Preparation and Characterization of Monoclonal Antibodies Directed against Four Structural Components of Canine Distemper Virus

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SUMMARY

Mouse hybridomas producing antibodies against structural proteins of canine distemper virus (CDV) were produced by fusion of Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with purified preparations of Vero cell-grown CDV. Ascites fluids collected after intraperitoneal inoculation with 149 CDV antibody-producing hybridoma cell lines were characterized by different serological tests. By immune precipitation tests with [35S]methionine-labelled extracellular virions and intracellular virus polypeptides, 57 clones were found to produce antibodies against the nucleocapsid protein (NP), 22 against the polymerase (P) protein, 10 against the fusion (F) protein and nine against the large uncleaved glycoprotein (named H in analogy with measles virus). By competitive binding enzyme-linked immunosorbent assay (ELISA) tests with monoclonal antibodies against each structural component, a minimum of 18, six, three and seven separate antigenic determinants were identified on the NP, P, F and H proteins, respectively. The reactions of clones directed against F and H surface components of the virus were tested for their ability to inhibit the infectivity of both CDV and measles virus in the absence and presence of anti-γ-globulin. In addition, the inhibitory activity of the clones on measles haemagglutinating (HA) and haemolysis (HL) activity were examined. Monoclonal antibodies against six of the seven antigenic determinants of the H protein could neutralize the infectivity of the virus. After addition of anti-γ-globulin to the test, increases of titres varying from twofold to several hundredfold were observed with the different clones. None of all the clones against H could block measles virus infectivity, HA or HL activity. The 10 clones directed against the F protein could not neutralize the infectivity of CDV even in the presence of anti-γ-globulin. Further, the antibodies could not inhibit measles HA and HL activity in the absence of anti-γ-globulin. However, after the addition of anti-γ-globulin, antibodies against two of the three sites were found to block measles virus HL activity. The reactions of all clones were tested in immune fluorescence, ELISA and immune precipitation tests with three strains of CDV. Each strain had a few unique antigenic sites. Variation was found in four, one and three different antigenic sites of the NP, P and H proteins, respectively.

INTRODUCTION

Canine distemper virus (CDV) is closely related to measles virus and belongs to the morbillivirus genus of the paramyxovirus family (Kingsbury et al., 1978). During recent years the polypeptide compositions of both measles virus (Mountcastle & Choppin, 1977; Tyrrell & Norrby, 1978; Rima et al., 1979) and CDV (Campbell et al., 1980; Hall et al., 1980; Örvell, 1980) have been investigated. The immunological relationships between homologous structural proteins have been studied by immune precipitation analysis of the two viruses with different sera against whole virions of CDV and measles virus (Stephenson & ter Meulen, 1979; Hall et
Monoclonal antibodies directed against measles virus have recently been described in a number of studies (McFarlin et al., 1980; Birrer et al., 1981a; Giraudon & Wild, 1981a; ter Meulen et al., 1981; Togashi et al., 1981; Bohn et al., 1982; Norrby et al., 1982). By the use of these monoclonal antibodies the antigenic structure of different strains of measles virus has been studied. Minor antigenic differences between the haemagglutinin (H) of different strains have been demonstrated (Birrer et al., 1981b; Giraudon & Wild, 1981b; ter Meulen et al., 1981; Trudgett et al., 1981). In a recent study by Sheshberadaran et al. (1983) it has been shown that the most extensive antigenic variation among different strains of measles virus was found on the matrix (M) protein. In the same study some variation was found on the H protein, whereas none was found on the nucleocapsid (NP), polymerase (P) or fusion (F) proteins.

Monoclonal antibodies against CDV have not been described and antigenic variation between different strains of this virus has not yet been reported. Different strains of CDV have been reported to vary in the electrophoretic mobility of the H and NP proteins (Orvell, 1980; Shapshak et al., 1982). Differences have been found between the NP, H and F protein of different strains (Shapshak et al., 1982) by peptide mapping following limited proteolysis with proteolytic enzymes and analysis by SDS-polyacrylamide slab gel electrophoresis. In the same study no differences were found between the M proteins of four different strains.

The aim of the present study was to prepare and characterize serologically a large number of monoclonal antibodies against every major structural protein of the Convac strain of CDV. With the aid of these reagents attempts were made to estimate the number of epitopes and relationships among them on each structural component of the homologous virus strain. Attempts were also made to identify these epitopes in heterologous strains of CDV.

