Canine Distemper Virus (CDV) Immune-stimulating Complexes (Iscoms), but Not Measles Virus Iscoms, Protect Dogs against CDV Infection

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SUMMARY

The potential of immune-stimulating complexes (iscoms), a novel form of antigenic presentation, for the induction of protective immunity against morbillivirus infection was shown by immunizing dogs with canine distemper virus (CDV) iscoms, which contained the fusion (F) protein and a minor amount of the haemagglutinin of the virus. The immunized dogs developed CDV-neutralizing antibodies but, in contrast to non-immunized dogs, did not develop viraemia or clinical signs of infection upon intranasal challenge with the virulent Snyder Hill strain of CDV. Immunization of dogs with measles virus (MV) iscoms, prepared either from affinity-purified MV F protein or from purified whole virus, resulted in partial protection against challenge with CDV. The data presented clearly show that the iscom form of antigenic presentation may be considered a serious candidate for subunit vaccines against morbillivirus infection.

INTRODUCTION

The haemagglutinin (H) and the fusion (F) membrane proteins of measles virus (MV) and canine distemper virus (CDV), both members of the genus morbillivirus of the family Paramyxoviridae, play an important role in the induction of antiviral immunity. The H protein of all paramyxoviruses is involved in the adsorption of the virus to cell receptors and in haemagglutinating activity. The F protein of paramyxoviruses in its biologically active form, in the case of CDV and MV two covalently coupled subunits, is responsible for virus-cell fusion, the formation of syncytia by infected cells, and the haemolytic activity of the virus (Scheid et al., 1972; Scheid & Choppin, 1974, 1977; Hardwick & Bussell, 1978; Hall et al., 1980). Antibodies directed against the H protein of these viruses show virus-neutralizing (VN) activity in vitro, whereas the affinity chromatography-purified F protein induces no or only very low VN antibody titres (Varsanyi et al., 1984; Orvell et al., 1985; Norrby et al., 1986; de Vries et al., 1988).

Although live vaccines protect well against CDV or MV infection and disease the use of subunit vaccines is still being considered, since the latter do not suffer from certain disadvantages associated with live vaccines. With the virtual disappearance of distemper and measles in vaccinated populations, the acceptability of side-effects due to live vaccination has been reduced considerably. Furthermore, live virus vaccine may be dangerous to other animal species or immunocompromised individuals, and in the case of CDV, reversal to virulence upon serial animal passaging has been shown to occur. The need for a well maintained cold chain and interference due to infection with other viral agents are also problems associated with the use of live vaccines (for reviews, see Norrby, 1985; Appel, 1987). Previous attempts to protect humans or dogs with Tween–ether- or formaldehyde-inactivated MV or CDV preparations, respectively, resulted in only partial protection against infection and disease (Gillespie, 1974; Norrby et al., 1975; Appel et al., 1984). The relative lack of protection

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associated with these inactivated vaccines was suggested to be due to an 'incomplete' antibody response to the F protein, since no haemolysis-inhibiting (HLI) antibodies were induced (Norrbys et al., 1975; Appel et al., 1984). Consequently, for the construction of effective subunit vaccines against MV or CDV infection, the presentation of the F protein in a proper immunogenic form is considered to be of major importance (Committee on Issues for New Vaccine Development, 1985).

A novel structure for the antigenic presentation of membrane proteins from enveloped viruses, the immune-stimulating complex (iscom), has been shown to be highly effective in inducing both humoral and cell-mediated immune responses (Morein et al., 1984; Morein & Simons, 1985; Osterhaus et al., 1985; Wahren et al., 1987; Howard et al., 1987; Varsanyi et al., 1987; de Vries et al., 1988). Iscoms prepared with whole MV or with affinity-purified MV F protein, were shown to induce biologically active HLI antibodies against the F protein, and to protect mice against fatal encephalopathy after intracerebral challenge with MV (de Vries et al., 1988).

In this paper we report a study of the potential of CDV iscoms to induce protective immunity in dogs against an experimental respiratory infection with the virulent Snyder Hill strain of CDV. Because of the cross-protection against CDV by vaccination of dogs with live MV vaccine, which was suggested to be due to the induction of MV F-specific antibodies (Appel et al., 1984), vaccination studies with MV iscoms and MV F iscoms were also carried out. It was found that CDV iscoms induce VN antibodies in dogs, and also protection against viraemia and disease upon challenge with virulent CDV, whereas immunization of dogs with MV iscoms or MV F iscoms results only in a reduced level of viraemia and less severe clinical signs.

