

Immune mechanisms against canine distemper

III. ROLE OF COMPLEMENT LYSIS IN THE IMMUNITY AND PERSISTENT INFECTION OF CANINE DISTEMPER VIRUS

C. K. HO & L. A. BABIUK *Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0*

Accepted for publication 10 September 1979

Summary. Antibody-mediated complement lysis of Vero cells and canine macrophages infected with canine distemper virus (CDV) was demonstrated in an *in vitro* ^{51}Cr release assay. This cytolytic activity was found to be highly efficient and was optimal under conditions which favoured the capping of redistribution of surface viral antigens. A prozone was observed in the presence of high antibody concentration and could not be eliminated by repeated washings. By tagging antibody-coated target cells with ^{125}I -labelled staphylococcus protein A, it was found that the extent of protein A binding was parallel to the degree of cytotoxicity suggesting that the mechanism of this prozone effect was similar to that of a precipitation test.

INTRODUCTION

Antibody-dependent complement-mediated lysis of viral infected target cells has been demonstrated in several paramyxoviruses (Minagawa & Yamada, 1971; Kielsing, Bandlow & Thomssen, 1972). This im-

mune mechanism is highly efficient since the process of complement lysis is normally completed in a short time if there is sufficient viral antigen expressed on the surface of the infected cell. In the presence of specific antibody, complement-mediated lysis can presumably limit viral spread by destroying infected cells prior to extensive virus maturation and thus block the progression of an infection. This would be especially important in infections such as canine distemper virus (CDV) where maturation and release occurs over a long period of time. Whether antibody and complement lysis indeed plays a very important role in reducing the degree of replication of CDV *in vivo* is not fully understood at present, especially in the light of recent findings that high serum concentrations, such as may be present *in vivo*, decrease the efficiency of complement-facilitated lysis of measles virus (Ehrnst, 1975; Kibler & Ter Meuler, 1975; Minagawa & Yamada, 1971). Whether such a prozone effect occurs in CDV remains to be elucidated.

Previously we have shown that specific CDV antibody alone can prevent the intercellular and extracellular spread of the virus among susceptible cells (Ho & Babiuk, 1979b). Furthermore, antibody was shown to modulate the viral antigens expressed on the surface of virus infected cells (Ho & Babiuk, 1979b) resulting in internalization and subsequent shedding of these antigens. The present study was designed to examine

Correspondence: Dr L. A. Babiuk, Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada S7N 0W0.

0019-2805/80/0200-0231\$02.00

© 1980 Blackwell Scientific Publications

the role of complement in viral spread, cytolysis of the infected cells in the presence and absence of antibody-induced capping of viral antigens as well as to determine whether a prozone effect of antibody-dependent complement lysis does occur in CDV infected cells.

MATERIALS AND METHODS

Cells and virus

Vero cells (African green monkey kidney) were propagated in Eagle's minimum essential medium (MEM) supplemented with 5% heat-inactivated (56° for 30 min) foetal bovine serum (FBS), 1% non-essential amino acids, 2mM glutamine and 25 µg/ml of gentamycin. Incubation was at 37° in a humidified incubator maintained at 5% CO₂. The green strain of canine distemper virus (CDV) was grown in Vero cells as described elsewhere (Ho & Babiuk 1979c).

Antiserum and complement

Canine anti-CDV antiserum was a serum pool obtained from six mongrel puppies (12 weeks old) which had been hyperimmunized with two doses of attenuated canine distemper vaccine (Norden, Lincoln, Neb.) given 2 weeks apart. These dogs were brought in from suburban areas and had no detectable anti-CDV antibody activity at the time of acquisition. At different times post-vaccination, the puppies were bled, the sera were heat-inactivated (56° for 30 min), absorbed twice with 1/5 packed Vero cells and kept at -20° until use. Normal serum was obtained from gnotobiotic dogs and had no detectable titre against CDV. Complement was obtained from the same puppies before vaccination. Blood was collected from the puppies within 48 h of arrival and allowed to clot for 1 h at room temperature then 2 h in ice after which time the sera were harvested, pooled, absorbed twice with 1/5 packed Vero cells at 4° and kept at -70° until use.

