Studies on Antigenic and Functional Characterization of the Structural Proteins of Rinderpest Virus by Monoclonal Antibodies

（モノクローナル抗体による牛疫ウイルス構成蛋白質の抗原的及び機能的解析に関する研究）

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INDEX

INTRODUCTION

Rinderpest is an acute, febrile, highly contagious viral disease of ruminants, particularly of cattle and buffalo, with high mortality. Clinically, this disease is characterized by a monophasic fever, nasal discharge, mucosal erosions, severe diarrhoea and death. In susceptible cattle, the mortality rate is often exceeding 90 percent (Plowright, 1965). There had been repeated big epidemics of this disease in Europe, Asia and Africa until the beginning of the twentieth century when the effective control measures were under developed. It is presently confined to the Africa and South Asia, where it persists in enzootic and occasionally epizootic form in spite of the widespread application of vaccines. The development of a more heat-stable vaccine is an urgent task for eradication of rinderpest, since the current live rinderpest vaccine is not stable enough for use in Africa and Asia with hot climates.

The causative virus of rinderpest belongs to the family Paramyxoviridae, genus Morbillivirus, in which measles virus (MV) of humans, canine distemper virus (CDV) of dogs and peste des petits ruminants virus (PPRV) of sheep and goats are included. Recently, it has been reported that many seals and dolphins died by infection of new morbilliviruses (Osterhaus et al., 1988; Kennedy et al., 1988; Domingo et al., 1990).

Morbilliviruses are composed of at least six proteins: the large (L) protein, polymerase (phospho, P) protein, and nucleoprotein (NP) inside the virion, and the haemagglutinin (H), fusion (F), and matrix (membrane, M) proteins of the envelope

(Bussell et al., 1974; Mountcastle & Choppin, 1977; Hall et al., 1980; Rima, 1983). The F polypeptide is cleaved to generate F_1 and F_2 during maturation, and is covalently linked to F_1 with a disulfide bond (Hardwick & Bussell, 1978; Sato et al., 1988). Several investigators analysed polypeptides of RPV in infected cells (Prakash et al., 1979; Sato et al., 1981; Diallo et al., 1987; Grubman et al., 1988).

In paramyxoviruses, the surface glycoproteins, the H and F proteins, mediate the attachment and penetration of viruses into host cells (Scheid et al., 1972; Homma & Ohuchi, 1973), and antibodies to these surface proteins probably play a key role in the development of immunity (Giraudon & Wild, 1985; Norrby et al., 1986). More recently the protection against rinderpest using recombinant vaccinia viruses containing the H or F gene of RPV which were candidates of heat-stable vaccine has been described (Yilma et al., 1988; Barrett et al., 1989; Belsham et al. 1989; Tsukiyama et al., 1989). In spite of such progresses in molecular biology of RPV, the proteins of RPV have not been analysed antigenically and functionally in detail. It is very important for a control of rinderpest to understand the antigenic and biological properties of RPV.

Antigenic correlations among morbilliviruses have been demonstrated by various serological tests such as virus neutralization (VN), indirect immunofluorescent antibody (IFA), complement fixation, neutralization-enhancement (NE), immunodiffusion, haemolysis-inhibition and haemagglutination inhibition (HI) tests (Imagawa, 1968; Örvell & Norrby, 1974).

However, studies have not always been in complete agreement because of variables resulting from differences in virus strains, experimental animals and other experimental methods used in comparative studies and no coherent picture has emerged from these studies.

There is only one serotype of rinderpest virus (RPV) although many strains have been isolated and some have been adapted to grow in different host species and cell types. The adaptation is considered not to alter the virus antigenicity. Recently an investigation with kinetic neutralization and competition enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies indicated small antigenic differences among RPV strains (Rossiter et al, 1988).

The hybridoma technique pioneered by Köhler & Milstein (1975) for the production of monoclonal antibodies (MAbs), has been applied to a variety of antigens. In virology, they have been used for numerous studies including the differentiation of rabies virus (Flamand et al., 1980a, b) and herpes simplex virus strains (Pereira et al., 1980). In the study of morbilliviruses, they have also been proven to be powerful tools, having revealed subtle antigenic difference and variability of MV and CDV proteins which were previously considered to be invariable (Birrer et al., 1981; Giraudon & Wild, 1981; Trudgett et al., 1981; ter Meulen et al., 1981; Örvell et al., 1985). MAbs against MV and CDV polypeptides have been used for comparison of proteins of MV, CDV and RPV (Norrby et al., 1985; Sheshberadaran et al., 1986), and it was suggested that RPV might be the archevirus of the morbillivirus group. The same approaches using

MAbs against RPV have never been attempted, but there have been few reports concerning MAbs against RPV, thus far (Bhavani et al., 1989).

The H, F, and NP genes of RPV were cloned, sequenced and compared with the other morbilliviruses (Tsukiyama et al., 1987, 1988; Yamanaka et al., 1988; Hsu et al., 1988; Rima, 1989; Kamata et al., 1990). These reports indicated that the similarity between RPV and MV appeared greater than that of CDV and MV. Although these data agree with those obtained by MAbs and mRNAs analysis (Sheshberadaran et al., 1986; Barrett & Underwood, 1985), an evolutionary relationship among morbilliviruses still remains inadequately defined.

In this study, I attempted first to establish the MAbs against the structural polypeptides of RPV. Subsequently, I analysed the antigenic structure and functional organization of RPV proteins and evaluated the antigenic correlations among morbilliviruses.