**METHODS**

**Viruses.** The Rockbom strain of CDV, kindly provided by Coopers Animal Health, New Zealand, was propagated in Vero cells, concentrated 75-fold from the culture supernatant by ultrafiltration in a hollow fibre system with an M, cut-off of 108 (Amicon) and purified according to methods previously described for MV (de Vries et al., 1988). During the concentration procedure infectivity titres increased from 106 to 109 TCID50/ml. The Snyder Hill strain of virulent CDV in dog brain suspension was kindly provided by Dr M. Weststrate (Duphar, Weesp, The Netherlands) and was used to challenge dogs. The CDV Bussell strain (Bussell & Karzon, 1965) was used for the VN test and radioimmunoprecipitation assay (RIPA). Concentrated culture supernatant of MV (Edmonston B strain)-infected Vero cells was used for the preparation of iscoms and for serological tests (de Vries et al., 1988).

**Preparation of CDV, MV F and MV iscoms.** CDV iscoms were prepared essentially according to the method previously described by Morein et al. (1984) for MV iscoms. Briefly, the membrane proteins of concentrated CDV of the Rockbom strain were solubilized with 2% Triton X-100. The insoluble debris was sedimented by ultracentrifugation (1.5 h, 35000 r.p.m. in an R40 Beckman rotor). Solubilized proteins were layered over a discontinuous gradient of 10, 20, 30, 40, 50 and 60% sucrose, of which the 10 to 30% sucrose solutions also contained 0.1% Quil A (Spikoside; Isocet, Sweden). After centrifugation in an SW50.1 rotor (Beckman) at 48000 r.p.m. for 4.5 h, gradient fractions were collected, dialysed against TN buffer (10 mM-Tris–HCl pH 7.6, 150 mM-NaCl) and analysed by SDS-PAGE and electron microscopy as described previously (de Vries et al., 1988). CDV iscom-containing fractions (Fig. 1, lanes 12, 13 and 14) were pooled and used in protection experiments. MV iscoms containing MV F protein and a minor amount of the MV H protein, and MV F iscoms containing affinity chromatography-purified MV F protein, were prepared according to methods described by Morein et al. (1984) and de Vries et al. (1988) respectively.

**Protection experiment in dogs.** Twelve 85-day-old beagle dogs purchased from the Centraal Proefdierenbedrijf colony (Zeist, The Netherlands) were housed under strict isolation conditions during the protection experiment. All dogs were free from CDV-specific antibodies, as shown in a VN test and ELISA (see below). Three groups of three dogs each were immunized intramuscularly twice at an interval of 4 weeks (days −35 and −7) with 7 µg CDV iscoms (dogs 4, 5 and 6), 7 µg MV F iscoms (dogs 7, 8 and 9) or 7 µg MV iscoms (dogs 10, 11 and 12) per dose respectively. Blood samples were collected before starting the experiment and at weekly intervals thereafter until the day of challenge (day 0). One week after the last immunization all animals including three non-immunized control dogs (dogs 1, 2 and 3) were challenged intranasally by aerosol with 5 × 103 TCID50 of the Snyder Hill strain of CDV. Clinical symptoms displayed by the dogs were recorded by measuring their body temperatures daily and by bi-weekly monitoring of their body weights. Heparinized blood samples were collected on days 4, 7, 11, 14, 18 and 21 after challenge for counting and differentiation of peripheral blood cells, serology and virus isolation. Peripheral blood mononuclear cells (PBMC) used for virus isolation were isolated by gradient
centrifugation on Ficoll-Isopaque (Pharmacia) and were stored in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 20% foetal calf serum (FCS) and 10% DMSO in liquid nitrogen (Appel, 1978).