Target cells

Vero cells persistently infected with CDV were prepared by a method described by Rice & Wolff (1978). This cell line had been maintained for over 4 months in our laboratory and 90% of cells showed membrane immunofluorescence when tested with anti-CDV serum. Subconfluent monolayers of these cells were dispersed by brief treatment with 0.025% trypsin in 0.01% EDTA, washed three times in MEM + 10% FBS, resuspended in MEM (2 × 10⁵ cells/ml) and incubated with 10 µCi/ml of [⁵¹Cr]-sodium chromate (New

England Nuclear, Dorval, P.Q.) for 90 min at 37°. Excess ⁵¹Cr was removed by washing and the cells were resuspended to the original volume in MEM + 2% FBS and were kept on ice until use.

Canine macrophages were prepared in ninety-six well microtitre plates (Falcon No. 3040) from peripheral blood as described in detail elsewhere (Ho & Babiuk, 1979a). Canine peripheral blood leucocytes prepared by ficoll hypaque flotation were cultured in MEM supplemented with 0.5% autologous red cells and 20% horse serum. After culturing for about 10 days with repeated washings the remaining cells were pure macrophages (Ho & Babiuk, 1979c, d). For use as target cells the macrophages were infected at a multiplicity of infection of 1 and simultaneously labelled with ⁵¹Cr (10 µCi/ml). After incubation for 60 min at 37° the cells were washed three times in HBSS and used as target cells in the cytotoxicity assay.

Cytotoxicity assays

Aliquots of CDV infected Vero cells (0.2 ml, 2 × 10⁵ cells/ml) were dispensed into plastic tissue culture tubes (Falcon No. 2054) and incubated with 50 µl of varying dilutions of antiserum in MEM + 5% FBS. Various incubation times and temperatures were used prior to the further addition of 50 µl of complement (final concentration eight lytic units). The tubes were once again incubated at 37° for different time periods before the addition of 0.2 ml of MEM + 5% FBS. The cells were pelleted by centrifugation at 400 g for 5 min and 0.2 ml of the supernatant was harvested for determination of the amount of radio-isotope released. In some experiments excess antibody was removed prior to the addition of complement. All tests were performed in triplicate and controls included target cells plus antiserum alone, target cells plus complement, non-infected Vero cells plus complement and non-infected cells plus antibody and complement. Cytotoxicity was calculated by the following formula:

$$\% \text{ Specific } ^{51}\text{Cr release} = \frac{T - C}{TR - C} \times 100$$

where *T* = counts per min of test culture, *C* = counts per min of control at specific antiserum concentrations, *TR* = total releasable counts per min—the amount of radioactivity released by treatment of cells with 3% Triton-X 100, which was 90–95% of the total ⁵¹Cr incorporated.

Complement was titrated by adding varying dilutions of complement to a standard number of cells in the presence of a constant antiserum concentration.

Kinetics of cytolysis of infected macrophages

Canine macrophage target cells were prepared in microtitre plates as described above. At different times post-infection with CDV, medium from each well was removed and replaced by 0.1 ml of diluted autologous antiserum and an equal volume of autologous complement. The plates were incubated at 37° for 2.5 h, then half the contents of each well was harvested for radioactivity counting.

Solid phase radioimmunoassay for CDV antibody

Protein A was labelled with carrier-free ¹²⁵Iodine (¹²⁵I, New England Nuclear, Dorval, P.Q.) by incubating 50 µg of protein A (Pharmacia) at 4° for 20 min with 1 µCi of ¹²⁵I and 10 µg of chloramine T. Unbound ¹²⁵I was removed by passage through a G-25 Sephadex column. The labelled protein A was then dialysed overnight against phosphate-buffered saline 0.05 M, pH 7.2, and stored frozen (-70°) until use. Titration of antibody was performed as described previously (Babiuk, Acres & Rouse, 1977). Briefly, Vero-infected cells were fixed to wells of a microtitre plate (Falcon No. 3040) prior to the addition of 50 µl of each dilution of antibody to quadruplicate wells followed by a 1 h incubation at 37°. The wells were washed four times and incubated for 1 h with 50 µl of ¹²⁵I protein A, washed and the radioactivity bound to each well was determined.

