

Myelin-Specific Autoantibodies Associated with Central Nervous System Demyelination in Canine Distemper Virus Infection

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Sera from dogs with spontaneously occurring and experimentally produced canine distemper virus-associated demyelinating encephalitis were examined for antibodies to central nervous system myelin by the complement fixation and indirect immunofluorescent methods. Complement-fixing immunoglobulin M antibodies and non-complement-fixing immunoglobulin G antibodies were found in 97% of the spontaneous cases. In comparison, only 28% of control sera contained these antibodies; furthermore, mean antibody titers in the control groups were significantly lower ($P < 0.005$) when compared to the distemper group. Complement-fixing antimyelin antibodies were also demonstrated in gnotobiotic dogs with experimentally induced distemper virus-associated demyelination. The antibody response could be correlated with clinicopathological features of the disease produced. Results of this study indicate that demyelination in canine distemper may proceed by immune mechanisms.

The role of the immune response in the etiology of demyelinating diseases such as multiple sclerosis (MS) and canine distemper (CD) remains to be clarified. It has been proposed and evidence presented that demyelination in MS may proceed by immune mechanisms (4-6). In this regard, experimental allergic encephalomyelitis (EAE) has been used as a model for MS on morphological and biochemical grounds (21, 28). This model is of limited value in that only myelin basic protein-sensitized lymphocytes are able to induce the disease. Recent reports indicate that an infectious agent in conjunction with a suspected sensitization to brain components may be involved in the etiology of MS (12, 17). Accordingly, the interplay between viruses, host tissues, and immune factors in chronic neurological disorders must be considered in the investigation of demyelinating disease.

Measles virus or closely related viral variant(s) are strongly implicated as the etiological agent in subacute sclerosing panencephalitis, an uncommon chronic demyelinating disease of man (23). Seroepidemiological surveys of MS patients and their families enhance speculation that measles virus is also associated with MS (25). Several investigators have presented evidence that CD virus (CDV), a paramyxo-

virus biochemically and antigenically closely related to measles virus, may initiate a brain-reactive immune response which could contribute to the demyelinating phase of the disease in dogs (1, 19, 30; A. Koestner, B. McCullough, G. S. Krakowka, J. F. Long, and R. G. Olsen, in press).

Complement-dependent serum demyelinating factors have been described by using the cerebellar explant culture technique in EAE, MS, and Guillain-Barré syndrome, a demyelinating disease of peripheral nerves (5, 9, 10, 15). Recently, we reported that some, but not all, distemper sera were capable of demyelinating explant cultures of canine cerebellum in vitro (A. Koestner et al., in press). The myelinotoxic effect was complement dependent in the more convincing cases.

These observations prompted us to investigate further these myelinotoxic serum factors. The present study was undertaken to determine whether antimyelin antibodies could be detected and characterized by serological means in the sera of dogs with naturally occurring demyelinating distemper encephalomyelitis. A further objective was to correlate this antibody response with clinicopathological features of experimental CDV-induced demyelination in gnotobiotic dogs.

MATERIALS AND METHODS

Canine experimental sera. CD sera were obtained from two sources. The first group of 34 sera was collected from naturally occurring cases of distemper-associated demyelinating encephalitis as confirmed by histological examination. Representative cases with definite and widespread central nervous system (CNS) lesions of demyelination were selected for detailed immunologic study.

A second group of CD sera consisted of weekly serial bleeds from 31 gnotobiotic dogs infected with R252-CDV, a neuropathogenic virus isolated from a naturally occurring case of distemper. Detailed clinical and pathological descriptions of these infected dogs are reported separately (B. McCullough, S. Krakowka, A. Koestner, and J. Shaddock, in preparation). Briefly, the infected dogs were separated into three groups based on histological and clinical criteria. Group I (11 dogs) became moribund at various intervals after infection and had microscopic lesions of noninflammatory distemper virus-induced demyelination with accompanying systemic lymphoid depletion.

Group II (5 dogs) survived the 12-week observation period with little or no clinical evidence of neurological involvement. In these dogs, lesions of demyelinating encephalitis, characterized by prominent hematogenous inflammatory cell infiltrates, were demonstrated in the CNS microscopically. Group III (15 dogs) remained asymptomatic throughout the observation period and had no detectable lesions in the nervous system.