**Virus isolation.** Virus isolation was carried out by co-cultivating PBMC from all dogs at day 4, 7 and 11 after challenge with primary cultures of dog lung macrophages as described by Appel & Jones (1967) with minor modifications. Briefly, the lungs of an anaesthetized dog were washed with phosphate-buffered saline (PBS) and washings were filtered through sterile cloth and centrifuged for 10 min at 1500 r.p.m. Sedimented cells were resuspended in Eagle’s MEM containing Hanks’ salts supplemented with 20% FCS, 5% lactalbumin, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mm-l-glutamine and seeded into 150 cm² plastic tissue culture bottles (Costar) at a density of 1 × 10⁶ to 2 × 10⁶ cells/ml. After 1 h at 37 °C the adherent cells were washed with PBS, incubated with 2 mm-EDTA in PBS for 15 min and collected by scraping with a rubber policeman. Cells were sedimented and resuspended in culture medium, then seeded at a concentration of 10⁶ cells/ml in eight-well Lab-Tek chambers (250 μl/well; Miles Laboratories). Cultures were maintained at 37 °C in a humidified atmosphere of 7% CO₂ in air. Tenfold dilutions of lymphocytes isolated from infected dogs were made in IMDM containing 10% FCS, and 0.1 ml of each dilution was added in duplicate to the primary lung macrophage cultures. After 5 days of cultivation the extent of formation of syncytiot was estimated by microscopy, and CDV infection was confirmed by immunofluorescence studies on cells fixed with 96% ethanol at −70 °C, using biotin-conjugated anti-CDV hyperimmune antibodies and an avidin–fluorescein isothiocyanate conjugate. The number of CDV-infected PBMC was calculated from titration endpoints and the total number of circulating lymphocytes.

**Serological tests.** CDV antibody ELISA (CDV ELISA) was carried out as previously described for the MV antibody ELISA (MV ELISA) with minor modifications (de Vries et al., 1988). Briefly, CDV-infected Vero cell lysate diluted in PBS was used as the coating antigen. An uninfected Vero cell lysate was used as a negative control antigen. Horseradish peroxidase (HRP)-conjugated anti-dog IgG (γ-chain specific) or HRP-conjugated anti-dog IgM (μ-chain specific) were used as second antibody (Kirkegaard & Perry Laboratories, Gaithersburg, U.S.A.). ELISA titres were expressed as the highest serum dilution that gave 50% of the maximal substrate reaction. CDV VN, MV VN and MV HLI tests were carried out according to methods described by Norrby & Gollmar (1972) with minor modifications. Briefly, CDV VN and MV VN tests were carried out by mixing 50 μl of serial twofold dilutions of serum and 50 μl of a CDV (Bussell strain) or MV (Edmonston B strain) dilution containing 10⁴ TCID₅₀ in 96-well microtitre plates and subsequently incubating for 1 h at 37 °C. Thereafter, 50 μl of a Vero cell suspension (2.5 × 10⁴ cells/ml) was added to each well. VN antibody titres were calculated from readings taken after 5 days incubation at 37 °C. The MV HLI test was carried out by mixing 100 μl of serial twofold dilutions of serum in PBS with 50 μl of MV diluted in PBS in V-bottomed, 96-well microtitre plates. After incubation for 1 h at 37 °C, 50 μl of a 4% (v/v) suspension of washed green monkey (Cercopithecus aethiops) erythrocytes was added. Mixtures were incubated for 3 h at 37 °C, after which erythrocytes were removed by centrifugation (5 min at 400 g). The optical density at 405 nm of 100 μl supernatant fluid was determined. HLI titres were expressed as the highest dilution of serum producing a 50% reduction of haemolysis.

**Radioimmunoprecipitation assay.** Immunoprecipitations were carried out as described by Rima et al. (1987) with minor modifications. Briefly, monolayers of Vero cells infected with CDV (Bussell strain) or MV (Edmonston B strain) were labelled 24 h after infection, when more than 50% of the monolayers consisted of syncytia, with about 20 μCi [35S]methionine per 10⁶ cells for 9 h. Thereafter cells were washed and lysed by adding RIPA buffer [1% Triton X-100, 1% sodium deoxycholate, 0-1% SDS, 0-15 M-NaCl, 1 mM-PMSF, 5% v/v aprotinin solution (Sigma) in 10 mM-Tris–HCl pH 8-0 (Lamb et al., 1978)]. The lysates were then centrifuged at 50 000 g for 20 min and the supernatants were used for immunoprecipitation. CDV- or MV-infected cell lysates (100 to 150 μl) were incubated with 5 μl antiserum or monoclonal antibody for 3 h at 4°C. Fifty μl of a 20% (v/v) suspension of Protein A–Sepharose beads (Pharmacia) in RIPA buffer was then added, followed by overnight incubation at 4 °C. The beads were washed four times with RIPA buffer and once with RIPA buffer minus detergents. The washed beads were mixed with an equal volume of double-strength sample buffer for SDS–PAGE. Samples were boiled for 5 min and subsequently analysed on 12.5% polyacrylamide gels. After electrophoresis gels were fixed in 8% acetic acid in 46% methanol, dried and fluorographed on X-Omat AR film (Kodak). The monoclonal antibodies against the MV F protein (F₁, 7-21) or MV H protein (C26-15) and a rabbit anti-MV hyperimmune serum used for control purposes, have been described previously (de Vries et al., 1987, 1988).