MATERIALS AND METHODS

Cells. Vero cells, an African green monkey kidney cell line, were maintained by the method of Hirayama et al. (1986). NA cells, a mouse neuroblastoma cell line, were grown in Eagle's minimum essential medium supplemented with 10 % fetal calf serum and antibiotics.

Viruses. The lapinized (L) strain at the 13th passage level in Vero cells (Vero-L, Ishii et al., 1986), the NA cell-adapted L (NA-L) strain and the LA strain of RPV were used for production of MAbs. One strain (RBOK, Plowright & Ferris, 1962) of RPV, five strains (FXNO, Hg, YSA, Hk and Snyder Hill) of CDV, four strains (Edmonston, Toyoshima, Ebihara and Mantooth) of MV and recombinant vaccinia virus expressing the H protein of RPV (RVV-H, Tsukiyama et al., 1989) were used in the IFA test. All of these viruses were propagated in Vero cells. RBOK vaccine strain was obtained from the National Institute of Animal Health, Japan.

Preparation of MAbs against RPV. RPV-infected cells showing 80-90 % cytopathic effects (CPE) were harvested, frozen and thawed, sonicated at 20 KHz for 30 seconds and clarified by centrifugation at 6000 r.p.m. to prepare immunizing materials. The technique for the production of MAbs was similar to that described previously (Sugiyama et al., 1989). Briefly, the spleen cells obtained from RPV-immunized BALB/c mice were fused with P3-X63-Ag-U1 or X63-Ag8-653 myeloma cells using polyethylene glycol. The hybridoma cells were screened for antibody activity against RPV by an indirect immunoperoxidase (IIP) test. In the

case of NA-L strain as a immunizing material, the hybridoma cells producing anti-RPV MAbs were screened by the membrane IFA (mIFA) test to isolate the MAbs against the surface proteins preferentially. The positive cells were cloned by limiting dilutions and inoculated intraperitoneally into pristane-primed BALB/c mice to collect antibody-rich ascitic fluids. The isotypes of the MAbs were determined by testing the hybridoma culture fluids using a commercial ELISA kit (Zymed Laboratories).

Radioimmunoprecipitation assay (RIPA). When about 80% of the RPV-infected NA cell monolayer showed CPE, the cells were incubated in methionine- or glucose-free Eagle's MEM for 1 hour and labelled with 740 KBq/ml [^{35}S] methionine (ICN Radiochemicals) for 4 hours or with 740 KBq/ml [^{3}H] glucosamine (American Radiolabeled Chemicals) for 6 hours, respectively. The RPV-infected NA cells were treated with tunicamycin (TM) by the method of Grubman et al. (1988) prior to radiolabelling to examine the reactivity of the MAbs to sugar residues on the surface proteins of RPV. RIPA and electrophoresis using 10 % polyacrylamide gel were carried out as described by Hirayama et al. (1985) and Laemmli (1970), respectively.

Competitive binding assay. IgG antibodies in the ascitic fluids were purified by precipitation with ammonium sulfate or by affinity chromatography on an Affi-Gel protein A column (Bio-Rad Laboratories) using the MAPS buffer system according to the directions by Bio-Rad. Then they were labelled with biotin by the method of Shaw et al. (1986). The unlabelled MAbs were

fourfold serially diluted starting from the dilution which corresponded to four times the dilution giving an optical density (O.D.) of 1.0 in the IIP test, and each 0.05 ml was put on the Vero-L-infected cells in the wells of microplates. After a 30 minutes incubation at 37°C, 0.025 ml of the biotinylated MAb diluted to an approximate O.D. of 1.0 was added to each well and allowed to bind for 30 minutes at 37°C. The plate was washed five times in 0.15 % Tween-20-phosphate-buffered saline (PBS) and the assay was processed with peroxidase-streptavidin (Zymed Laboratories) and o-phenylenediamine. The competition was determined to be positive when the dilution of the unlabelled antibody required for 70 % inhibition was less than four times the dilution of the unlabelled antibody necessary to inhibit the homologous biotinylated antibody to a similar extent.

Serological tests. VN, complement-required neutralization (CRN) and NE tests were carried out by plaque assay on Vero cells using the L strain of RPV as challenge virus. In brief, 0.05 ml of serial twofold dilutions of inactivated ascitic fluid was mixed with an equal volume of the virus suspension containing approximate 50 plaque forming units per 0.05 ml, incubated at 4°C overnight. Then 0.1 ml of the virus-antibody mixture was inoculated onto the cell monolayer in a 24-well plate. After adsorption for 60 minutes at 37°C, each culture received 1 % methyl cellulose medium, and virus-induced plaques were counted by adding 0.017 % neutral red 4 days later. The neutralizing antibody titers were expressed as reciprocals of the highest ascitic fluid dilution causing a 90 % reduction in plaque counts.

The CRN and NE tests were conducted similarly as the VN test described above except the addition of a 1:15 dilution of fresh guinea-pig serum and a 1:24 dilution of anti-mouse immunoglobulin (Cedarlane Laboratories), respectively, to the virus-antibody mixture 4 hours after mixing them at a ratio of 1:2 and the inoculation of cells with 0.15 ml of this mixture per well. HI test was carried out by testing the ascitic fluids using commercial HA antigen prepared from the Toyoshima strain of MV (Denka Seiken). The ascitic fluids were treated with kaolin and then absorbed with green monkey erythrocytes before testing.