Control sera were obtained from three sources. Twenty sera were obtained from a colony of conventional dogs ranging in age from 6 months to 7 years. The dogs had been vaccinated for CD with a modified live virus vaccine according to published recommendations (2). The second control group consisted of serum samples from 33 surgically derived colostrum-deprived gnotobiotic dogs raised according to the techniques of Griesemer and Gibson (14). Serial bleeds from nine uninfected gnotobiotic dogs constituted the normal controls for the experiments with R252-CDV.

Preparation of myelin antigen and reference antisera. CNS myelin was prepared from canine spinal cord by using discontinuous sucrose density ultracentrifugation by the method of Autillo et al. (3). A sample of the preparation was examined by electron microscopy.

High-titered hyperimmune antiserum was produced in rabbits and in gnotobiotic dogs by bimonthly 10.0-mg subcutaneous or intramuscular injections, or both, of myelin antigen suspended in 1.0 ml of saline and emulsified in an equal volume of complete Freund adjuvant. Complement fixation (CF) titers of 1: ≥ 256 were obtained after four injections.

Serological methods: (i) titration of antigen and reference antibody. Lyophilized myelin antigen was resuspended to 5.0 mg/ml in Veronal buffer with 0.5% sodium azide and stored at 4 C. A sample of this stock suspension was block titrated against selected bleeds

of CF reference antisera in microtiter plates (Cooke Engineering, Alexandria, Va.). The CF test consisted of incubating serial twofold dilutions of antigens (0.025 ml) with an equal volume of twofold dilutions of heat-inactivated sera and three hemolytic units of complement ($C'H_{50}$) for 12 h at 4 C.

Amboceptor-coated sheep red blood cells (0.025 ml of 2.5% suspension in Veronal buffer) were then added, and the plates were incubated an additional 30 minutes at 37 C. One CF antigen-antibody unit was designated as that final dilution of serum and antigen that permitted less than 50% of the lysis in indicator cells. Routinely, reference antisera gave titers of 1: ≥ 256 when used with antigen excess (3 units). With 3 U of antibody, CF occurred with myelin antigen dilutions of 1: ≥ 2048 .

(ii) CF/CFI. The CF and CF inhibition (CFI) test as adapted to the microtiter system by Olsen and Yohn (20) was employed to evaluate myelin-binding antibodies in test sera.

Sera were evaluated for CF antibody with 3 U of antigen. Titers were expressed as the last dilution of serum resulting in less than 50% lysis of the indicator cells.

For the CFI test, 2 U of antigen was incubated with serial twofold dilutions of serum for 1 h at 37 C. This was followed by a 12-h incubation period at 4 C with 2 added U of hyperimmune antimyelin antibody. CFI activity was indicated by the absence of reference antibody-antigen associated complement activation and was detected visually when greater than 50% lysis of indicator cells was seen.

Test sera were examined for CF and CFI activity simultaneously with controls for anticomplementary activity of test serum, antigen, and nonspecific interaction of reference antiserum with test serum.

(iii) Absorption experiments. Serological specificity was investigated by absorption experiments using lyophilized canine myelin, spleen, and liver powders, rabbit liver powder, and heterologous rat myelin. A 5.0-mg amount of saline-moistened organ powder was incubated with 2.0 ml of test serum at 37 C for 1 h and then for an additional 12 h at 4 C. Insoluble material was removed by low-speed centrifugation. When necessary, lyophilized guinea pig complement was added to remove anticomplementary activity. The treated sera were heat inactivated and filtered with 0.45 and 0.22- μ m pore size membrane filters (Millipore Corp., Bedford, Mass.), respectively, before being tested. Absorbed and unabsorbed sera were also tested for CF antibody to suspensions of liver, spleen, and rat myelin in Veronal buffer.

(iv) Whole-serum fractionation. Ion exchange chromatography of CD sera was performed on columns of diethylaminoethyl (DEAE)-cellulose (Sigma Chemical Co., St. Louis, Mo.) equilibrated with 0.01 M phosphate buffer, pH 8.0 (7). The immunoglobulin (Ig) G-containing fraction was eluted with 0.05 M NaCl in phosphate buffer. The remaining serum proteins, including IgM, were eluted with 0.6 M NaCl in phosphate buffer. Samples with 0.05% glycine added were concentrated back to starting volume with a Diaflo apparatus (Amicon, Lexington, Mass.) using

a membrane exclusion size of 10,000 molecular weight. Residual anticomplementary activity was removed by absorption with lyophilized guinea pig complement.

