IgM, the second peak reacted with

TABLE 2. Protein A-Sepharose 4B-CL column adsorption of antibody

Serum from:	Days post CDV exposure	VN titer (log ₁₀) tested in:					
		DLM			Vero cells		
		Untreated	Protein A		Untreated	Protein A	
Dog no.			Bound	Not bound		Bound	Not bound
1	14	2.9	2.9	<0.8	<0.8	<0.8	<0.8
2	14	1.6	1.3	<0.8	<0.8	NT ^a	NT
3	14	2.5	1.3	<0.8	<0.8	NT	NT
4	14	2.5	2.2	<0.8	<0.8	NT	NT
7	18	4.4	4.3	1.0	2.2	2.5	<0.8
8	20	2.9	3.2	1.2	1.3	1.3	<0.8
CSF no.							
1	30	1.7	1.5	<0.8	<0.8	<0.8	<0.8
6	55	2.0	2.1	<0.8	1.0	0.8	<0.8

^a NT, Not tested.

FIG. 2. Serum protein elution pattern of dog serum 1 from G200 Sephacryl Superfine. VN titers of pooled fractions tested in DLM cultures were: fractions 36 to 38, <0.8 ($\log_{10}$); fractions 42 to 44, 2.0 ($\log_{10}$); fractions 48 to 50, <0.8 ($\log_{10}$).

anti-canine IgG, and the third peak reacted with anti-canine albumin. VN activity was only found in the second peak fraction (Fig. 2). Similar results were obtained when the protein A bound and eluted fraction of serum 1 was fractionated in G200 Sephacryl gel. Although IgM and IgG fractions were obtained, VN activity was found only in the IgG fraction.

Humoral cytotoxicity. Sera with VN activity in DLM cultures or in Vero cell cultures were cytotoxic to CDV-infected dog kidney target cells in the presence of complement (Table 3). Antibody that was adsorbed to protein A and eluted or IgG that was fractionated by G200 Sephacryl filtration retained the cytotoxic activity. Sera with VN activity in either DLM cultures or Vero cell cultures lost their ability to lyse CDV-infected target cells after Fc portions had been removed by pepsin digestion (Table 3).

Reaction of F(ab)₂ fragments. When the Fc portion was removed from CDV antibody by pepsin digestion, the macrophage-restricted VN activity of sera or CSF was greatly reduced or abolished (Table 4). Only a slight reduction was

noticed when F(ab)₂ fractions prepared from sera with VN activity in Vero cells were tested in either Vero cell or DLM cultures. Removal of the Fc portion from sera or CSF with high levels of VN activity in DLM cultures and low levels of VN activity in Vero cell cultures resulted in a reduction of titer in DLM cultures to approximately the level of Vero cell titers (Table 4).

Blocking of Fc receptors. When macrophage Fc receptors were blocked by addition of heated serum from SPF dogs, CDV-antibody complexes became infectious for macrophages if the VN activity of the antibody was restricted to DLM cultures. Of six serum samples tested, VN antibody titers that ranged from $10^{1.7}$ to $10^{2.5}$ in DLM cultures were reduced to $<10^{1.0}$ when Fc receptors were blocked. VN antibody titers of four sera with VN activity in Vero cells were not reduced by Fc receptor blocking.

Addition of protein A to virus-antibody complex. Virus infectivity of CDV-antibody complexes was fully restored in DLM cultures when protein A (40 μ g/ml) was added (Fig. 3; Table 5). The infectivity was restored only when VN activity was restricted to DLM cultures. Sera with VN activity in Vero cell cultures still neutralized CDV after the addition of protein A. When sera with a high VN titer in DLM cultures and a low VN titer in Vero cell cultures were tested for VN activity in the presence of protein A in DLM cultures, the VN titer was reduced to approximately the Vero cell titer (Table 5).

Effect of weak bases on inactivation of virus-antibody complexes. The addition of weak bases (NH₄Cl and chloroquine) caused only a slight reduction in VN titers in DLM cultures (Table 6). Replication of test virus in DLM cultures was not blocked by the addition of the same weak bases.

DISCUSSION

The influence of the indicator cell on variation in residual infectivity of virus after interaction with antibody has been observed repeatedly (9, 15, 17). However, the mechanism involved in these variations has not been explained. We are reporting here that a certain population of antibody in dogs renders CDV noninfectious for macrophages but not for epithelial or fibroblast cells or for mitogen-stimulated lymphocytes. The explanation was found to be an "opsonization" of virus by antibody, which had an effect for macrophages but not for cells without Fc receptors or cells without phagocytic capacity. When the attachment of virus-antibody complex to Fc receptors of macrophages was blocked, the complex became infectious for macrophages as well.