IFA test. Vero cells grown on a heavy Teflon-coated slide (Bokusui Brown) were infected with various strains of morbilliviruses. When the CPE developed to a moderate degree, the cells were fixed with the mixture of 50 % acetone and 50 % methanol for 10 minutes at -20°C. Serial fourfold dilutions of ascitic fluids from 1:100 were added, and then reacted with fluorescein-conjugated anti-mouse IgG (ICN Immuno Biochemicals). For the mIFA test using RPV strain L-infected living cells, the cells were trypsinized and single cells were mixed with a 1:100 dilution of the ascitic fluid in a 1.5 ml microtube. After incubation at 37°C for 40 minutes, the cells were washed by centrifugation at 3000 r.p.m. for 1 minute, and treated with fluorescein-conjugated anti-mouse IgG.

ELISA. To prepare the antigen for ELISA, RPV-infected NA cells showing 80 to 90 % CPE were harvested. After freezing and thawing followed by sonication at 20 KHz for 30 seconds, the cell extract was clarified by centrifugation at 3000 r.p.m. for 10

minutes. The antigen was treated with 0.2 % sodium dodecyl sulfate (SDS) or 10 % 2-mercaptoethanol (2ME) by incubation at 37°C for 30 minutes. One-fifth volume of 1M iodoacetamide was added to 2ME-treated antigen and the mixture was dialyzed against PBS. The treated antigens were diluted at a final concentration of 1:160 in 0.05 M carbonate buffer (pH 9.6) and absorbed onto microtiter plate (Dynatech Laboratories) by incubation overnight at 4°C (0.05 ml/well). The plate was rinsed once with PBS, coated with PBS containing 2 % fetal bovine serum (0.1 ml/well) and incubated at 37°C for 60 minutes. The ascitic fluids were serially fourfold diluted starting from 1:800 and each 0.05 ml was put in the well. After incubation for 60 minutes at 37°C, the plate was washed five times in 0.15 % Tween-20-PBS. The assay was processed with biotinylated anti-mouse IgG (EY Laboratories), peroxidase-streptavidin (Zymed Laboratories) and o-phenylenediamine. The ELISA titers were expressed as reciprocals of the highest dilution of ascitic fluids giving an O.D. of over 0.5 at 490 nm.

RESULTS

Specificity of MAbs

One to 2 weeks after fusion the different hybridoma cell lines were tested for production of antibodies by the IIP test. The medium from each clone was tested against normal and RPV-infected Vero cells. It was found that antibodies from some clones reacted with all two cells. Antibodies from the other clones reacted with RPV-infected cells but not with normal cells. The latter clones were considered to react with virus-specified antigenic determinants and were therefore selected for further work.

A total of 40 hybridomas producing antibodies against RPV-proteins were isolated. Twenty four of 40 MAbs reacted with RPV-infected cell surface as demonstrated by capping in mIFA test (Fig. 1). From this finding, these 24 MAbs were considered to be specific for one of the surface proteins, H or F, and the remaining 16 MAbs for the inner proteins of RPV. Nineteen of these 24 MAbs showed positive fluorescence on the RVV-H-infected cells (Fig. 2). Moreover, the specificity of all the MAbs was determined by RIPA. Results from representative MAbs are shown in Fig. 3. All the 19 MAbs which reacted with RVV-H precipitated a methionine- and glucosamine-labelled viral protein of the molecular weight (M.W.) 76 to 80K (Fig. 3 and 7b). The remaining 5 MAbs against the surface protein reacted with both of M.W. 45K- and less than 24K-proteins regarded as the F_1 and F_2 proteins, respectively. One anti-F MAb (clone 100) precipitated 60K-protein which corresponded to the uncleaved F_0 protein (Fig. 8).

Ten of 16 MAbs against the inner proteins of RPV precipitated a 66K-band and the remaining six MAbs did a 80K-protein. The proteins of M.W. 66K and 80K corresponded to the NP and P protein, respectively, in accordance with the data relating to RPV proteins described by some investigators (Sato et al., 1981; Diallo et al., 1987; Grubman et al., 1988). Thus, 10 MAbs (B11, C22, D12, G11, K11, M22, N21, P31, Q24 and Y13) were considered to be specific for the NP, six (2-1, H21, O32, R23, U32 and W21) for the P protein, 19 (1d, B-1, E-1, S-1, 19, 20, 29, 30, 31, 32, 39, 41, 47, 50, 53, 59, 61, 71 and 80) for the H protein, and five (2a, 7-1 100, 122 and 135) for the F protein.

As shown in Table 1, 18 MAbs were derived from the mice given materials of the Vero-L strain, 20 MAbs of the NA-L strain and two MAbs of the LA strain.

IFA staining of RPV-infected cell cultures by MAbs against four different RPV proteins

Ascitic fluid containing MAbs of any one of the four different RPV proteins gave a bright staining of infected cells. The pattern of the IFA staining varied. Granular and sometimes more coalescent inclusions were stained predominantly by MAbs against the NP and P protein in the cytoplasma of multinucleated giant cells which were formed by fusion of infected cells (Fig. 4a and b). Antibodies against the H and F proteins gave IFA staining with small dot-like granular in the whole cytoplasma of infected cells (Fig. 5a and b).