Gel filtration of selected sera was accomplished on a Sephadex G-200 column (2.5 by 93 cm) equilibrated with 0.4 M NaCl-0.01 M phosphate buffer (pH 7.2) adjusted to a flow rate of 50 ml/h (29). Eluates were monitored at 280 nm and collected in 5.0-ml volumes. Protein peaks were concentrated back to original volume and tested for CF/CFI antibody.

(v) **Reduction of CD sera with mercaptoethanol.** The effect of a reducing agent on CF-positive sera was evaluated by pretreating the sera with an equal volume of 0.2 M 2-mercaptoethanol for 1 h at 37 C before testing (18).

(vi) **Immunofluorescence microscopy.** The indirect immunofluorescent method was used to visually confirm the binding of immunoglobulins to myelin. Rabbit anticanine globulin with strong anti-IgM activity for use as the fluorescein-conjugated secondary reagent was prepared in the following manner. A dog was injected intravenously with sheep red blood cells and exsanguinated 10 days later. This serum had a mercaptoethanol-sensitive sheep red blood cell hemagglutinating (HA) titer of 1:512. After euglobulin precipitation, the redissolved protein solution was applied to a Sephadex G-200 column, and the exclusion peaks (HA 1:16) from two runs were pooled and rechromatographed on the same column. Immunoelectrophoretic analysis of the leading shoulder of the first peak showed a single precipitin line consistent with the described position and electrophoretic mobility of canine IgM (22). Rabbits were immunized with 5.0 mg of this material in complete Freund adjuvant. Immunoelectrophoretic analysis of these antisera against whole canine serum showed a single heavy precipitin line in the IgM region and several precipitin lines in the IgG region. The antiglobulin reagent was conjugated to fluorescein isothiocyanate (FITC) by the dialysis technique of Clark and Shephard (8) except that unbound dye was removed by passing the conjugate through a column of Sephadex G-25.

Blocks of canine spinal cord were frozen in liquid nitrogen. Sections (4.0 μ m) were cut on a cryostat at -20 C, mounted on ovalbumin-treated cover slips, and air dried. After washing, the primary reagent (CD sera or control sera prediluted 1:10 in 3.0% isotonic rhodamine-conjugated bovine serum albumin counter-stain) was applied, and the cover slips were incubated for 1 h at room temperature. After the second washing, a previously determined optimal dilution of fluorescein-conjugated antiglobulin was added, and all the cover slips were incubated at 37 C for 1 h. Each test was accompanied by control cover slips treated with conjugate alone and cover slips treated with known positive primary (antimyelin reference serum), and a known negative serum (heterologous normal rabbit serum).

The specificity of the test was established by showing: (i) the conjugate alone did not stain myelin, (ii) normal dog serum did not stain myelin, and (iii) unconjugated rabbit anti-canine globulin serum, but not pooled normal rabbit serum, successfully blocked

myelin fluorescence when applied to spinal cord sections previously treated with myelin-binding CDV sera.

RESULTS

Electron microscopy of myelin antigen. Examination of isolated myelin revealed a preparation consisting almost entirely of multilaminated membranes and fragments characteristic of myelin (Fig. 1). Occasional synaptosomal structures were seen. No intact organelles were identified.

Reference antisera and antigen. The stock suspension of myelin was stable for several months when stored at 4 C. Furthermore, no significant differences in antigenicity in CF/CFI were noted with similar preparations from five different dogs.

CF antibody to myelin was detected in all animals 4 weeks after immunization and reached maximal titers of 1: \geq 256 by 6 weeks. No significant differences in the titers were noted when a myelin preparation of rat origin was substituted. Several animals also reacted in low titers (1:4 to 1:16) with a reconstituted liver powder preparation, but this activity could be removed by absorption with liver powder. The above absorption decreased antimyelin titers from 1: \geq 256 to 1:64. In reference antisera used to measure CFI antibody, two units of antigen and reference antiserum were contained in dilutions well beyond cross-reactivity with liver. Reference antisera reacted strongly with myelin membranes when examined by indirect immunofluorescence.