To detect antibodies bound to the cell surface, CDV infected Vero cells were incubated in suspension at 37° with different dilutions of antiserum for 60 min. Unattached antibody was removed by three washes in MEM and bound antibody was determined after further incubation of the cells with ¹²⁵I-protein A for a further 60 min. The amount of protein A bound was calculated by the following formula:

% Protein A bound =

$$\frac{\text{Mean c.p.m. bound by infected cells} - \text{Mean c.p.m. bound by uninfected cells}}{\text{Mean c.p.m. unbound to infected cells}} \times 100$$

Serum neutralization assays were conducted on the same antibody samples as described previously (Ho & Babiuk, 1979c).

RESULTS**Complement mediated cytotoxicity**

Complement destroyed Vero cells infected with canine

distemper in the presence of specific antibody but failed to do so in the absence of specific antibody or if uninfected Vero cells were used as controls. The degree of cytotoxicity was very low at low serum dilutions, increased rapidly to reach a maximum level and then once again decreased as the amount of antibody was reduced (Fig. 1). This prozone effect was not only evident when the antibody was maintained in the cultures during the cytotoxicity assay but also if the unbound antibody was removed by washing. In contrast, higher concentrations of complement resulted in a greater degree of cytotoxicity (Fig. 2). Thus, at a 1/5 dilution of serum containing complement but no specific antibody, cytotoxicity was maximal if a low level of antibody was added. Under these same conditions, complement alone (no added antibody) was not toxic, suggesting that the cytotoxicity observed required both complement and antibody directed against specific membrane antigens.

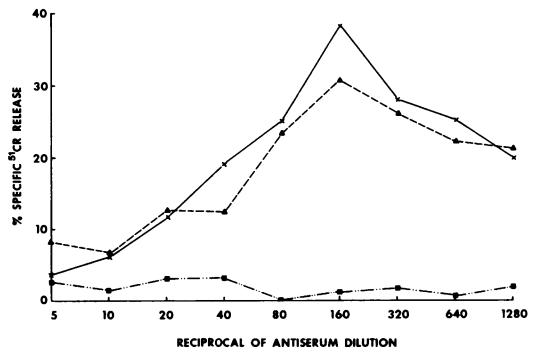


Figure 1. Titration of antiserum in the presence of eight units complement (x); antibody was first absorbed onto target cells, washed and then incubated with complement (Δ). Control included target cells plus antibody alone (■). Spontaneous release was about 1% of total. All tests were performed in triplicate.

The destruction of target cells occurred rapidly and the degree of cytotoxicity increased exponentially with increased incubation times until it plateaued at approximately 2.5 h post-incubation (Fig. 3). The degree of cytotoxicity was also influenced by the incubation temperature such that at all antibody dilutions there was a significantly higher level of cytotoxicity at 37° than at 25° with only low levels occurring at 4° (Fig. 4). These results parallel those previously

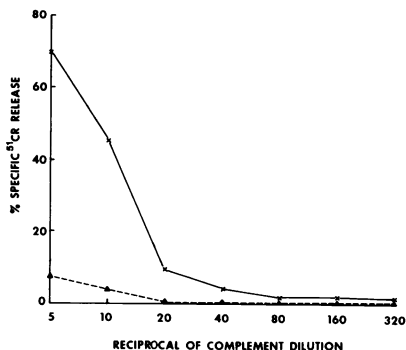


Figure 2. Titration of complement in the presence of 1/50 dilution of antiserum (x) and in the absence of antiserum (Δ). A 1/10 dilution of complement was equivalent to eight complement units.

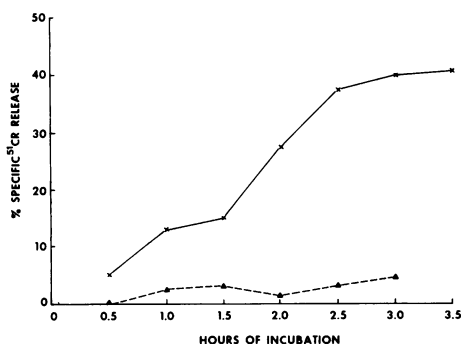


Figure 3. Kinetics of antibody-complement lysis. Canine distemper virus (CDV)-infected Vero cells (2×10^5 cells/ml) were incubated at 37° for different time intervals with a 1/100 dilution of antiserum and eight units of complement. Non-infected Vero cells (Δ) were assayed under the same conditions as a control.