**RESULTS**

**Production and characterization of CDV iscoms**

The H and F glycoproteins of CDV were used for the production of CDV iscoms after solubilization of the virus in Triton X-100 and separation of these proteins from the matrix (M) protein and nucleoprotein (N) by ultracentrifugation. Centrifugation of the H and F proteins into a Quil A-containing sucrose gradient resulted in a sedimentation profile of the two proteins,
as analysed by SDS-PAGE, which closely resembled that of the two MV membrane proteins after a similar density centrifugation (Fig. 1a). Electron microscopy of the fractions in the lower part of the gradient, containing the majority of the F component of CDV and about 5% of the total amount of H protein as estimated by densitometry, revealed the presence of iscom-like particles of 40 to 60 nm in diameter (Fig. 1b), whereas no iscoms were found in the fractions
Table 1. Effect of immunization with CDV iscoms, MV F iscoms or MV iscoms on clinical signs after exposure of dogs to virulent CDV

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Diarrhoea</th>
<th>Weight loss</th>
<th>Elevated body temperature*</th>
<th>Lymphopenia†</th>
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<tbody>
<tr>
<td>Control</td>
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<td>1</td>
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<td>2</td>
<td>+</td>
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<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>CDV iscoms</td>
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<td>4</td>
<td>−†</td>
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<td>5</td>
<td>−</td>
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<td>6</td>
<td>−</td>
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<tr>
<td>MV F iscoms</td>
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<td>7</td>
<td>−</td>
<td>+</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>−</td>
<td>−</td>
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<td>−</td>
</tr>
<tr>
<td>MV iscoms</td>
<td></td>
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<tr>
<td>10</td>
<td>+</td>
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<td>12</td>
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* +, Body temperature ≥ 39.5°C.
† +, Circulating lymphocytes <1·5 × 10⁶/ml; □, circulating lymphocytes ≤ 0·75 × 10⁶/ml.
†† No clinical signs of infection.

containing the majority of the H protein. The efficiency of incorporation of the F protein of CDV into the iscom matrix was estimated to be over 90% of the total amount of F present in the purified virus preparation.

Clinical signs upon challenge with virulent CDV

The clinical signs observed in the dogs upon challenge with virulent CDV are summarized in Table 1. The non-immunized control animals (dogs 1 to 3) all developed diarrhoea in the 2nd week after challenge. Dogs 1 and 3 also lost weight and showed a biphasic elevation (monophasic in dog 2) of their body temperatures. Counting the PBMC revealed lymphopenia in dogs 1 and 3 on day 4 after challenge and in dog 2 on day 7.

In contrast, the dogs immunized with CDV iscoms (dogs 4 to 6) did not show any clinical reaction upon challenge.

Dogs immunized with MV F iscoms (dogs 7 to 9) all developed clinical signs and lymphopenia. However, the symptoms proved to be less severe than those in the control dogs, since none of these dogs developed diarrhoea, the elevation of the body temperature was only monophasic and the lymphopenia lasted for a shorter period.

Two of the three dogs immunized with MV iscoms (dogs 10 and 11) developed clinical signs, and all three suffered from lymphopenia. The symptoms in these dogs were also less severe than in the control dogs, since only one dog (dog 10) developed diarrhoea, the elevation of their body temperatures was monophasic and lymphopenia was only observed in these dogs on day 7 after the challenge.