Characterization of the antigenic sites by competitive binding assay

Forty MAbs recognizing the NP, P, H or F proteins were purified and used to examine for antigenic sites on the proteins in a competitive binding assay. Representative results for the six NP-specific MAbs are shown in Fig. 6. The biotinylated antibody B11 competed effectively with an unlabelled homologous antibody, but not with any of the other five antibodies (Fig. 6a). The biotinylated antibody M22 competed not only with homologous antibody but also significantly with MAb N21 (Fig. 6b).

The results of the competitive binding assay are summarized in Tables 2 to 5. All of the purified MAbs significantly inhibited the binding of homologous biotinylated antibodies. By testing heterologous combinations, the NP, P, H and F proteins could be assigned to at least five, four, seven and three antigenic sites, respectively. Antigenic sites I (NP-I) and II (NP-II) on the NP overlapped with the site recognized by MAb K11 (Table 2) and sites H-III and -IV did by MAbs 59 and 1d (Table 4). The competition was not always reciprocal, one-way competition being observed among the NP-I and -II antibodies (Table 2) and the H-V, -VI and -VII antibodies (Table 4). Namely, the bindings of MAbs against NP-I and -II were inhibited by competitor K11, but MAb K11 was not inhibited by the NP-I and -II antibodies except the homologous antibody. Among the NP-II antibodies, MAb G11 was inhibited by competitor P31, but MAb P31 was not by G11. Similarly MAb 71 to H-VI was inhibited by competitors B-1 to H-V and E-1 to H-VII, but B-1 and E-1 were not

by MAb 71. The antigenic site which was recognized by the anti-F MAb 100 was not determined as reproducible data were not obtained.

Biological activities of MAbs

Antibody activities determined by mIFA, VN, CRN, NE and HI tests are summarized in Table 6. The mIFA activity in the form of the capping on the cell surface was found in all the antibodies against the H and F proteins, but in none of the antibodies against the NP and P protein as described above. The anti-F MAb 100 showed very weak fluorescence on it.

None of the MAbs against the NP and P protein of RPV showed VN, CRN, NE and HI activities. All the anti-H MAbs which bound to sites II to VII neutralized virus infectivity but MAb 50 to site I did not. Most of the MAbs to the epitopes in sites H-II, III, IV and VII showed strong VN activity, whereas those to sites V and VI had the relatively weak activity. After the addition of guinea-pig complement or anti-mouse immunoglobulin to the VN assay mixture, a large variation in the increase in titers was observed among the anti-H MAbs. Non-neutralizing MAb 50 to site H-I exhibited CRN and NE activities. The MAbs 29, 20, 53, 41, B-1 and 71 exhibited more than tenfold higher titers in the presence of complement. The MAbs 30 and 31 exhibited a significant increase in titers only in the presence of anti-mouse immunoglobulin. In general, the MAbs with low neutralizing activity tended to show a marked increase in titers in the CRN and NE tests. HI activity was found only in the MAbs to site H-V. All of the anti-F MAbs lacked VN and HI activities. Two of

three MAbs to sites F-I and -II exhibited low CRN or NE activities.

Structural requirement for each antigenic site

The MAbs were examined using the RIPA for their reactivities with the antigen treated with TM to define whether or not they were directed to the N-linked oligosaccharide chains on the envelope proteins of RPV. Anti-H MAbs precipitated a M.W. 76 to 80K band of [^{3}H] glucosamine-labelled RPV-infected NA cell lysates, but this band was not detected with TM-treated cell lysates (Fig. 7b). In methionine-labelled cells, anti-H MAbs precipitated a M.W. 76 to 80K-native protein of RPV, whereas they precipitated a major M.W. 70K-protein and a minor M.W. 54K-protein in cells treated with TM (Fig. 7a). These findings indicated that asparagine-linked glycosylation was inhibited by TM. The anti-F MAbs precipitated M.W. 60K-, 45- and less than 24K-bands of native proteins of RPV, whereas they precipitated M.W. 54K- and 45K-bands of TM-treated antigen (Fig. 8). The molecular weight of 45K-protein was not altered by TM-treatment. This result indicated that the sites of glycosylation existed only in the F_2 protein of RPV in agreement with the result of sequence analysis (Tsukiyama et al., 1988). These 70K-protein directed by anti-H MAbs and 54K-protein by anti-F MAbs in TM-treated antigen were corresponded to the molecular weight calculated from the deduced amino acid sequences of the nonglycosylated form of RPV-H and -F_0, respectively (Tsukiyama et al., 1987, 1988).

To examine the role of the disulfide bonds and three-

dimensional structure in antigenicity, the ELISA antigen was treated with 2ME or SDS. The MAbs to five sites (I, III, IV, V and VII) on the H protein and the MAb to one site (IV) on the NP showed a significant decrease in the ELISA titers by 2ME-treatment, whereas the remaining MAbs against the NP, P, H and F proteins clearly reacted with 2ME-treated antigen(Table 7 and 8). Although none of the MAbs against the H and F proteins except anti-F MAb 100 reacted with the SDS-treated antigen completely, those against two sites (I and III) on the NP and three sites (I, II and III) on the P protein reacted with it. Although the anti-F MAb 100 failed to react with the native antigen of RPV, it exhibited a high ELISA titer to the antigen treated with SDS.