observed for capping of CDV-specific cell-surface antigens (Ho & Babiuk, 1979b). Thus, at 4° the rate of capping was lower than at 37° . These observations are compatible with the increased membrane fluidity at 37° which would then favour migration of virus membrane proteins in the membrane to allow redistribution of the antigen which could then be cross-linked by antibody with IgG doublet formation and complement-mediated lysis. Further support for the involvement of viral antigen movement and capping in antibody complement-mediated lysis or lack of lysis is given in Table 1. Thus, prolonged incubation in the presence of antibody, which favoured capping and subsequent internalization or shedding of surface

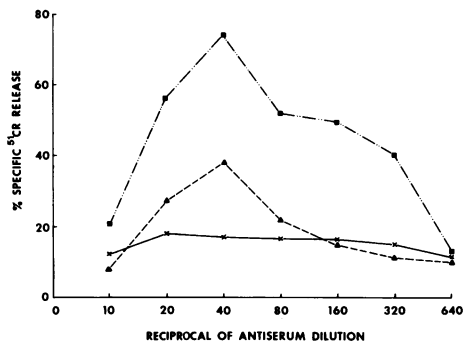


Figure 4. Effect of temperature on antibody-dependent complement cytotoxicity. CDV-infected Vero cells (1×10^5 cells/ml) were labelled with ^{51}Cr and incubated with various concentrations of antiserum (680 TCID₅₀/ml) for 1 h at 4° (x); 25° (Δ); and 37° (\blacksquare). Complement was then added at a concentration of 1/4 and the supernatants were assayed after incubation at 37° for 2.5 h.

Table 1. Relationship between antibody-complement lysis of target cells, antigenic modulation and ^{125}I -protein A binding

Reciprocal of antiserum dilution	% Specific ^{51}Cr release \pm SD*		Counts per min $\times 10^{-3} \pm$ SD†	
	1 h	24 h	1 h	24 h
5	4 \pm 2	4 \pm 3	—	ND†
10	6 \pm 3	ND†	1.3 \pm 0.4	2 \pm 0.8
20	19 \pm 6	6 \pm 2	1.5 \pm 0.6	ND†
40	25 \pm 7	ND†	1.7 \pm 0.8	1.9 \pm 0.8
80	30 \pm 10	14 \pm 6	2.8 \pm 1.0	ND†
160	42 \pm 6	12 \pm 4	6.5 \pm 1.6	1.8 \pm 0.2
320	35 \pm 7	—	3.4 \pm 1.2	ND†
640	33 \pm 5	19 \pm 7	2.0 \pm 0.7	ND†
1280	20 \pm 6	21 \pm 6	1.5 \pm 0.4	1.7 \pm 0.7

* Complement-antibody lysis of Vero cells persistently infected with CDV were performed as described in Materials and Methods. After the target cells were incubated with different concentrations of antiserum at 37° for 1 or 24 h the cells were washed twice in cold MEM + 5% FCS and eight units of complement was added followed by incubation at 37° for 3 h. Non-infected Vero cells treated in the same manner were set up as controls to measure non-specific release. Values represent specific release \pm SD of triplicate cultures.

† ND = not done.

‡ ^{125}I -labelled protein A was added to persistently infected Vero cells which had been pre-incubated with different concentrations of antiserum for 1 or 24 h followed by washing three times in cold MEM. Cell bound ^{125}I -protein A was determined following a 60 min incubation period at 37° . c.p.m. of non-infected Vero cell controls was 352. SD of quadruplicate cultures.

antigens, reduced the degree of cytotoxicity. These results all support the previous suggestion that antigenic modulation occurs (Ho & Babiuk, 1979b), and indicate that such modulation may influence the degree of antibody-complement cytotoxicity.

In an attempt to quantify the number of antibody molecules bound to the cell surface of infected cells that are required for maximal cytotoxicity, we conducted a preliminary experiment to determine whether the level of protein A binding to antibody-treated cells could be used as a method of quantitation. Using a solid phase radioimmunoassay we demonstrated that the amount of bound antibody was proportional to the amount of ^{125}I -protein A bound (Table 2). Based on this assumption, attempts to quantify the degree of antigenic modulation were initiated. The data in Table 1 show that after incubation for only 1 h there was a low level of ^{125}I -protein binding if high levels of antibody were reacted with infected cells. The level of ^{125}I protein A binding then increased to a peak at a dilution (1/160), similar to that of peak cytotoxicity. At greater dilutions both cytotoxicity and ^{125}I -protein A

binding declined. The comparable results of cytotoxicity and ^{125}I -protein A binding suggest that even within 1 h at 37° there is modulation of the membrane antigens if high levels of antibody are present. This explains both the prozone effect which followed peak cytotoxicity and a subsequent reduction in cytotoxicity with progressive dilution of antiserum. If the target cells were incubated for 24 h then modulation could occur even at lower concentrations of antibody and there was also a gradual increase in cytotoxicity with more dilute antiserum.