METHODS

Virus strains and preparation of virus materials. Three strains of CDV were used. One strain was a vaccine strain, referred to as the Convac strain of CDV (Orvell & Norrby, 1980). The second strain of CDV was a rapidly growing variant of the Onderstepoort strain, kindly provided by Dr M. Appel, Cornell University, Ithaca, N.Y., U.S.A. (Martin & ter Meulen, 1976). The third strain of CDV was the Rockborn strain previously studied in this laboratory (Rockborn, 1958; Orvell & Norrby, 1974). In addition to these three strains of CDV the LEC strain of measles virus was used (Sheshberadaran et al., 1983). All strains were propagated in Vero cells maintained in Eagle’s MEM containing 2% foetal calf serum. When the cultures showed pronounced cytopathic effects, the medium was harvested and the extracellular virions were purified according to the method described previously for mumps virus (Orvell, 1978).

Production of hybridoma cell lines. Purified virions of the Convac strain of CDV (protein concentration 0.5 to 1 mg/ml) were mixed with an equal volume of Freund’s complete adjuvant and a 0.4 ml portion of this mixture was inoculated intramuscularly into each hind leg of BALB/c mice. Five weeks to 2 months later the mice were given a booster of 1 ml of the same material without Freund’s adjuvant intraperitoneally. The injections were repeated on the 2 following days and the animals were sacrificed 4 days after the first booster; the cells from their spleens were used for fusions. The procedure for establishment of mouse hybridoma cell lines was similar to that described previously for studies on Sendai virus (Orvell & Grandien, 1982), with the exception that most fusions were performed with the myeloma cell line Sp2/0.

Enzyme-linked immunosorbent assay (ELISA). The technique first described by Engvall & Perlmann (1972) was used. Purified virions of all three CDV strains that had been freeze-thawed ten times and sonicated (protein concentration 20 μg/ml) were used to coat plastic plates. The titre with the different clones was expressed as the highest 10-fold dilution that gave an absorbance of more than 0.2 (Orvell & Grandien, 1982). All 1000-fold or higher differences in titres between different strains of CDV were considered as significant.

Determination of immunoglobulin class and subclass produced by individual hybridoma cell lines. Ascites material from mice inoculated intraperitoneally with different hybridoma cell lines were used for Ig class and subclass determination by ELISA as described previously (Orvell, 1984). Each ascites material at a dilution of 1:10 in a volume of 0.1 ml was added to five different wells of plastic plates coated with the Convac strain of CDV. After incubation at 37 °C for 1 h the plates were washed and 0.1 ml samples of five different peroxidase-labelled rabbit anti-mouse Ig classes and subclasses at a carefully titrated dilution were added separately to each of the five wells. The procedure of incubation and washing was repeated, after which 0.1 ml of the substrate 5-aminosalicylate (5-AS) was added. The plates were read at 450 nm 30 min later with a Titertek Multiskan apparatus (Flow Laboratories).
Monoclonal antibodies against CDV components

Radioimmune precipitation assay (RIPA). [35S]Methionine-labelled purified virions and intracellular viral antigens prepared as described previously were used as antigens in RIPA (Örvell & Norrby, 1980). In some experiments infected cell cultures were labelled with 100 μCi/ml [35S]methionine for 2 h. The extracellular [35S]methionine-labelled virions were adjusted to RIPA buffer composition [0.1% SDS, 1% sodium deoxycholate (DOC), 1% Triton X-100 in 0.15 m-NaCl, 0.01 m-Tris-HCl pH 7.4] in an ice-bath in order to preserve antigenic determinants. Twenty μl samples of each ascites material diluted 1:10 were added to 0.5 ml of 1 × 10^6 c.p.m. [35S]methionine-labelled purified virions at 0°C. Hereafter, the samples were processed as described previously (Örvell & Norrby, 1980). The details of the procedure for polyacrylamide gel electrophoresis and scintillation autoradiography have been described previously (Örvell, 1978).

Immune fluorescence analysis. The technique for immunofluorescence has been described previously (Norrby et al., 1982). Vero cells on coverslips were infected with the three strains of CDV. When cytopathic effects started to appear the coverslips were fixed in cold (−20°C) acetone at room temperature for 10 min and then air-dried. The different ascites materials derived from each clone were used at a dilution of 1:10 to stain coverslips infected with each of the three CDV strains. In some experiments ascites materials were examined at dilutions of 1/100 and 1/1000.

Other serological tests. The techniques used for determination of haemagglutination inhibition (HI) and haemolysis-inhibiting (HLI) antibodies with the LEC strain of measles virus have been described previously (Norrby & Gollmar, 1972, 1975). In haemolysis-inhibition enhancement tests, antigen–antibody mixtures were incubated at room temperature for 1 h and at 4°C overnight. After this incubation, anti-mouse Ig (Dako, Copenhagen, Denmark) at a final concentration of 1:40 was added to each tube. After incubation at room temperature for 1 h, monkey erythrocytes were added. Neutralization (NT) and neutralization-enhancement (NE) antibodies were determined as described previously (Örvell, 1980). In NE tests anti-mouse Ig was added at a final dilution of 1:24.