Virus isolation from PBMC upon challenge with virulent CDV

CDV was isolated from PBMC of the non-immunized dogs (dogs 1 to 3) on days 4 and 7 after challenge, whereas no virus was isolated from dogs immunized with CDV iscoms (dogs 4 to 6, Fig. 2). Immunization with MV iscoms or MV F iscoms did not protect the dogs from developing CDV viraemia, since as in the non-immunized dogs CDV could be isolated from their PBMC on days 4 and 7. However, the percentage of CDV-infected lymphocytes in the circulation at day 4 after the challenge was lower than the percentage of infected cells in the non-immunized dogs.
Fig. 2. Viraemia in (a) non-immunized dogs (dogs 1 to 3), or in (b) dogs immunized with CDV iscoms (dogs 4 to 6), (c) MV F iscoms (dogs 7 to 9) or (d) MV iscoms (dogs 10 to 12) at 4, 7 and 11 days after challenge with virulent CDV. Numbers of circulating CDV-infected PBMC per 10⁶ lymphocytes are expressed as \( \log_{10} \) of the mean values of independent measurements within each group of three dogs. Minimal and maximal values measured within each group are indicated.

**Antibody responses after immunization and after challenge with virulent CDV**

The development of CDV- and MV-specific IgG and IgM antibodies was monitored in the respective ELISAs and the development of biologically active antibodies by CDV VN, MV VN and by MV HLI tests during the course of the experiment (Fig. 3 and 4).

The non-immunized dogs (dogs 1 to 3) developed CDV-specific IgG and IgM ELISA antibodies (Fig. 3a) between days 7 and 11 after challenge which coincided with the development of CDV VN antibodies (Fig. 4a). In these dogs no MV-specific IgG and IgM antibodies were found in the MV ELISA (Fig. 3b) and low MV-neutralizing antibody titres were found from day 18 onwards (Fig. 4b). MV HLI antibodies were observed from day 11 onwards (Fig. 4b). CDV could not be isolated from their PBMC beyond the time at which CDV-neutralizing antibodies were observed.

The dogs immunized with CDV iscoms (dogs 4 to 6) developed high titres of CDV-specific IgG ELISA antibodies (Fig. 3c) within 1 week of the second immunization, as well as CDV VN antibodies (Fig. 4c). Low levels of antibodies which were cross-reactive with MV were induced, as shown by MV ELISA (Fig. 3d), and MV VN and MV HLI tests (Fig. 4d). After challenge with CDV neither ELISA nor VN or HLI antibody titres increased in dogs 4 and 5. Only dog 6 showed an increased level of MV VN antibodies between day 11 and 18. These observations together with the inability to isolate CDV from their PBMC strongly suggests that no CDV replication occurred in dogs 4 and 5, and only limited replication occurred in dog 6 after CDV challenge.

The dogs immunized with MV F iscoms (dogs 7 to 9) developed low CDV-specific and high MV-specific IgG ELISA antibody titres after the second immunization (Fig. 3e, f). Neither CDV VN nor MV VN antibodies were observed after immunization, whereas high titres of MV HLI antibodies were shown to be induced (Fig. 4e, f). Challenge of these dogs with CDV resulted in an increase of CDV-specific IgG and IgM ELISA antibody titres to about the same levels and at about the same time as in the control dogs (Fig. 3e). The levels of CDV-neutralizing antibody titres induced upon challenge were similar to those observed in the control dogs (Fig. 4e). These observations confirmed that dogs immunized with MV F iscoms were not protected against CDV infection. Although the levels of MV ELISA antibody titres after immunization with MV F iscoms were high, challenge with CDV resulted in an increase in MV IgG antibody titres between day 7 and 11 (Fig. 3e, f). No MV-specific IgM antibodies were found. Furthermore MV HLI antibody titres increased and MV VN antibodies were also shown to be induced by CDV challenge (Fig. 4f). CDV was isolated from their PBMC at days 4 and 7.
CDV iscoms protect dogs against CDV infection

![Graph showing antibody titres over time](attachment:image)

**Fig. 3.** Development of CDV ELISA (a, c, e, g) and MV ELISA (b, d, f, h) antibody titres in non-immunized dogs (dogs 1 to 3: a, b), or in dogs immunized with CDV iscoms (dogs 4 to 6: c, d), MV F iscoms (dogs 7 to 9: e, f) or MV iscoms (dogs 10 to 12: g, h) after challenge with virulent CDV. IgG or IgM antibody titres are shown for the individual dogs. Open bars represent IgG, stippled bars represent IgM.