Complement-mediated cytotoxicity of CDV-infected macrophages

Since canine macrophages are one of the cell types involved in replicating CDV *in vivo*, this cell population was assessed for its susceptibility to lysis by antibody and complement. As illustrated in Fig. 5, a low level of cytotoxicity was observed immediately post-infection, possibly due to viral antigens absorbed to the surface of the cells prior to virus entry. The cytolytic activity declined after virus entry such that there was minimal cytotoxicity at 6 h which began to increase rapidly thereafter as new antigens were being expressed. A comparison of the kinetics of virus replication, release and susceptibility to lysis demonstrated that cytotoxicity does occur early in the infection cycle

Table 2. Correlation between CDV serum neutralization antibody titre and radioimmunoassay (RIA) titre involving ^{125}I -labelled Staphylococcus protein A (SPA) binding to CDV infected target cells coated with specific antibody

Days post-immunization*	Serum titre	
	TCID ₅₀ †	RIA‡
0	0	50
7	32	500
14	256	2500
21	1280	2600
28	2560	3800
35	1920	2000
42	960	ND§

* Sera were collected from a puppy (2 months old) at weekly intervals after a subcutaneous injection of a single dose of CDV vaccine. Corresponding serum samples were then pooled, and heat-inactivated before use in titration.

† Titrations were performed in Vero cells as described in text.

‡ The end point was considered to be the last serum dilution that resulted in ^{125}I binding twice that of the control cultures receiving non-immune serum.

§ ND = not done.

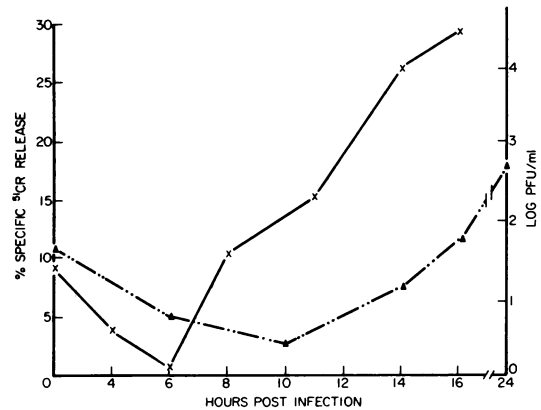


Figure 5. Kinetics of antibody-dependent complement lysis and virus production of CDV-infected canine macrophages. Values represent specific lysis in the presence of antibody and complement (\times). Each point represents the mean of triplicate wells. Non-infected macrophages plus antiserum and complement were used as controls to calculate % specific release. CDV released from infected macrophages (Δ) were titrated in Vero cells as described in the text.

and may prevent establishment of a CDV infection if antibody is present (Fig. 5).

DISCUSSION

This study shows that antibody and complement are highly efficient in destroying canine distemper-infected target cells. Cytolytic activity can be detected at low antibody concentrations such as occur early in the disease process and requires only a few hours for completion. These findings suggest that complement may play an important role in controlling the spread of CDV by destroying infected cells as soon as surface antigens are expressed and prior to or when virus is being released (Fig. 5). Thus, cytotoxicity to canine macrophages could be detected 9 h after infection, a time when minimal virus release was occurring. These observations suggest that antibody and complement could reduce the amount of virus produced and therefore reduce the severity of the disease.