A similar effect could have been observed in earlier studies. For example, Kjellen and Schlesinger (15) found

TABLE 3. Complement-dependent humoral cytotoxicity

Serum from dog no.:	Days post CDV exposure	VN titer ($\log_{10}$) tested in:		Cytotoxicity titer			
		DLM	Vero cells	Untreated	Protein A ^a	G200 ^b	F(ab) ₂ ^c
1	14	2.9	<0.8	1:64	1:64	1:64	$<1:4$
2	14	1.6	<0.8	1:16	1:16	NT ^d	$<1:4$
3	14	2.5	<0.8	1:32	1:32	1:16	$<1:4$
8	20	3.2	1.3	1:32	1:16	1:16	$<1:4$
9	24	3.6	2.7	1:128	NT	NT	$<1:4$
12	54	4.4	3.6	$>1:256$	NT	NT	$<1:4$

^a Antibody bound and eluted from protein A-Sepharose 4B-CL column.

^b IgG fraction after separation on G200 Sephacryl gel column.

^c F(ab)₂ fractions prepared by pepsin digestion of antibody.

^d NT, Not tested.

TABLE 4. VN with F(ab)₂^a fractions of antibody

Serum from:	Days post CDV exposure	VN titer (log ₁₀) tested in:					
		DLM			Vero cells		
		Before digestion	After digestion	Control ^b	Before digestion	After digestion	Control ^b
Dog no.							
1	14	3.2	1.0	2.5	<0.8	<0.8	<0.8
2	14	1.7	<0.8	1.5	<0.8	<0.8	<0.8
3	14	2.5	<0.8	1.7	<0.8	<0.8	<0.8
6	18	3.9	1.5	3.4	2.0	1.5	1.5
7	18	4.4	2.0	4.1	2.0	1.7	2.0
8	20	3.2	1.0	3.4	1.3	1.0	1.3
9	24	3.6	1.7	3.4	2.7	1.5	2.2
10	37	4.2	1.5	4.1	2.0	1.7	1.7
11	54	3.4	2.5	3.9	2.9	2.5	2.5
12	54	4.4	2.2	2.9	3.6	2.7	2.9
CSF no.							
4	50	1.5	<0.8	1.3	<0.8	<0.8	<0.8
5	50	1.7	<0.8	1.3	0.8	<0.8	<0.8
7	55	2.9	1.5	2.5	1.9	1.7	1.7
9	60	2.5	1.0	1.9	1.5	1.0	1.2

^a Prepared by pepsin digestion.^b Pepsin omitted in procedure.

vesicular stomatitis virus to be neutralized by antibody in a cell line derived from leukemia bone marrow but not in chicken embryo fibroblasts. The bone marrow-derived cell line may have been macrophage derived and neutralization may have occurred via Fc receptors. Similarly, Lafferty (17) observed a reduction by antibody of rabbit poxvirus infectivity to 0.1 to 0.5% of total virus infectivity when titrations were made in the skin of rabbits, but only a 10% reduction when titrations were made on chorioallantoic membranes of embryonated chicken eggs. Macrophages in the rabbit skin may have been responsible for the results.

Antigenic differences, as reported for measles virus (1), between CDV strains that were used for VN tests in different cell systems could not account for the VN in macrophages. Although minor variations were observed in VN tests among

CDV strains, the principal difference between VN in macrophages and other cells remained unchanged.

Separation of serum immunoglobulins on protein A-Sepharose 4B-CL and Sephacryl G200 columns resulted in IgG fractions that had VN activity in macrophages but not in other cells. Although both IgG and IgM fractions were

TABLE 5. Addition of protein A to virus-antibody complex

Serum from:	Days post CDV exposure	VN titer (log ₁₀) tested in:			
		DLM		Vero cells	
		Un-treated	Protein A added ^a	Un-treated	Protein A added ^a
Dog no.					
1	14	2.9	<0.8	<0.8	NT ^b
2	14	1.6	<0.8	<0.8	NT
3	14	2.5	<0.8	<0.8	NT
4	14	2.5	<0.8	<0.8	NT
5	14	2.7	<0.8	<0.8	NT
6	18	3.9	2.2	2.5	2.2
7	18	4.4	1.5	2.2	1.9
8	20	2.9	1.0	1.3	1.1
9	24	3.9	2.5	2.9	2.6
10	37	4.2	1.8	2.0	2.0
11	54	3.9	2.5	2.9	2.7
12	54	3.9	2.5	3.2	2.9
CSF no.					
1	30	1.7	<0.8	<0.8	NT
2	30	1.5	<0.8	<0.8	NT
3	45	1.3	<0.8	<0.8	NT
4	50	1.5	<0.8	<0.8	NT
5	50	1.7	<0.8	0.8	NT
6	55	2.0	0.8	1.0	0.8
7	55	2.9	2.0	1.9	1.7
8	60	1.5	<0.8	<0.8	NT
9	60	2.5	1.5	1.5	1.5
10	54	2.5	1.5	1.0	0.8

^a 1 µg per well.^b NT, Not tested.