Antigenic variations in the proteins of morbillivirus

All of the 40 MAbs were examined for their cross-reactivity with various strains of morbillivirus using an IFA test (Table 9). Nine antibodies against antigenic sites NP-I, -II, -III and -IV, cross-reacted with all the MV and CDV strains tested, whereas the antibody against site NP-V failed to cross-react with any of these strains. The anti-P MAbs showed three different patterns of cross-reactivity. The MAbs against antigenic sites P-I and P-II reacted only with RPV, those against P-III cross-reacted with all the MV strains tested, and the one antibody against P-IV cross-reacted with all the morbilliviruses tested. Only the MAbs to one site of seven sites on the H protein cross-reacted with all the morbillivirus strains tested, whereas the remaining MAbs were specific to RPV. All the MAbs against antigenic sites H-VI and -VII, and five of those against sites H-

II, -III and -IV failed to cross-react with the RBOK strain of RPV. Moreover, two of five MAbs against site H-VII failed to cross-react with the LA strain, whose passage history was different that of the L strain, and the IFA titers to the LA strain of the remaining three MAbs were lower than those to the L strain. The MAbs against site F-I cross-reacted with MV except MAb 7-1 which did not react with Mantooth strain. The anti-F MAb to site II reacted with two of five strains of CDV and two of four strains of MV tested. The IFA titers of this MAb to CDV were lower than those to RPV and MV. The MAb 135 against site F-III reacted with only L and LA strains of RPV.

DISCUSSION

An Understanding the antigenic and functional properties of viral structural proteins is important for the development of a diagnosis method and a recombinant vaccine to control rinderpest and for the evolutionary studies of morbilliviruses. The present study defines the antigenic structure and functional organization of the proteins of RPV and provides a more complete picture of the antigenic variations in the structural proteins of the other morbilliviruses.

A total of 40 MAbs reacting either with NP, P, H or F proteins was obtained. MAbs against the M and L proteins were not obtained. It is possible that these proteins of RPV have low immunogenicity in BALB/c mice. In IFA tests, it has been reported that the anti-NP MAbs stained both of the intracytoplasmic and intranuclear inclusions (Norrby et al., 1982; Orvell et al., 1985), but all the MAbs in this study gave a staining only in the cytoplasma of RPV-infected cells.

By competitive binding assays, non-reciprocal interactions were found in the NP and H protein. These interactions were similar to those previously noted for influenza virus and MV (Lubeck & Gerhard, 1981; Sato et al., 1985). A possible mechanism of such one-way interactions has been suggested: binding of an antibody might be hindered due to steric constraints resulting from the size of the competing antibody molecules and it might allosterically alter a second antigenic site (Lubeck & Gerhard, 1981).

Only the MAbs to epitopes on the surface proteins exhibited

some biological activities similarly to results reported in MV and CDV (ter Meulen et al., 1981; Birrer et al., 1981; Sheshberadaran et al., 1983; Giraudon & Wild, 1985; Sato et al., 1985; Örvell et al., 1985). Six of seven sites on the H protein were able to induce neutralizing activity. Similar results had been reported in the other morbilliviruses; two of three sites in MV (Sato et al., 1985) and six of seven sites in CDV (Örvell et al., 1985). It is therefore suggested that the epitopes relating to neutralization exist on a wide range of the antigenic domain on the H protein of morbillivirus.

The neutralizing activity of the MAbs to non- or weak neutralization sites could be augmented by complement. Similarly, some MAbs against the F protein exhibited this CRN activity. Although the mechanism responsible for this enhancement is currently unknown, the successful immunization with recombinant vaccinia virus expressing RPV-H or -F may partly dependent on this enhancement of neutralizing activity by complement (Yilma et al., 1988; Barrett et al., 1989; Belsham et al., 1989; Tsukiyama et al., 1989).

HI activity of the anti-H MAbs to site V may be related to the fact that only this site is completely conserved among morbilliviruses. Waterson et al. (1963) has reported that ether-tween-80-treated measles virus shared common haemagglutinating antigens with RPV and CDV. However, these common sites have never been identified, thus far. The present results strongly indicate that the antigenic similarity in the H protein exists among the three viruses.

One anti-F MAb (100) reacted weakly with the RPV-infected cell surface, precipitated the F_0 protein clearly and exhibited a significantly higher titer in ELISA to SDS-treated antigen than untreated one. This antibody may be directed to the sequential epitope of the F_0 precursor before posttranslational conformational changes, and will be useful as a tool in understanding the processing of the F protein of RPV.

I attempted to define whether the structural basis required for the formation of antigenic determinants on the proteins of RPV was dependent on sugar moieties and on protein conformation or not. All the MAbs against RPV-H and -F reacted with the TM-treated protein. This result indicates that the antigenic sites defined in the present study are independent of N-linked oligosaccharide chains. These findings may suggest that the sugar is not very important for inducing neutralizing activity against RPV.

The anti-H MAbs precipitated both H apoprotein and 54K-protein which corresponds to nonglycosylated F_0 protein in the cells treated with TM. Similar findings have been reported for the Newcastle disease virus proteins after treatment with TM: coprecipitation of 52K- or 56K-protein with nonglycosylated HN protein was observed in RIPA using anti-HN MAbs and extracts of TM-treated cells (Long et al., 1986; Nishikawa et al., 1986). It has been suggested that the coprecipitation might be due to interactions of 52K protein, the presumed nonglycosylated precursor of the F protein, with regions on the nonglycosylated HN protein that were not available on the native HN (Long et al., 1986).