It has been previously reported for complement lysis of measles virus infected cells that there is a prozone at high antibody concentrations which cannot be abolished by repeated washings to remove excess antiserum (Kibler & Ter Meuler, 1975). These observations suggest that complement inactivating factors that may be present in the serum are not responsible for this phenomenon. Our results support these findings and extend them to illustrate that the prozone is directly related to the amount of ^{125}I -labelled protein bound. Since protein A has affinity for both canine IgM and IgG (Goudswaard, Van der Donk, Noordzij, Van Dam & Vaerman, 1978), the amount of protein A should correlate well with the number of antibody molecules binding to cell surface antigens (Table 1). Therefore, the mechanisms of the prozone effect seems to be due to the lesser amount of cell bound antibody at higher concentrations. However, the subclasses and avidity of the antibody should also be taken into consideration since the presence of a relatively small amount of non-complement fixing, high avidity antibody could effectively block complement lysis. This is particularly true when hyperimmune serum containing mostly IgG is used. Finally, prozone is greatest at higher temperatures which favour membrane fluidity (Fig. 1). These results suggest that aggregation of viral antigens bound to antibody is essential for IgG doublet formation with consequent complement fixation and cell lysis. However, excess antigen movement with subsequent internalization and shedding (Ho &

Babiuk, 1979b) are detrimental to complement mediated lysis (Table 1).

Previously we have shown that antibody is highly efficient in preventing both the extracellular and intercellular spread of CDV as well as modulating surface viral antigens (Ho & Babiuk, 1979b). This redistribution and subsequent disappearance of surface antigens could render the cells refractory to host defence mechanisms such as antibody-dependent cell cytotoxicity (Ho & Babiuk, 1978) and direct T-cell cytotoxicity (Ho, unpublished data). We have now shown that antibody complement lysis is abrogated by antigen internalization induced by overnight incubation with antiserum (Table 1). Whether the same phenomenon occurs *in vivo* is a matter for speculation since in the presence of complement, infected cells should be quickly destroyed before the disappearance of viral antigens. There are, however, two situations whereby some target cells may escape. First, during the recovery phase when there is a high level of circulating virus-antibody complexes, the alternate pathway of complement may be activated leading to a transient decrease of the C3 component of complement. Second, a secondary infection with bacteria may also lead to similar activation and lowering of complement levels. This is especially feasible in canine distemper since bacterial infections are always associated with canine distemper infection (Hsiung & Stafseth, 1952). Finally, the absence of demonstrable events of lytic complement in the central nervous system (Albrecht, Barnstein, Klutch, Hicks & Ennis, 1977) would favour persistence in this site. These are the ideal conditions for both prozone and rapid capping to occur and thus favour persistence of infection. It is therefore tempting to speculate that in some cases of distemper, animals develop high levels of antibody and recover rapidly despite secondary infections. However, a small amount of virus might persist and could develop post-distemper encephalitis if conditions were favourable. Such conditions may be very complex (Norrby & Vandick, 1974) but may involve the suppression of immune mechanisms and transportation of the virus via lymphocytes to the central nervous system (Summers, Greisen & Appel, 1978).

Persistent CDV infection of dogs may occur at any age either as a result of a natural infection or vaccination with attenuated vaccines. In the case of infection of young puppies which still possess passively acquired antibody, the vaccine virus or field strains may cause an infection of some cells. If the virus is a field strain it may only cause a sub-clinical infection due to the pas-

sive antibody present. Unfortunately, this passively acquired antibody could also maintain the virus in cells, protected from immune clearance, due to antigenic modulation. It is also possible that sufficient antigenic stimulation of the immune response has not occurred due to very low levels of virus replication. As the passively acquired antibody disappears the virus could conceivably become reactivated, replicate and rapidly spread to the brain before an adequate cell-mediated immune response developed. A similar situation could occur in adult animals who have actively acquired immunity to CDV. Reinfection of these animals with high levels of antibody, may favour persistence of the virus and subsequent late disease. This type of antigenic modulation has been proposed to occur with respect to a very closely related human virus, measles, in man (Joseph & Oldstone, 1975). Our present findings support this possibility. The prozone effect of complement lysis of measles target cells (Ehrnst, 1975; Kibler & Ter Meuler, 1975; Minagawa & Yamada, 1971), the immunosuppressive nature of the virus (Starr & Berkovich, 1964) and antigen modulation by measles antibody (Oldstone & Tishon, 1978) would favour persistent infection in the two situations described above. Furthermore, chronic measles or subacute sclerosing panencephalitis (SSPE) commonly develop in immunized individuals shortly after a measles episode in the community as well as when measles virus and antibody are injected simultaneously into animals (Wear & Rapp, 1971). The latter situation is analogous to weaning puppies with passively acquired antibody exposed to CDV.