Competition experiments using peroxidase-conjugated monoclonal antibodies in ELISA. The periodate method was used to conjugate horseradish peroxidase to ammonium sulphate-precipitated antibodies (Wilson & Nakane, 1978). The immunological reactivity of the conjugated antibodies was monitored and calibrated in tests with viruscoated plates (Convac strain, protein concentration 20 μg/ml) by ELISA to give an absorbance at 450 nm of 0.4 to 0.5: competition experiments were performed as described (Örvell & Grandien, 1982; Örvell, 1984). The highest 10-fold dilution of unlabelled clonal Ig that could inhibit 50%, or more of the A50 value caused by the labelled clonal Ig was considered as the end titre.

RESULTS

Specificity of mouse hybridoma cell lines

One to 2 weeks after fusion the different hybridoma cell lines were tested for production of antibodies by ELISA. The medium from each clone was tested against purified virions of CDV and antigenically unrelated respiratory syncytial (RS) and parainfluenza 2 virus, all produced in Vero cells. It was found that antibodies from some clones reacted with all three viruses. Antibodies from other clones reacted with CDV but not with RS or parainfluenza 2 viruses. The latter clones were considered to react with virus-specified antigenic determinants and not with contaminating Vero cell products and were therefore selected for further work. The cells of 149 clones were successfully passaged in BALB/c mice and the antibodies were collected as ascites materials. Ascites materials from 137 of the clones had an ELISA titre of ≥ 10^3 and these were selected for further work. The monoclonal nature of the antibodies produced by the individual hybridoma cell lines was verified by the presence of only one immunoglobulin class or subclass and by their ability to precipitate only one viral protein in RIPA. The distribution of Ig class and subclass specificities in the total material was similar to that obtained in corresponding materials directed against Sendai and mumps viruses (Örvell & Grandien, 1982, Örvell, 1984), the only exception being that IgM antibodies were represented to a lesser extent in this material. In RIPA experiments the specificity of more clones could be determined by the use of extracellular [35S]methionine-labelled extracellular virions than by the use of labelled intracellular viral antigens. Of the total material, 76 clones were found to precipitate only one virus protein band each. Fifty-seven of these clones were directed against the NP protein, 10 against the F protein and nine against the H protein. Ascites material from 22 other clones exhibited an identical precipitation pattern. These antibodies precipitated several protein bands, the largest one of 78000 (78K) molecular weight, indicating that the antibodies might be directed against the 78K P protein (Örvell, 1980), the lower molecular weight bands representing breakdown products. The four different precipitation patterns with representatives from each group can be seen in Fig. 1.
Immune precipitation of \(^{35}\)S\)methionine-labelled purified virions of the Convac strain of CDV with monoclonal antibodies directed against major virus structural proteins. Lanes 1, 6, 10, purified CDV virions; lanes 2, 4, 5, 8, 9, 12, purified virions precipitated with two different monoclonal antibodies directed against NP (2 and 8), F (4 and 9) and H (5 and 12). Note that antibodies against F also precipitate a higher mol. wt. band which may represent dimers of the protein. A fourth group of clones represented by three monoclonal antibodies precipitated more than one protein band (lanes 3, 7 and 11).

The 22 monoclonal antibodies that immunoprecipitated several protein bands were reexamined in a modified RIPA. Intracellular antigen from infected cell cultures labelled for 2 h with 100 \(\mu\)Ci/ml \(^{35}\)S\)methionine was prepared in 2% Triton X-100, 0-6 M-KCl, 0-15 M-NaCl, 5 mM-EDTA, 3 mM-phenylmethylsulphonyl fluoride, 2-5 mM-iodoacetamide, 1% aprotinin, 0-01 M-Tris–HCl pH 7-8; RIPA was performed in the same buffer. Under these conditions the 22 monoclonal antibodies were found to immunoprecipitate only the 78K polymerase protein (Fig. 2; Örvell, 1980). The reaction in RIPA with 39 clones could not be determined. Ascites materials from mice inoculated with Sp2/0 myeloma cells did not precipitate any protein band (data not shown).