FIG. 3. Mean values (O) of CDV (SH strain) neutralizing antibody of sera 1 to 5 tested in DLM cultures in the presence of various amounts of protein A (*S. aureus*).

TABLE 6. VN in DLM in the presence of weak bases^a

Serum from dog no.:	Days post-exposure	VN titer (log ₁₀)		
		Untreated	20 mM NH ₄ Cl	0.01 mM chloroquine
2	14	1.9	1.7	1.5
3	14	2.2	1.7	1.3
5	14	2.5	2.0	2.3
7	18	4.4	4.1	4.1
8	20	3.2	2.9	2.7

^a Virus titers (in log₁₀) in test without neutralizing antibody were as follows: untreated, 1.6; 20 mM NH₄Cl, 1.3; 0.01 M chloroquine, 1.8.

bound to protein A as reported for canine immunoglobulins (34), the IgM fraction had no VN activity.

The formation of CDV-antibody complexes in dogs with persistent CDV infection was not sufficient to neutralize virus when tested in epithelial or fibroblast cells or lymphocytes. Virus was neutralized in macrophages but only if the Fc receptors were operative and if the antibody Fc protein was free to attach. When Fc receptors were blocked by heated normal dog serum, when Fc portions were removed from antibody by pepsin digestion, or when Fc portions were blocked by protein A, the virus-antibody complex became infectious even for macrophages. We are assuming that virus-antibody complex enters the macrophage via a specific virus receptor, when attachment to Fc receptors is blocked.

The attachment to Fc receptors alone could not explain the neutralization effect, because certain lymphocyte populations have Fc receptors and the virus-antibody complex remained infectious for them. After attachment to macrophage Fc receptors, it can be speculated that either virus-antibody complex was phagocytosed and inactivated or the attachment stimulated complement release from macrophages that inactivated the complex.

We tested the proposition of phagocytosis and enzymatic lysis by inhibiting lysosomal fusion with weak bases (13, 20, 26). Because only a slight reduction in virus inactivation in the presence of antibody was noticed, it appears unlikely that phagocytosis and lysosomal fusion are involved in the virus inactivation process. An unrelated observation in that test was the almost undiminished virus replication in the presence of weak bases, which would suggest that CDV replication itself in macrophages is not initiated by phagocytosis and lysosomal fusion.

An alternate explanation for virus inactivation in complexes might be by complement release from macrophages after complex attachment to Fc receptors. Macrophages have been shown to synthesize all of the components of the alternate pathway and C3 (35), C1 subcomponents (21), C2 (18), and C4 (10).

It is intriguing to speculate on the possible role of the CDV-antibody complex in inducing persistent infection. If macrophages are protected from infection by this complex but lymphatic, epithelial, and brain cells are not, it may be sufficient to prevent mortality from acute disease but not sufficient to eliminate virus. Perhaps macrophages "armed" with immunoglobulin destroy CDV-infected cells or other target cells in different combinations (31). A similar situation has been described in myelin breakdown in multiple sclerosis (29). It is also possible that the CDV-antibody complex has an effect on cellular immune responses. In a previous study we have found that dogs lacking in VN antibody activity in Vero cells had a suppressed or delayed immune specific cytotoxic T-cell response (5). These dogs had anti-

body with VN activity in macrophages. the antibody may be capable of "modulating" viral envelope glycoproteins on cell membranes, which could interfere with humoral immune responses (11). Humoral cytotoxic responses in the presence of complement were found in vitro with the CDV antibody and they should be expected to take place in vivo. Exceptions may be found in the central nervous system and eye where CDV persists (3, 5, 32, 33).

The macrophage Fc receptor-dependent inactivation of CDV-antibody complex is in contrast to certain togavirus and bunyavirus-antibody complexes which cause a macrophage Fc receptor-dependent enhancement of infectivity (6, 12, 25). It is presently not known why some virus-antibody complexes remain infectious and others are inactivated via this pathway.

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