The dogs immunized with MV iscoms (dogs 10 to 12) developed antibody responses similar to those observed in the dogs immunized with MV F iscoms (dogs 7 to 9) and developed additional high titres of MV VN antibodies (Fig. 3g, h and 4g, h). Immunization experiments with MV iscoms and MV F iscoms previously carried out in monkeys, rabbits and mice had shown essentially the same anti-MV responses as observed in the dogs immunized with these preparations (de Vries et al., 1988). After challenge with CDV these dogs developed antibody responses that closely resembled those observed in the dogs immunized with MV F iscoms (Fig. 3 and 4). This confirmed that these dogs also were not protected against CDV infection.

**Protein specificities of the antibody responses measured in RIPA**

The presence of antibodies to MV or CDV antigens in the sera of the dogs after the second immunization were also determined by RIPA (Fig. 5). Analysis of the MV-infected cell lysate with the F-specific and H-specific monoclonal antibodies resulted in the precipitation of proteins related to the MV F protein (\(M_r\) values 55K, 40K and 15K, F0, F1, and F2 respectively) and the H protein respectively. The anti-MV serum also precipitated the M protein and the N protein. Certain degradation products, most probably of the N protein, were also precipitated (Fig. 5a). Analysis of the CDV-infected cell lysate with the pre-infection sera and the convalescent sera of the non-immunized dogs showed precipitation of degradation products of
Fig. 4. Development of CDV VN (a, c, e, g) and MV VN (●) or MV HLI (○) (b, d, f, h) antibody titres in non-immunized dogs (dogs 1 to 3; a, b) or in dogs immunized with CDV iscoms (dogs 4 to 6; c, d), MV F iscoms (dogs 7 to 9; e, g) or MV iscoms (dogs 10 to 12; g, h) after challenge with virulent CDV. Antibody titres are expressed as the mean values of independent measurements within each group of three dogs. Minimal and maximal values measured within each group are indicated.
the N protein and also the F protein (Fig. 5b). Precipitation of the M protein was not observed, as has also been reported by Rima et al. (1987), and there was relatively little precipitation of the H protein, which may have been due to either a low expression of this protein with the Bussell strain or the use of convalescent sera as early as 3 weeks after infection.

One week after the first immunization with CDV iscoms (dogs 4 to 6), the CDV F protein and minor amounts of the MV F protein were shown to be precipitable. Immunization with MV F iscoms (dogs 7 to 9) resulted in the exclusive induction of precipitating antibodies to the MV F protein, which were shown to cross-react with the CDV F protein. Immunization with MV iscoms (dogs 10 to 12) resulted not only in the induction of antibodies that precipitated the MV F protein, but also in antibodies that precipitated the MV H protein. After challenge with virulent CDV, sera of the dogs that proved to be infected with CDV showed the most prominent increase of antibodies that precipitated the MV N protein. In contrast, in sera from dogs immunized with CDV iscoms, which were shown to be protected against infection, this increase was not observed.

DISCUSSION

In the present study we have shown that an inactivated CDV preparation, which contained both the F and H membrane proteins incorporated into iscoms, clearly protects dogs both from disease and infection after intranasal challenge with the virulent Snyder Hill strain of CDV. In contrast, it has been shown in previous studies that Tween–ether- or heat-inactivated CDV preparations induce only partial protection against disease and infection (Gillespie, 1974; Appel et al., 1984).

The importance of the F protein in the induction of protective immunity against CDV infection was stressed by Norrby et al. (1986). It was shown that dogs immunized with affinity chromatography-purified CDV F protein administered in complete Freund’s adjuvant (CFA) were more efficiently protected against disease and infection than dogs immunized with purified CDV H protein in CFA. However, two out of three dogs immunized with CDV F protein and from which no virus could be isolated did contract CDV infection upon challenge, as judged from the rise in their antibody titres. Although these data suggest an important role for the F protein in the induction of protective immunity, they also indicate that, in addition, a subunit CDV vaccine should also contain other virus proteins. The standard procedure applied for the preparation of CDV iscoms resulted in an efficient incorporation of the F protein into iscoms, whereas a minor amount of the H protein was also incorporated. The relatively low amounts of CDV or MV H proteins incorporated into CDV iscoms or MV iscoms respectively may be due to the less hydrophobic character of the H protein compared to the F protein. The use of Triton X-100 as a detergent for the preparation of iscoms may be another reason for this poor incorporation, since iscoms prepared in the presence of a milder detergent, like β-octylglucoside, contained larger amounts of the viral H protein (data not shown).