**Immune fluorescence (IF) staining of lytically infected cell cultures by monoclonal antibodies directed against four different CDV proteins**

Ascites fluid containing monoclonal antibodies of any one of the four different specificities gave a bright staining of infected cells. The character of the IF staining varied. Cytoplasmic inclusions were stained predominantly by monoclonal antibodies against the NP and P components but, similar to the finding with measles virus-specific hybridoma products (Norrby et al., 1982), only monoclonal antibodies against the NP component of CDV stained intranuclear inclusions. However, a fraction of the hybridomas producing antibodies with this
Monoclonal antibodies against CDV components

Fig. 2. Immune precipitation of $[^{35}S]$methionine-labelled intracellular antigens of the Convac strain of CDV labelled with 100 μCi/ml of the isotope for 2 h. Lane 1, antigen precipitated with antibodies of clone 3.766 directed against NP; lanes 2, 3 and 4, antigen precipitated with antibodies of clone 4.051, 4.088 and 3.695, respectively. The latter three antibodies precipitated a 78K P protein.

specificity lacked the ability to stain intranuclear inclusions (Fig. 3a, b). Thus, cytoplasmic nucleocapsid structures have epitopes that are absent on intranuclear nucleocapsids. Antibodies against the H and F components gave IF staining of similar distribution in cells. Besides some cytoplasmic staining there was a pronounced fluorescence associated with the cytoplasmic membrane (not illustrated).

Competition experiments using peroxidase-conjugated monoclonal antibodies against the P protein in ELISA

The 22 clones directed against the P polypeptide were coupled with peroxidase and used in competition experiments by ELISA. In these experiments a minimum of six different antigenic determinants were identified. Representative data are shown in Table 1. The antigenic determinant represented by the reaction of clones 3.698 and 3.801 (Group 1) was immunodominant on the molecule, as eight more clones reacted similarly to these two clones. The remaining antigenic determinants were identified by reaction with three clones or fewer.

Competition experiments using monoclonal antibodies against the NP protein in ELISA

Of the 57 clones directed against the NP polypeptide, 27 could be coupled with peroxidase and used in competition experiments. The 27 clones were found to react with a minimum of 18
Fig. 3. IF staining of Vero cells infected with the Convac strain of CDV by monoclonal antibodies against the nucleocapsid component. The antibody used in (a) reacts with an epitope present on both intranuclear and cytoplasmic nucleocapsids, whereas in (b) only cytoplasmic inclusions are stained.

different antigenic determinants (data not shown). Two of the antigenic determinants were recognized by groups of clones consisting of three members per group. The remaining 16 antigenic determinants were identified by the unique inhibition pattern obtained with one or at most two clones.

Identification of antigenic sites on the P and NP protein in other strains of CDV by IF, ELISA and RIP

Among the clones directed against P, all six antigenic determinants were also identified on the heterologous Onderstepoort strain, but one of the six determinants, recognized by clone 3.695 (Group 5) could not be identified on the Rockborn strain in IF and ELISA tests (Fig. 4). For the clones reacting against P, the RIP test was not applied as these clones in most experiments precipitated more than one band. Among the 57 clones directed against the NP polypeptide, one did not react with the Rockborn strain in immune fluorescence, ELISA or RIP test. With three other clones, antigenic differences could only be detected by RIP test but not by immune fluorescence or ELISA tests (see Discussion). One of the three clones did not react with the heterologous Onderstepoort strain, the second clone did not react with the heterologous strain Rockborn and the third clone did not react with either the Onderstepoort or the Rockborn strain. The antigenic differences found in all clones directed against P and NP proteins are summarized in Table 2.

Characterization of mouse ascites material from nine clones directed against the H protein

The results from the tests on nine clones directed against the H protein of CDV are summarized in Tables 3 and 4. As can be seen from Table 3 a minimum of seven antigenic sites could be identified by competitive ELISA tests. Antibodies against six of the seven antigenic determinants could neutralize the infectivity of the homologous Convac strain (Table 4). After
Table 1. *Competition experiment using ELISA with mouse ascites material from ten clones producing antibodies against the P protein of CDV in tests with ten peroxidase-conjugated clones of similar specificity*