Treatment with 2ME destroyed antigenicity of five of seven sites on RPV-H, but none of the three sites on RPV-F, -P and -NP except site NP-IV. With regard to disulfide bridges in the RPV proteins, the existence of 13 and 10 cystein residues in the H and F proteins, respectively, was found previously (Tsukiyama et al., 1987, 1988) in contrast with two in NP (Kamata et al., 1990). It is unknown why the antigenic sites of F protein were not affected by 2ME-treatment in spite of the cystein-rich protein. Further studies of protein antigenicity at a molecular level are required to resolve this problem. In this study, all the epitopes on the envelope proteins of RPV were sensitive to SDS, whereas more than half of the sites on NP and P protein were resistant to it. The disulfide bond or the three-dimensional structure was indicated to be important to the antigenicity of the H and F molecules of RPV. The localization of the antigenic sites on MV-NP expressed in Escherichia coli was achieved by using MAbs, but similar attempts on the H and F proteins were not successful (Buckland et al., 1989). The authors indicated that the epitopes on the H and F proteins were conformation-dependent and those on NP were linear to account for this difference. Moreover, Mäkelä et al. (1989) indicated that the small peptides synthesized had little resemblance to natural antigenic sites from the analysis of MV-H at the molecular level. Therefore, it appears likely that the antigenic sites on the envelope proteins of morbilliviruses are not predominantly dependent on the primary amino acid sequence but require posttranslational conformational changes.

The NP appears to be the most antigenically stable viral protein, because four out of five antigenic sites were completely conserved among the morbillivirus strains. The amino acid sequences deduced from the nucleotide sequences of the NP of MV and CDV indicated highly conserved nature except for 100 amino acids at the carboxyl end (Rozenblatt et al., 1985). Moreover our recent data on the NP sequence of RPV showed high level of homology in the NP of MV, CDV and RPV strains (Kamata et al., 1990). The results of this study agree with these findings and extend them to at least four highly conserved sites among the three morbilliviruses.

The P protein of three morbilliviruses appears to have distinct epitopes by the IFA test. Norrby et al. (1985) have proposed that RPV was the archevirus of morbillivirus group and CDV branched from RPV earlier than MV. One MAb (R23) against P-III cross-reacted with all the strains of MV but not with CDV. This may support their proposal, since the antigenic relationship between RPV and MV seemed to be closer than that between RPV and CDV.

The H protein of the structural proteins have been reported to be the most distinct among morbilliviruses using MAbs against MV and CDV (Sheshberadaran et al., 1986). The anti-H MAbs directed to the conserved sites among morbilliviruses have not been established, thus far. In this study, only one (site V) of seven sites on the H protein was completely conserved. When the amino acid sequences deduced from the nucleotide sequences of the H protein of RPV and MV are compared (Tsukiyama et al., 1987; Yamanaka et al., 1988), one hydrophilic region (amino acid 87-

155) is highly conserved although the amino acid divergence between the two viruses is about 40 %. It is possible that the site V may be present within this conserved region of the H protein. There was a moderate degree of antigenic variation in the RPV-specific sites on the H proteins of the L and RBOK strains. Yamanaka et al. (1988) demonstrated that the H proteins of L and Kabete O strains (Plowright & Ferris, 1959), from which the RBOK strain was derived, differed in 12.2 % of their amino acid residues. The present results may have some relationships to this difference.

The epitopes of the F proteins have been shown to be mainly group-specific among morbilliviruses (Sheshberadaran et al., 1986). In the present study, the two sites on RPV-F were relatively conserved between RPV and MV, whereas the remaining site was unique to the L and LA strains of RPV. Similarly, one of four sites on MV-F was reported to cross-react with RPV (Sato et al., 1985). These findings may indicate that the homology of the F proteins between RPV and MV is greater than that between CDV and MV, similar to the results in comparison of these amino acid sequences (Hsu et al., 1988, Rima, 1989).

In general, the NP of the morbilliviruses showed a high degree of epitopic homology; the P and F proteins showed a partial epitopic homology; the H protein showed a low degree of it. Interestingly, all the sites of the NP and P protein were conserved between the L and RBOK strain, whereas more than half of sites of the H and F proteins were not. These results suggest that the inner proteins were highly conserved than the outer

proteins among various strains of RPV. In this aspect, it appears necessary to analyse the P protein more in detail for elucidating the evolutionary relationship among morbilliviruses, since the P protein may have group-specific epitopes.

SUMMARY

A total of 40 monoclonal antibodies (MAbs) against the four structural proteins of rinderpest virus (RPV) were characterized for their antigenic structures and biological properties, and were used to analyse natural variations of the proteins of morbilliviruses.

Of the 40 MAbs, 10 recognized the nucleoprotein (NP), six the phosphoprotein (P), 19 the haemagglutinin (H), and five the fusion (F) proteins as determined by radioimmunoprecipitation assay. From a competitive binding assay using the MAbs against each structural protein, at least five, four, seven and three separate antigenic sites were identified on the NP, P, H and F proteins, respectively.

None of the MAbs against the NP and P protein exhibited any biological activities. The MAbs to six of seven sites on the H protein were able to neutralize the infectivity of RPV. By adding fresh guinea-pig serum or anti-mouse immunoglobulin, virus neutralizing antibody titers were increased in most of the anti-H MAbs including those lacking neutralizing activity. Only the MAbs to one of the antigenic sites on the H protein had haemagglutination inhibition activity against measles virus (MV). The anti-F MAbs lacked neutralizing activity, but two of five MAbs exhibited this activity in the presence of guinea-pig complement or anti-mouse immunoglobulin.