In summary, our studies show that antibody and complement are very effective in destroying CDV infected cells and that an excess of antibody prevents this phenomenon. Under conditions which allow the internalization of viral antigens, complement lysis does not occur. The prozone and antigen modulation phenomena are possible mechanisms for persistent infection.

REFERENCES

- ALBRECHT P., BURNSTEIN T., KLUTCH M.J., HICKS J.T. & ENNIS F.A. (1977) Subacute sclerosing panencephalitis: experimental infection in primates. *Science*, **195**, 64.
- BABIUK L.A., ACRES S.D. & ROUSE B.T. (1977) Solid phase radioimmunoassay for detecting bovine (neonatal calf diarrhoea) rotavirus antibody. *J. clin. Microbiol.* **6**, 10.
- EHRNST A. (1975) Characterization of measles virus-specific cytotoxic antibodies by use of a chronically infected cell line. *J. Immunol.* **114**, 1077.
- GOUDSWAARD J., VAN DER DONK J.A., NOORDZIJ A., VAN DAM R.H. & VAERMAN J.P. (1978) Protein A reactivity of various mammalian immunoglobulins. *Scand. J. Immunol.* **8**, 21.
- HO C.K. & BABIUK L.A. (1978) Isolation of various canine leucocytes and their characterization by surface marker analysis. *Immunology*, **35**, 733.
- HO C.K. & BABIUK L.A. (1979a) Immune mechanisms against canine distemper I. Identification of K cells against canine distemper virus infected target cells *in vitro*. *Immunology*, **37**, 231.
- HO C.K. & BABIUK L.A. (1979b) Immune mechanisms against canine distemper II. Role of antibody in antigen modulation and prevention of intercellular and extracellular spread of canine distemper virus. *Immunology*, (in press).
- HO C.K. & BABIUK L.A. (1979c) Development and utilization of a plaque system for canine distemper virus: characteristics of the Green strain of canine distemper virus. *Can. J. Microbiol.* **25**, 680.
- HO C.K. & BABIUK L.A. (1979d) Long term culture of canine peripheral blood monocytes *in vitro*. *Can. J. Comp. Med.* **43**, 223.
- HSIUNG G. & STAFSETH H.J. (1952) Canine distemper. I. Bacteriological studies. *Cornell Vet.* **42**, 223.
- JOSEPH B.S. & OLDSTONE M.B.A. (1975) Immunologic injury in measles virus infection. II. Suppression of immune injury through antigenic modulation. *J. exp. Med.* **142**, 864.
- KIBLER R. & TER MEULER V. (1975) Antibody-mediated cytotoxicity after measles virus infection. *J. Immunol.* **114**, 93.
- KIELING F., BANDLOW G. & THOMSEN R. (1972) Cytotoxische Reaktionen an Sendai-Virus infizierten Zellen. *Arch. Gesamte Virusforsch.* **36**, 123.
- MINAGAWA T. & YAMADA M. (1971) Studies on the persistent infection with measles virus in HeLa cells. III. Immunolysis of cells in carrier state by anti-measles sera. *Jap. J. Microbiol.* **15**, 341.
- NORRBY E. & VANDICK N. (1974) Measles and multiple sclerosis. *Proc. R. Soc. Med.* **67**, 1129.
- OLDSTONE M.B.A. & TISHON A. (1978) Immunologic injury in measles virus infection. IV. Antigenic modulation and abrogation of lymphocyte lysis of virus infected cells. *Clin. Immunol. Immunopathol.* **9**, 55.
- RICE J.M. & WOLFF D.A. (1978) Establishment of a persistent measles virus infection in Hep-2 cells. *Microbiology* **18**, 178.
- STARR S. & BERKOVICH S. (1964) Effects of measles, gammaglobulin-modified and vaccine measles on the tuberculin test. *N. Eng. J. Med.* **270**, 386.
- SÜMMERS B.A., GREISEN H.A. & APPEL M.J.G. (1978) Possible initiation of viral encephalitis in dogs by migrating lymphocytes infected with distemper virus. *Lancet*, **ii**, 187.
- WEAR D.J. & RAPP F. (1971) Latent measles virus infection of the hamster central nervous system. *J. Immunol.* **107**, 1593.