<table>
<thead>
<tr>
<th>Designation of clone</th>
<th>Designation Antibody of clone</th>
<th>Group</th>
<th>Designation of peroxidase-conjugated clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.698</td>
<td>IgG1</td>
<td>1</td>
<td>3.698 <em>(10^5)</em> + (10^4)</td>
</tr>
<tr>
<td>3.801</td>
<td>IgG3</td>
<td>1</td>
<td>3.801 <em>(10^4)</em> + (10^3)</td>
</tr>
<tr>
<td>3.568</td>
<td>IgG3</td>
<td>2</td>
<td>3.568 <em>(10^3)</em> + (10^2)</td>
</tr>
<tr>
<td>3.802</td>
<td>IgG2b</td>
<td>2</td>
<td>3.802 <em>(10^2)</em> + (10)</td>
</tr>
<tr>
<td>3.630</td>
<td>IgG2b</td>
<td>3</td>
<td>3.630 *(10) + (10^3)</td>
</tr>
<tr>
<td>4.051</td>
<td>IgG1</td>
<td>3</td>
<td>4.051 *(10^4) + (10^3)</td>
</tr>
<tr>
<td>3.768</td>
<td>IgG1</td>
<td>4</td>
<td>3.768 *(10^3) + (10^2)</td>
</tr>
<tr>
<td>4.088</td>
<td>IgG2b</td>
<td>4</td>
<td>4.088 *(10^4) + (10)</td>
</tr>
<tr>
<td>3.695</td>
<td>IgG2b</td>
<td>5</td>
<td>3.695 *(10^2) + (10^3)</td>
</tr>
<tr>
<td>4.215</td>
<td>IgG1</td>
<td>6</td>
<td>4.215 *(10^3) + (10^4)</td>
</tr>
</tbody>
</table>

* + , Inhibition of binding of peroxidase-conjugated clone.
† Titre of inhibition with inhibiting clone.
‡ - , No inhibition of binding of peroxidase-conjugated clone.

Monoclonal antibodies against CDV components
Table 2. Recognition differences found in ascites materials from five clones producing antibodies against the P and NP proteins in tests with three strains of CDV

<table>
<thead>
<tr>
<th>Designation of clone</th>
<th>Specificity</th>
<th>Antibody subclass</th>
<th>Convac</th>
<th>Onderstepoort</th>
<th>Rockborn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IF ELISA RIPA</td>
<td>IF ELISA RIPA</td>
<td>IF ELISA RIPA</td>
<td></td>
</tr>
<tr>
<td>3.695</td>
<td>P</td>
<td>IgG2b</td>
<td>+ + NT</td>
<td>+ + NT</td>
<td>+ + + NT</td>
</tr>
<tr>
<td>4.188</td>
<td>NP</td>
<td>IgG2b</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>3.552</td>
<td>NP</td>
<td>IgG2b</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>3.958</td>
<td>NP</td>
<td>IgG3</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>4.317</td>
<td>NP</td>
<td>IgG2b</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

* +, Reaction with antigenic determinant.
\[\dagger\] NT, Not tested.
\[\ddagger\] -, No reaction with antigenic determinant.

Table 3. Competition experiment using ELISA with mouse ascites material from nine clones producing antibodies against the H protein of CDV in tests with nine peroxidase-conjugated clones of similar specificity

<table>
<thead>
<tr>
<th>Designation of peroxidase-conjugated clone</th>
<th>Designation of antibody subclass</th>
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<tbody>
<tr>
<td>1.347 IgG1</td>
<td>1.347 (10^5)</td>
</tr>
<tr>
<td>2.267 IgG2a</td>
<td>2.267 (10^4)</td>
</tr>
<tr>
<td>3.734 IgG1</td>
<td>3.734 (10^4)</td>
</tr>
<tr>
<td>3.900 IgG1</td>
<td>3.900 (10^4)</td>
</tr>
<tr>
<td>3.775 IgG1</td>
<td>3.775 (10^3)</td>
</tr>
<tr>
<td>4.043 IgG1</td>
<td>4.043 (10^4)</td>
</tr>
<tr>
<td>4.074 IgG1</td>
<td>4.074 (10^4)</td>
</tr>
<tr>
<td>4.275 IgG1</td>
<td>4.275 (10^4)</td>
</tr>
<tr>
<td>4.941 IgG2a</td>
<td>4.941 (10^4)</td>
</tr>
</tbody>
</table>

* +, Inhibition of binding of peroxidase-conjugated clone.
\[\dagger\] Titre of inhibition with inhibiting clone.
\[\ddagger\] -, No inhibition of binding of peroxidase-conjugated clone.

Table 4. Determination of different antibody activities in ascites material from nine clones producing antibodies against the H protein of CDV