All of the sites of the envelope proteins defined in this study were sensitive to sodium dodecyl sulfate, whereas more than half of the sites of the inner proteins were resistant to it.

Although the sites on the F and inner proteins were hardly affected by 2-mercaptoethanol treatment, five of seven sites on the H protein were destroyed by it. These results suggest that the epitopes on the H and F proteins are conformation-dependent and especially those on the H protein are mostly formed by posttranslational organization.

The reactivities of each of the MAbs for the other strains of morbillivirus were tested using an indirect immunofluorescent antibody assay. The MAbs against four out of five antigenic sites on the NP showed cross-reactivity among all the strains of morbillivirus tested whereas the fifth antibody reacted only with RPV. Of the antibodies specific for the P protein, the antibody against one site was cross-reactive with all the strains of RPV, MV and canine distemper virus (CDV), the antibody against another site was reactive with RPV and MV but not with CDV, and the antibodies against other two sites were specific for RPV. Two antibodies against one site on the H protein were cross-reactive with all the strains of morbilliviruses tested and had HI activity described above. The remaining MAbs against the H protein appeared to be specific for RPV and antigenically variable among the strains of RPV. The MAbs to two sites on the F protein were, on the whole, cross-reactive with some of MV but not CDV. The remaining MAb was reactive only with the L and LA strains of RPV.

Table 1. Hybridoma clones obtained from mice immunized with various antigens of RPV

Immunizing material	No. of hybridoma clones producing antibody against				Total
	NP	P	H	F	
Vero-L	10	5	2	1	18
NA-L	0	0	17	3	20
LA	0	1	0	1	2
Total	10	6	19	5	40

Table 2. Summary of competitive binding assays of anti-NP MAbs*

Unlabelled MAb	Isotype	Antigenic site	Biotinylated MAb									
			M22	N21	K11	P31	G11	C22	D12	Q24	B11	Y13
M22	IgG1	I	+	+								
N21	IgG1	I	+	+								
K11	IgG1	I & II	+	+	+	+	+					
P31	IgG1	II				+	+					
G11	IgG1	II					+					
C22	IgG1	III						+	+	+		
D12	IgG1	III						+	+	+		
Q24	IgG1	III						+	+	+		
B11	IgG2a	IV									+	
Y13	IgG1	V										+

* +, MAbs bind competitively (70 to 100% competition).

Table 3. Summary of competitive binding assays of anti-P MAbs*

Unlabelled MAb	Isotype	Antigenic site	Biotinylated MAb H21	U32	W21	032	R23	2-1
H21	IgG1	I	+	+	+			
U32	IgG1	I	+	+	+			
W21	IgG1	I	+	+	+			
032	IgG1	II				+		
R23	IgG1	III					+	
2-1	IgG2b	IV						+

* +, MAbs bind competitively (70 to 100% competition).

Table 4. Summary of competitive binding assays of anti-H MAbs*

Purified MAb	Isotype	Antigenic site	Biotinylated MAb: 50	30	S-1	29	59	1d	19	47	20	53	41	B-1	71	31	E-1	32	39	61	80
50	IgG2b	I	+																		
30	IgG2a	II		+	+																
S-1	IgG3	II		+	+																
29	IgG2a	III				+	+														
59	IgG3	III & IV				+	+	+													
1d	IgG1	III & IV					+	+	+	+	+	+									
19	IgG2a	IV						+	+	+	+	+									
47	IgG1or3	IV						+	+	+	+	+									
20	IgG2a	IV						+	+	+	+	+									
53	IgG2a	IV						+	+	+	+	+									
41	IgG2a	V											+	+							
B-1	IgG1	V											+	+	+						
71	IgG1	VI													+	+					
31	IgG2a	VI													+	+					
E-1	IgG3	VII													+		+	+	+	+	+
32	IgG3	VII															+	+	+	+	+
39	IgG2a	VII															+	+	+	+	+
61	IgG2a	VII															+	+	+	+	+
80	IgG1	VII															+	+	+	+	+

* +, MAbs bind competitively (70 to 100% competition).

Table 5. Summary of competitive binding assays of anti-F MAbs*

Purified MAb	Isotype	Antigenic site	Biotinylated MAb 7-1	122	2a	135
7-1	IgG1	I	+	+		
122	IgG2a	I	+	+		
2a	IgG1or3	II			+	
135	IgG2a	III				+

* +, MAbs bind competitively (70 to 100% competition).