Myelin antibodies in sera from cases of naturally occurring CD. Serum from distemper-infected dogs contained antibodies that are capable of reacting with CNS myelin in vitro (Table 1). Twenty-one of 34 CD sera had CF titers ranging from 1:16 to 1: \geq 256, with a group mean titer of 1:62.5. CFI activity was detected in 12 of 34 CD sera and varied from 1:4 to 1:16 with a mean titer of 1:8. Ninety-seven percent of CD sera tested reacted to myelin in either CF or CFI. None of the sera examined showed simultaneous CF and CFI activity. In contrast, only 30% of sera from conventional normal dogs and 39% of gnotobiotic sera contained myelin antibodies. Moreover, mean CF titers in the control groups (1:8 and 1:4.2, respectively) were significantly lower ($P < 0.005$) when compared to the distemper group. CFI antibody was detected in only 2 of 20 conventional dogs and in none of the gnotobiotic sera.

Representative CD sera were chosen for further immunological characterization on the basis of completeness of clinicopathological rec-

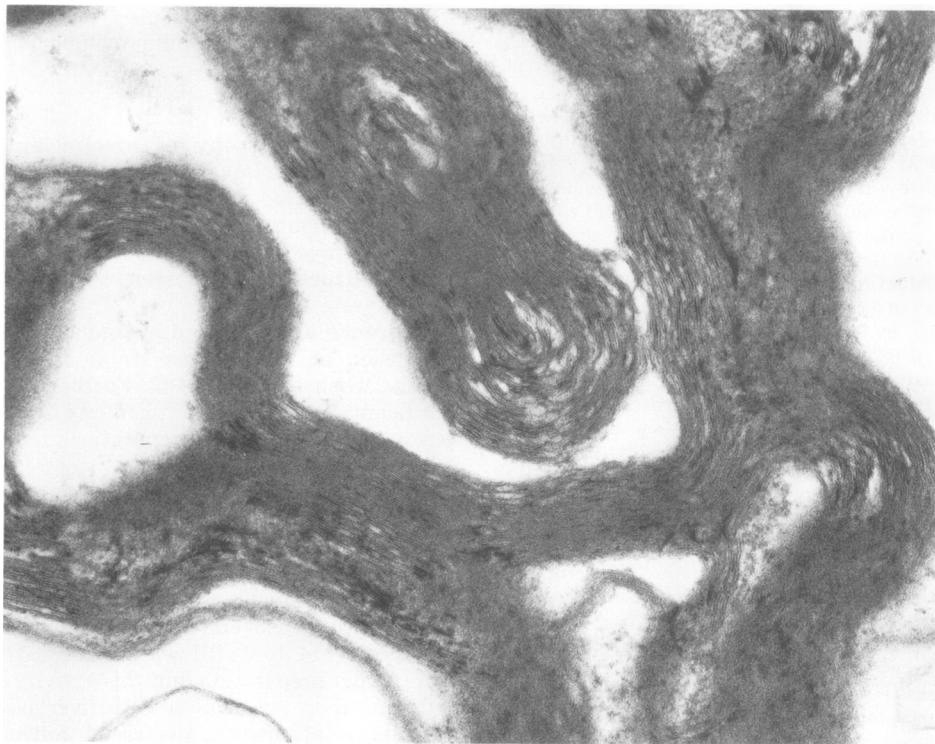


FIG. 1. *Electron micrograph of CNS myelin. $\times 57,500$.*

ords and amount of serum available for use. These cases represented the well established clinical spectrum of findings in neurological CD (13).

CD serum absorption. The nonneural tissue distribution of myelin membrane antigen(s) under investigation was evaluated by absorption experiments and additional direct CF tests (Table 2). Myelin of rat origin reacted with all CF-positive sera. In contrast, Veronal buffer suspensions of canine liver and spleen, equivalent on a per weight basis to 6 antigenic U of myelin, reacted with only two of seven sera. Absorption of CD sera with heterologous or homologous myelin decreased CF antibody titers two to sevenfold, whereas absorption with homologous liver or spleen did not reduce antimyelin activity significantly (Table 2).

CD serum fractionation. Further investigation of CD sera by anion exchange chromatography and gel filtration identified specific antibody activity in two immunoglobulin classes (Table 3). CF antibody was eliminated by treatment with 2-mercaptoethanol and appeared only in the 19S peak after passage through a Sephadex G-200 column. CFI antibodies were found only in the 7S peak after gel filtration. 7S CFI activity was uncovered in several CF CD sera after separation of IgM and

IgG immunoglobulin classes. Protein losses due chiefly to visible aggregation accounted for the marginal recovery of antibody activity in the eluates.