<table>
<thead>
<tr>
<th>Designation of clone</th>
<th>Convac</th>
<th>Onderstepoort</th>
<th>Rockborn</th>
<th>Measles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT NE</td>
<td>NT NE</td>
<td>NT NE</td>
<td>NT NE</td>
</tr>
<tr>
<td>1.347</td>
<td>32</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>2.267</td>
<td>256</td>
<td>2048</td>
<td>(\leq) 4</td>
<td>1024</td>
</tr>
<tr>
<td>3.734</td>
<td>128</td>
<td>4096</td>
<td>16</td>
<td>2048</td>
</tr>
<tr>
<td>3.900</td>
<td>256</td>
<td>8192</td>
<td>32</td>
<td>8192</td>
</tr>
<tr>
<td>3.775</td>
<td>16</td>
<td>2048</td>
<td>32</td>
<td>1024</td>
</tr>
<tr>
<td>4.043</td>
<td>16</td>
<td>4096</td>
<td>16</td>
<td>8192</td>
</tr>
<tr>
<td>4.074</td>
<td>64</td>
<td>32000</td>
<td>32</td>
<td>32000</td>
</tr>
<tr>
<td>4.275</td>
<td>(&lt;4)</td>
<td>(&lt;8)</td>
<td>(&lt;4)</td>
<td>(&lt;8)</td>
</tr>
<tr>
<td>4.941</td>
<td>8</td>
<td>1024</td>
<td>16</td>
<td>1024</td>
</tr>
</tbody>
</table>

Measles: NT NE HI HLI
Fig. 4. Titration of clone 3.695 directed against the P protein of the Convac strain of CDV in tests with three different strains of CDV in ELISA. CDV strains used to coat plastic plates were Convac (●), Onderstepoort (■) and Rockborn (○).

Fig. 5. Titration of clone 2.267 directed against the H protein in tests with three different strains in ELISA. CDV strains used to coat plastic plates were Convac (●), Onderstepoort (■) and Rockborn (○).

Fig. 6. Titration of clone 3.775 directed against the H protein in tests with three different strains in ELISA. CDV strains used to coat plastic plates were Convac (●), Onderstepoort (■) and Rockborn (○).
addition of anti-γ-globulin to the test, a large variation between the increases of titres with
different clones was observed. Clone 1.347 exhibited no significant increase of titre, clone 2.267
an eightfold increase and clones 3.775, 4.043, 4.074 and 4.941 a hundredfold or higher increase
of titres. In neutralization tests with the two heterologous strains, some differences were noted.
Clone 2.267 could not neutralize the infectivity of the Onderstepoort strain in the absence of
anti-γ-globulin, but in the presence of anti-γ-globulin an efficient neutralization was observed.
In ELISA tests this monoclonal antibody reacted significantly less well with the Onderstepoort
strain as the slope of the titration curve was less steep (Fig. 5). The absolute titre, on the other
hand, was similar to that measured with strains Convac and Rockborn. From Table 4 it can also
be seen that clone 3.775 could not neutralize the infectivity of the Rockborn strain either in the
absence or presence of anti-γ-globulin. The lack of this antigenic determinant on the Rockborn
strain was confirmed by IF and ELISA (Fig. 6). Somewhat lower titres of clones 3.734 and
3.900 were observed in neutralization tests with the Onderstepoort strain in the absence of anti-
γ-globulin, but this possible difference could not be confirmed by IF and ELISA tests. Clone
4.275, which could not neutralize the infectivity of the homologous virus strain, reacted with
the Convac and Onderstepoort strain, but not with the Rockborn strain in IF and ELISA (data not
shown). All clones directed against the H protein lacked the ability to react in NT, NE, HI and
HLI tests against measles virus (Table 4).

Characterization of mouse ascites material from ten clones directed against the F protein

Ten clones were found to produce antibodies against the F protein of CDV. The results from
testing of eight ascites materials from representative clones are summarized in Table 5. The ten
clones formed three groups by competitive ELISA: the largest group which is represented by
clones 3.551, 3.584 and 4.068 (Group 2) contained five different clones. Clones from Groups 1
and 2 could not inhibit each other’s binding to the F protein, but both groups exhibited some
capacity to block the binding of clones of Group 3 to the protein. All clones reacted in ELISA
with CDV to high titres, but none of the clones could neutralize the infectivity of the homologous virus strain, reacted with the Convac and Onderstepoort strain, but not with the Rockborn strain in IF and ELISA (data not
shown). All clones directed against the H protein lacked the ability to react in NT, NE, HI and
HLI tests against measles virus (Table 4).

DISCUSSION

In the present study the ascites materials from 137 clones were studied in immune
precipitation assays. The reactivity of 98 clones could be determined. The proportion of clones
with defined reactivity in RIPA was significantly higher than that in corresponding materials
directed against Sendai virus and mumps virus (Orvell & Grandien, 1982; Örvell, 1984). The
reasons for this may be that in the present study only clones reacting with virus-specified protein
determinants were selected for further work. Clones reacting with the NP, F and H proteins
were readily identified by RIPA. Antibodies against the P protein could only be identified when
antigens that had been labelled for short periods of time were used in RIPA tests. The P protein
of paramyxoviruses is very susceptible to proteolysis (Rima, 1983), which may explain the
failure to recognize the protein in RIPA tests under standard experimental conditions. In IF
tests the staining characteristics of clones directed against the P protein of CDV were similar to
that obtained with the corresponding measles virus clones (Norrby et al., 1982).