Dogs immunized with CDV iscoms (dogs 4 to 6) developed CDV-neutralizing antibodies and, in contrast to the control dogs (dogs 1 to 3), showed neither clinical signs nor viraemia after challenge. Furthermore, no increase in CDV- or MV-specific antibody titres measured by ELISA (IgG or IgM), VN or HLI tests was found in two out of these three dogs (dogs 4 and 5), indicating that no CDV replication took place in these animals after challenge. In dog 6 only a small, short-lived increase of MV VN, but not of CDV VN, antibodies was observed. Taken together, these experiments showed that the CDV membrane proteins that became incorporated into iscoms induced protective immunity against CDV infection. For the MV F protein we have recently shown that the iscom form of antigenic presentation is more immunogenic than a protein–micelle preparation (de Vries et al., 1988). Since the role of the F protein in the induction of protective immunity is important (Norrby et al., 1986), the CDV iscom preparation consisting mainly of the CDV F protein incorporated in the iscom matrix indeed seems to be a promising candidate subunit vaccine. The duration of immunity to CDV after immunization with CDV iscoms has not yet been investigated. However, immunization studies with MV F iscom in monkeys have shown that iscoms induce long-persisting antibody titres. Antibody titres found 1 year after vaccination did not differ significantly from those found after 1 month (de Vries et al., 1988).
Fig. 5. Analysis of protein specificities of the antibody responses in dogs after immunization or challenge with virulent CDV, in MV RIPA (a) and CDV RIPA (b). (a) Lane 1, rabbit pre-infection serum; lane 2, hyperimmune rabbit anti-MV serum; lane 3, monoclonal antibody to MV H protein; lane 4, monoclonal antibody to MV F protein. Sera of the non-immunized dogs (lanes 5 to 10: dogs 1 to 3), dogs immunized with CDV iscoms (lanes 11 to 17: dogs 4 to 6), with MV F iscoms (lanes 18 to 24: dogs 7 to 9) or with MV iscoms (lanes 25 to 31: dogs 10 to 12) were tested at the day of the first immunization (day -35, results of one serum out of three are given), at the day of challenge (day 0) and at day 21. (b) Sera of non-immunized dogs (lanes 1 to 6: dogs 1 to 3), dogs immunized with CDV iscoms (lanes 7 to 10: dogs 4 to 6) or with MV F iscoms (lanes 11 to 14: dogs 7 to 9) were tested in a similar fashion to sera in (a). M, markers (×10^-3) and CDV- or MV-specific proteins are indicated.
Although MV vaccines have been shown to induce partial protection against canine distemper, they do not induce protection against CDV infection in dogs (Norrby & Appel, 1980; Appel et al., 1984). Appel et al. (1984) suggested that this partial cross-protection was due to MV F protein-specific antibodies which cross-react with CDV F protein. We vaccinated dogs with MV F iscoms or MV iscoms, which have been shown to induce high MV HLI antibody titres in monkeys, rabbits and mice (de Vries et al., 1988), and found that although high titres of MV HLI antibodies were induced with both preparations, these dogs were not protected against CDV infection. Because the clinical signs in these dogs, as in the dogs vaccinated in the past with live and inactivated MV vaccines, were less severe than those observed in the non-immunized dogs, the suggestion by Appel et al. (1984) that the partial cross-protection induced by MV vaccination is caused by epitopes shared by the F protein of each virus was confirmed. The serological data also confirmed observations by Appel et al. (1984) that MV HLI and MV VN antibody titres develop or increase after a heterologous boost with CDV. Antibodies induced by vaccination with CDV iscoms, which were directed against the CDV F protein, did not show a major cross-reactivity with the MV F protein in RIPA. This confirmed the observation that anti-CDV iscom antibodies did not cross-react with MV proteins in ELISA. It seems unlikely that a partial denaturation of type-common antigenic sites of the CDV F protein in iscoms was responsible for this phenomenon, since type-common sites of the MV F protein proved to be preserved during incorporation into iscoms as was shown by CDV RIPA. Recently we have shown that immunization with MV iscoms and MV F iscoms may protect mice against fatal MV encephalopathy after intracerebral challenge (de Vries et al., 1988). The present study shows that immunization of dogs with CDV iscoms protects against experimental CDV infection in the respiratory tract, which is the natural route of morbillivirus infection.

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