Indirect immunofluorescence with CD sera. The results obtained with indirect immunofluorescence correlated well with serological data (Table 4). Three of five CD sera with CF titers above 1:16 gave myelin-positive fluorescence. One of four CFI CD sera (titer \leq 1:16) reacted with myelin in this test system. Cross and longitudinal sections of myelin reacted with specific antibody in an intense and homogeneous manner (Fig. 2). Dull green background nuclear and cytoplasmic staining was easily distinguished from positively stained myelin. CD sera also reacted positively with air-dried smears of isolated myelin; control sera were negative. A variety of commonly employed tissue fixatives (cold acetone, methanol, picric acid formaldehyde, and Formalin) eliminated myelin-specific fluorescence.

Sera CF/CFI tests for myelin antibodies in experimentally produced CD. Weekly serum samples from 31 gnotobiotic dogs infected with R252-CDV along with 9 uninfected gnotobiotic controls were tested by CF/CFI. CF antibody predominated in all dogs tested; CFI antibodies occasionally were detected late in the observa-

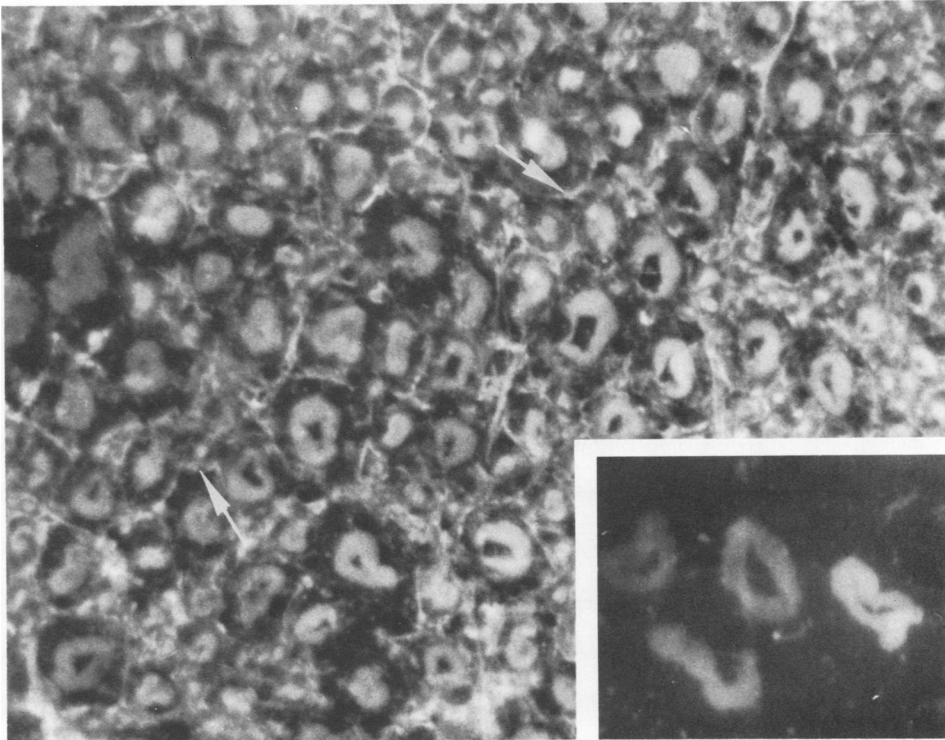


FIG. 2. Stained cross-section of canine spinal cord that demonstrates myelin-specific immunofluorescence by the indirect method. Nonmyelin tissues stained nonspecifically (arrows) only with rhodamine-albumin counterstain ($\times 520$). Insert: immunofluorescent positive isolated CNS myelin ($\times 840$).

TABLE 1. Distribution of myelin antibody titers in naturally infected canine distemper (CD) dogs and control dogs

Source of test serum	Total no. tested	Complement fixation titer						Complement fixation inhibition (CFI) titer		
		<1:2	1:2	1:4	1:8	1:16-64	$\geq 1:128$	1:4	1:8	1:16
CD-infected dogs	34	13 ^a	0	0	0	16	5	4	5	3
Normal vaccinated conventional dogs	20	16	0	2	1	1 ^b	0	2	0	0
Normal gnotobiotic dogs	33	20	5	5	3	0	0	0	0	0

^a Twelve of thirteen sera had CFI titers of 1:4 to 1:16, as indicated in right-hand column.