The reasons why no clones producing antibodies against the M protein were obtained is not
known. In similarly produced materials directed against Sendai virus and mumps virus, four and
ten anti-M clones were identified (Örvell & Grandien, 1982; Örvell, 1984). It is possible that the
M protein has a low immunogenicity, or is insufficiently exposed to the antibody-producing cells
when the protein is located inside purified virions used for immunization.
Table 5. Characterization of mouse ascites material from eight clones directed against the F protein of CDV in competitive ELISA and other serological tests

<table>
<thead>
<tr>
<th>Designation of clone</th>
<th>Group</th>
<th>Antibody subclass</th>
<th>Designation of peroxidase-conjugated clone</th>
<th>CDV</th>
<th>Measles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.608 3.633 5.086 3.551 3.584 4.068 4.985 5.148</td>
<td>ELISA NT NE HI HLI HLIE§</td>
<td></td>
</tr>
<tr>
<td>3.608</td>
<td>1</td>
<td>IgG2a</td>
<td>+(10^5) t  + (10^5) + (10^5) - - - + (10^3) + (10^3)</td>
<td>10^6</td>
<td>&lt;4 &lt;4 &lt;4 5-120</td>
</tr>
<tr>
<td>3.633</td>
<td>1</td>
<td>IgG2b</td>
<td>+ (10^4) + (10^4) + (10^5) - - - + (10^3) + (10^3)</td>
<td>10^6</td>
<td>&lt;4 &lt;4 &lt;4 1-280</td>
</tr>
<tr>
<td>5.086</td>
<td>1</td>
<td>IgG2a</td>
<td>+ (10^3) + (10^3) + (10^3) - - - -</td>
<td>10^3</td>
<td>&lt;4 &lt;4 &lt;4 256</td>
</tr>
<tr>
<td>3.551</td>
<td>2</td>
<td>IgG2b</td>
<td>- - - - - - +(10^5) + (10^5) + (10^5) + (10^3) + (10^3)</td>
<td>10^6</td>
<td>&lt;4 &lt;4 &lt;4 32</td>
</tr>
<tr>
<td>3.584</td>
<td>2</td>
<td>IgG2a</td>
<td>- - - - - - + (10^4) + (10^4) + (10^4) + (10^4) + (10^4)</td>
<td>10^6</td>
<td>&lt;4 &lt;4 &lt;4 64</td>
</tr>
<tr>
<td>4.068</td>
<td>2</td>
<td>IgG1</td>
<td>- - - - - - - + (10^3) + (10^3) + (10^3) + (10^3)</td>
<td>10^5</td>
<td>&lt;4 &lt;4 &lt;4 4</td>
</tr>
<tr>
<td>4.985</td>
<td>3</td>
<td>IgG2a</td>
<td>- - - - - - - - + (10^3) + (10^3)</td>
<td>10^5</td>
<td>&lt;4 &lt;4 &lt;4 &lt;4</td>
</tr>
<tr>
<td>5.148</td>
<td>3</td>
<td>IgG1</td>
<td>- - - - - - - - - - + (10^3) + (10^3)</td>
<td>10^5</td>
<td>&lt;4 &lt;4 &lt;4 &lt;4</td>
</tr>
</tbody>
</table>

* + , Inhibition of binding of peroxidase-conjugated clone.
† Titre of inhibition with inhibiting clone.
‡ - , No inhibition of binding of peroxidase-conjugated clone.
§ HLIE, HLI enhancement test.
Intracytoplasmic and intranuclear nucleocapsids showed different antigenic properties. This should be related to the previously observed morphological difference between structures in these two compartments (Koestner & Long, 1970). The cytoplasmic nucleocapsids are more fuzzy due to their association with additional material. Such an association might conceal epitopes, but since the cytoplasmic nucleocapsids carried epitopes which were absent on nuclear nucleocapsids this explanation does not appear to apply. Instead, there may be a difference in the folding of the nucleoprotein, possibly due to interaction with other proteins or because of different interactions with RNA. In fact the nature of the RNA assumed to be associated with morbillivirus intranuclear inclusions has not been unequivocally defined.

By competitive ELISA, attempts were made to estimate the number and relationship between different antigenic determinants of each structural component. The rationale was that if two clones were directed against separate antigenic sites they would be inhibited in a different way by a panel of monoclonal antibodies directed against the same protein. Six antigenic determinants, one of which was immunodominant, were identified on the P protein. Eighteen different antigenic determinants were identified among 27 different clones directed against the NP protein. As almost all antigenic determinants were identified by reaction with only one or two clones, it is likely that the real number of antigenic determinants on the protein is considerably higher than 18. Among all 79 clones directed against P and NP only two clones did not react with the Rockborn strain in IF and ELISA.

Three other clones directed against NP did not react with one or two of the heterologous strains in RIPA but cross-reacted in IF and ELISA tests. The absence and presence of reactions with heterologous strains in RIPA and ELISA tests, respectively, is known from the measles virus system (Sheshberadaran et al., 1983). Also, the limited proteolysis pattern obtained with proteolytic enzymes has been shown to be different for the NP protein of different CDV strains (Shapshak et al., 1982).

In a recent study on measles virus by the use of monoclonal antibodies against this virus, it has been shown that the most extensive strain variation of antigenic determinants exists on the M protein (Sheshberadaran et al., 1983). The antigenic differences between the M proteins of different CDV strains could not be investigated in the present study due to the lack of clones directed against this protein.

The nine clones directed against the H protein were found to react with seven different antigenic determinants by competitive ELISA. These antibodies interacted with each other in a more complex pattern than the corresponding clones directed against the F and P proteins. Only the non-neutralizing clone 4.275 was not blocked in its binding to the protein by any other clone. Antibodies against six of the seven sites could neutralize the infectivity of the virus. This result was in contrast to recently obtained results on Sendai virus and mumps virus, because with these viruses a relatively smaller proportion of antibodies against the corresponding protein was found to neutralize infectivity (Örvell & Grandien, 1982; Örvell, 1984). After addition of anti-\(\gamma\)-globulin to the neutralization test a large difference in the increases of titres between different clones was observed. These pronounced differences were not due to different avidity in binding to the protein, as the clones showed a similar slope of the curve in titration against the virus by ELISA. Two of the seven antigenic determinants were not present on the Rockborn strain of CDV. One clone against a third determinant only showed a partial fit to the corresponding structure on the Onderstepoort strain. This conclusion was based on the finding that this antibody could neutralize the infectivity of the Onderstepoort strain in the presence but not in the absence of anti-\(\gamma\)-globulin. The slope of the titration curve in ELISA also indicated that this monoclonal antibody had lower avidity in its binding to the Onderstepoort strain.

In competition experiments by ELISA the ten clones directed against the F protein were found to be directed against three different antigenic sites. Two groups of antibodies cross-reacted with measles virus in HLI enhancement tests. One clone, 4.068, which appeared to be directed against the same antigenic determinant as four other clones by competitive ELISA, has been shown to be directed against a separate antigenic site due to its abberant staining characteristics of measles virus strains by IF (E. Norrby, unpublished data). All clones directed against the F protein lacked the capacity to neutralize the infectivity of CDV either in the
absence or presence of anti-γ-globulin. In recent studies on mumps and measles viruses, it has been shown that antibodies against their F proteins do not contain demonstrable NT or NE antibody activities (Örvell, 1984; E. Norrby, unpublished data). Also, monoclonal antibodies against the F protein of CDV, measles virus and mumps virus do not prevent cell-to-cell spread of infection in tissue culture (C. Orvell & E. Norrby, unpublished data). These results are in contrast to results obtained with polyclonal sera against the F protein of simian virus 5 and measles virus which efficiently prevent cell-to-cell spread of infection with these viruses (Merz et al., 1980; Norrby et al., 1984). The monoclonal antibodies against the F protein were used in the 'Western blot' technique (Burnette, 1981) in an attempt to see if the clones reacted with the F₁ or the F₂ polypeptide chain of the F protein. These experiments were unsuccessful except for one clone, 3.633, which was found to react with F₁ (H. Sheshberadaran, unpublished data).

In recent studies it has been shown by the use of monoclonal antibodies that all paramyxoviruses studied exhibit antigenic differences between different strains (ter Meulen et al., 1981; Giraudon & Wild, 1981b; Server et al., 1982; Russell & Alexander, 1983; Nishikawa et al., 1983; Sheshberadaran et al., 1983; Örvell, 1984). In the present study it has been shown that antigenic variations exist also in CDV strains. Of the two surface components the H protein appeared to exhibit more antigenic variability than the F protein. Similar results have been obtained in recent studies on measles and mumps viruses (Sheshberadaran et al., 1983; Örvell, 1984).

The large number of monoclonal antibodies described in the present study should be useful for the study of antigenic composition of different wild strains of CDV. Also, the antigenic relationships between different viruses of the morbillivirus genus should be amenable to more detailed studies.

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