^b Titer 1:16.

tion period in infected dogs only.

Throughout the 5-week survival period, mean antimyelin antibody titers in dogs of group I did not differ significantly ($P > 0.1$) from control levels (Fig. 3). Dogs of group II produced statistically significant ($P < 0.01$) antibody responses by postinfection day (PID) 21. Rising antibody titers at the time of sacrifice is also indicated ($P < 0.01$).

The serological response to myelin in group III dogs was surprising in view of the absence of neural lesions in these dogs. Highly significant ($P < 0.005$) antibody levels were evident on PID

21. Titers gradually declined toward control levels. This peak response is significantly greater ($P < 0.01$) than the peak response noted in group II.

DISCUSSION

Experiments reported here describe antibodies that bind to myelin in sera of dogs with CDV-induced demyelinating encephalomyelitis. Low levels of antibodies ($< 1:8$) were also detected in control sera. In this regard, the findings of Edgington and Dalessio (11) in man are relevant. They reported low levels of IgG mye-

lin-binding antibodies (range 1: ≤4) in 88% of normal individuals. Since these "autoantibodies" appeared at the time of active myelination in the brain, they speculated that antibody appears in response to myelination. This conclusion is supported in the present study by noting that appearance of antibody coincides roughly with the time of CNS myelination in the dog.

Edgington also found that sera of all MS patients examined contained IgM antimyelin antibody in relatively high titer, analogous to the findings reported here. Other investigators have reported CF antibodies of the IgM class in

EAE (3), MS (8), and Guillain-Barré syndrome (7). All three diseases are characterized by demyelination of a suspected immunological nature.

Absorption of CD sera with several tissues including myelin indicates that the membrane antigen, while present in the greatest amounts on myelin, may be a component of other plasma membranes. Preliminary quantitative absorption experiments (unpublished data) indicate that this antigen, or one similar to it, is found in lymphoid tissues, especially thymus. In certain strains of mice, a shared brain-thymocyte antigen, designated theta antigen, is well documented (27).

The evidence that IgM is responsible for the CF observed is threefold. (i) CF activity is lost

TABLE 2. Organ specificity of myelin antibodies in canine distemper (CD) sera; evaluation by tissue absorption

CD serum no.	Loss of CF antibody units ^a to dog myelin after absorption ^b with:			
	Myelin ^c	Myelin ^d	Liver ^e	Spleen ^e
1 ^e	4	3	2	2
2 ^e	7	6	2	2
3	6	5	1	2
4	5	4	1	2
5, 6, 7	4 (3)	3 (3)	0 (3)	0 (3)

^a One CF antibody unit defined as 1 log₂ dilution of CD sera.

^b Absorbed with 2.5 mg of tissue powder per 1.0 ml of CD serum.

^c Dog origin.

^d Rat origin.

^e Two of seven sera reacted with canine liver and spleen powders in Veronal buffer, equivalent to 6 U of myelin antigen on a weight basis, with titers of 1:8 and 1:32 (liver) and 1:8 and 1:4 (spleen), respectively.

TABLE 4. Comparison of the complement fixation test and indirect immunofluorescence for myelin antibodies in canine distemper (CD) sera and controls

CD serum no.	Complement fixation titer	Complement fixation inhibition titer	Myelin-specific immunofluorescence ^a
1	≥1:256	<1:2	+
2	1:128	<1:2	+
3	1:64	<1:2	+
4	1:32	<1:2	-
5	1:16	<1:2	-
6	<1:2	1:16	+
7	<1:2	1:16	-
8	<1:2	1:8	-
9	<1:2	1:4	-
Controls (7)	<1:2 (7)	<1:2 (7)	- (7)

^a Sera prediluted 1:10 in 3% rhodamine albumin (Difco) counterstain.

TABLE 3. Fractionation of myelin antibodies in canine distemper (CD) sera by gel filtration^a and anion exchange chromatography^b

CD serum no.	Unfractionated antibody titer		Antibody titer after sephadex G-200 gel filtration ^a				Antibody titer after DEAE chromatography ^b			
	CF	CFI	Peak I (19S)		Peak II (7S)		IgG fraction		IgM fraction ^c	
			CF	CFI	CF	CFI	CF	CFI	CF	CFI
1	1: ≥256	<1:2	1:16	<1:2	<1:2	1:2	<1:2	1:4	1:16	<1:2
2	1:128	<1:2	1:8	<1:2	<1:2	<1:2	<1:2	1:2	1:8	<1:2
3	1:64	<1:2	1:2	<1:2	<1:2	<1:2	<1:2	1:2	1:8	<1:2
4	1:32	<1:2	1:4	<1:2	<1:2	1:2	<1:2	<1:2	<1:2	<1:2
5	<1:2	1:16	<1:2	<1:2	<1:2	1:16	1:2	1:16	<1:2	<1:2
6, 7, 8	1:16 (3)	<1:2 (3)	<1:2 (3)	<1:2 (3)	<1:2 (3)	<1:2 (3)	<1:2 (3)	<1:2 (3)	<1:2 (2 ^d)	<1:2 (3)

^a Sephadex (Pharmacia)—equilibrating buffer: 0.4 M NaCl in 0.01 M phosphate buffer, pH 7.2

^b DEAE-cellulose (Sigma)—starting buffer: 0.05 M NaCl in phosphate buffer, pH 8.0; limiting buffer: 0.6 M NaCl in phosphate buffer, pH 8.0.

^c Serum fraction containing IgM after elution with the limiting buffer.

^d One of three fractions tested had a CF titer of 1:4.

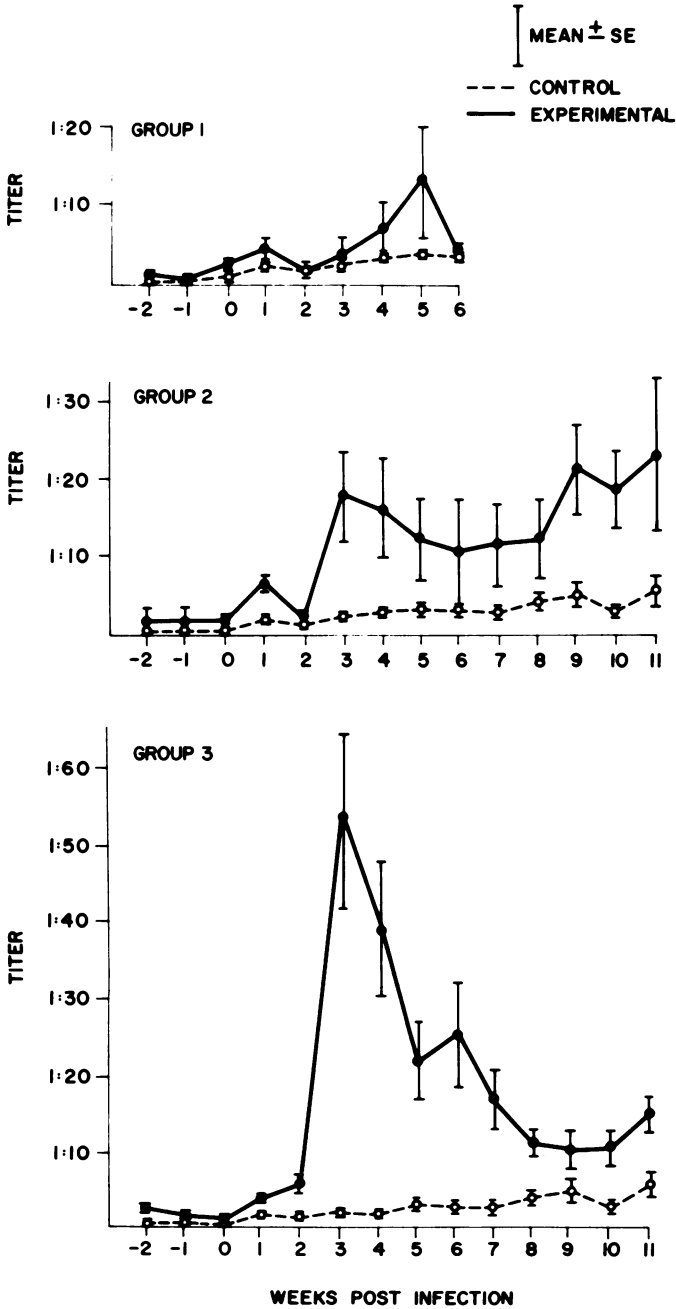


FIG. 3. Mean antimyelin antibody titers in gnotobiotic dogs infected with R252-CDV versus mean antibody titers of nine control dogs. Criteria for group separation are given in the text.

after mercaptoethanol treatment, (ii) CF was seen only in the 19S peak of Sephadex G-200, and (iii) in the immunofluorescence experiments, only a FITC-conjugate with strong anti-canine IgM specificity was effective in demon-

strating myelin-binding antibodies in CD sera.

CFI antibody uncovered after fractionation appears to be IgG based upon its electrophoretic mobility in immunoelectrophoresis and behavior on DEAE-cellulose. IgM-mediated CF can

obscure CFI antibody in whole serum (Table 3). Thus, the type of antibody measured by the CF/CFI test depends upon which antibody class contains the most specific activity at the time of sampling.

Indirect immunofluorescence provided morphological conformation of the myelin-binding capacity of these antibodies. This technique was relatively insensitive when compared to the serological test. Also, positive myelin staining was obtained only when a secondary reagent with strong anti-IgM activity was employed. Experiments using a secondary reagent with only anti-IgG specificity failed to demonstrate antimyelin antibody. Thus, the choice of reagent was critical. Finally, preservation of tissue sections in a variety of fixatives eliminated myelin-specific immunofluorescence.

Study of experimentally infected gnotobiotic dogs provided additional pertinent information. Antibody response to myelin in the subacutely infected dogs of group I did not differ significantly from controls. An exact counterpart of this group is not represented in the naturally occurring cases of distemper since all dogs studied here had inflammatory cell infiltrates in areas of demyelination and presented a chronic clinical course of illness.

In contrast, dogs with inflammatory infiltrates associated with demyelination (group II) showed marked differences from controls. These dogs probably represent infected dogs that appear to recover from the acute respiratory and enteric forms of distemper and yet eventually die with demyelinating encephalitis at a later date (16). It appears that these dogs remain persistently infected, even though clinically asymptomatic. In this group, viral invasion and persistence in the CNS may provide the stimulus necessary to incite an anti-brain response.

No satisfactory explanation can be given for the serological response observed in the dogs of group III. The paradox of a vigorous antimyelin response in the absence of demyelination is difficult to reconcile with information available at this time. Short-term viral replication in the brain, terminated by the onset of active antiviral immunity, may expose myelin to invading immunocompetent cells with resultant antibody production. Alternatively, the previously noted lymphoid tissue-myelin cross-antigenicity may provide the necessary antigenic stimulus for the response in the absence of viral persistence in the brain.

The possibility that the antigen involved is a normal constituent of all plasma membranes and that the degree of specificity observed with

myelin represents a fortuitous concentration of antigen on this membrane cannot be excluded at this time. Further sequential pathogenesis studies are necessary to resolve these points.

Data from these dogs indicate another possible effect of CDV on susceptible dogs, that of immunosuppression. Immunosuppressive effects have been well documented for measles and rinderpest viruses, which are other members of the paramyxovirus group (24, 26). It appears that, based on the magnitude of humoral antibody response, infected dogs of group I (Fig. 3) are the most severely compromised, whereas those of group III show the least evidence of depressed humoral immune responsiveness. Group II dogs occupy the intermediate position between these extremes. This impression is further strengthened by noting that cellular infiltrates in the brain usually associated with immunocompetence (i.e., lymphocytes and plasma cells) are not a feature of disease in group I, but are a prominent histological finding in the demyelinating lesions of group II. The role of specifically sensitized lymphocytes (i.e., cell-mediated immunity) in distemper-associated demyelination has not been investigated. Morphological changes in lymphoid tissues induced by R252-CDV are described elsewhere (B. McCullough, S. Krakowka, and A. Koestner, in preparation). Experiments designed to investigate viral effects on both cellular and humoral immunocompetence are in progress with particular emphasis on how this may relate to chronic infectious neurological disease.

The precise role of these antibodies in the etiology of demyelinating disease is still unknown. The demonstration of CNS-bound immunoglobulin in demyelinating lesions of CDV-infected dogs (19) provides tentative support for an active role in the disease process. We presently believe that these antibodies are contributing factors in the pathogenesis of demyelination associated with CDV and serve to amplify the basic lesion of noninflammatory virus-induced demyelination in the brain.

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