Table 6. Biological activities of anti-RPV MAbs

Antigenic specificity	Antigenic site	MAb	Antibody titer (log_{10})*					
			IFA	mIFA	VN	CRN	NE	HI
NP		M22, N21, K11, P31 G11, C22, D12, Q24 B11, Y13	3.8～5.0	−	<1.0	<1.0	<1.0	<1.0
P		U32, 032, 2-1	4.4	−	<1.0	<1.0	<1.0	<1.0
		H21, W21, R23	3.8	−	<1.0	<1.0	<1.0	<1.0
H	I	50	3.8	+	<1.0	3.2	3.8	<1.0
	II	30	5.0	+	4.4	4.7	5.6	<1.0
	II	S-1	3.8	+	3.8	4.1	4.7	<1.0
	III	29	5.0	+	4.4	5.7	5.9	<1.0
	III&IV	59	5.0	+	4.7	5.0	5.3	<1.0
	III&IV	1d	3.8	+	2.6	3.2	3.2	<1.0
	IV	19	5.0	+	5.6	5.6	5.6	<1.0
	IV	47	4.4	+	4.7	5.0	5.6	<1.0
	IV	20	5.0	+	3.2	5.6	≧5.9	<1.0
	IV	53	5.0	+	2.9	4.7	4.7	<1.0
	V	41	5.0	+	3.5	5.6	≧5.9	2.3
	V	B-1	4.4	+	3.5	5.0	5.3	2.6
	VI	71	4.4	+	2.9	4.1	4.7	<1.0
	VI	31	5.0	+	2.9	3.2	5.3	<1.0
	VII	E-1	3.8	+	5.0	5.0	5.0	<1.0
	VII	32	4.4	+	4.4	4.4	4.4	<1.0
	VII	39	5.0	+	4.7	5.6	5.6	<1.0
	VII	61	5.0	+	≧5.9	5.6	5.6	<1.0
	VII	80	4.4	+	5.6	5.3	5.0	<1.0
F	I	7-1	3.8	+	<1.0	2.6	2.0	<1.0
	I	122	4.7	+	<1.0	<1.0	2.0	NT†
	II	2a	3.8	+	<1.0	2.0	<1.0	<1.0
	III	135	4.4	+	<1.0	<1.0	<1.0	<1.0
	ND‡	100	4.7	±	<1.0	<1.0	<1.0	NT

* Reciprocal of the highest dilution of ascitic fluid.
† NT, Not tested.
‡ ND, Not determined.

Table 7. Titers* of anti-NP and -P MAbs on the RPV-antigen treated with 2ME or SDS in ELISA

Antigenic specificity	Antigenic site	MAb	2ME		SDS	
			−	+	−	+
NP	I	M22	4.7	4.7	4.1	3.5
	I	N21	4.7	4.7	4.1	3.5
	I & II	K11	5.9	5.9	5.3	4.1
	II	P31	4.1	4.1	4.1	2.9
	II	G11	4.1	3.5	4.1	−
	III	C22	3.5	2.9	3.5	2.9
	III	D12	4.1	4.1	4.1	4.7
	III	Q24	3.5	3.5	3.5	4.1
	IV	B11	6.5	4.7	5.3	−
	V	Y13	≧7.1	≧7.1	6.5	2.9
P	I	H21	4.1	4.1	4.1	4.7
	I	U32	4.1	4.1	4.1	4.7
	I	W21	4.7	4.7	4.7	4.7
	II	O32	4.1	4.1	3.5	3.5
	III	R23	5.9	5.9	5.9	5.9
	IV	2-1	3.5	3.5	3.5	−

* Log_{10} reciprocal of the highest dilution showing ≧0.5 at OD_{450}.

† −, < 2.9.

Table 8. Titers* of anti-H and -F MAbs on the RPV-antigen treated with 2ME or SDS in ELISA

Antigenic specificity	Antigenic site	MAb	2ME		SDS	
			−	+	−	+
H	I	50	5.9	−	5.3	−
	II	30	6.5	6.5	5.9	2.9
	II	S-1	5.3	4.7	4.7	−
	III	29	≧7.1	−	6.5	−
	III & IV	59	5.3	−	5.3	−
	III & IV	1d	5.3	−	5.3	−
	IV	19	6.5	4.7	6.5	3.5
	IV	47	5.9	−	5.9	2.9
	IV	20	6.5	−	6.5	−
	IV	53	6.5	−	5.9	−
	V	41	6.5	−	6.5	2.9
	V	B-1	5.9	−	5.9	−
	VI	71	5.9	4.7	5.3	−
	VI	31	6.5	5.9	5.9	−
	VII	E-1	4.7	−	4.7	−
	VII	32	4.7	−	4.1	−
	VII	39	6.5	−	5.9	2.9
	VII	61	6.5	−	5.9	−
	VII	80	5.3	−	4.7	−
F	I	7-1	5.3	5.3	4.7	−
	I	122	6.5	6.5	5.3	−
	II	2a	5.3	5.3	4.1	−
	III	135	5.9	5.9	4.7	−
	ND	100	−	−	−	4.1

* Log_{10} reciprocal of the highest dilution showing ≧0.5 at OD_{460}.

† −, < 2.9.

Table 9. Cross-reactivity* of anti-RPV MAbs with morbilliviruses in IFA test

Antigenic specificity	Antigenic site	MAb	RPV			CDV†					MV‡			
			L	LA	RBOK	FXNO	YSA	SH	Hk	Hg	TY	EBI	EDM	MAN
NP	I	M22, N21	++	++	++	++	++	++	++	++	++	++	++	++
	I & II	K11	++	++	++	++	++	++	++	++	++	++	++	++
	II	P31, G11	++	++	++	++	++	++	++	++	++	++	++	++
	III	C22, D12, Q24	++	++	++	++	++	++	++	++	++	++	++	++
	IV	B11	++	++	++	++	++	++	++	++	++	++	++	++
	V	Y13	++	++	++	−	−	−	−	−	−	−	−	−
P	I	H21, U32, W21	++	++	++	−	−	−	−	−	−	−	−	−
	II	O32	++	++	++	−	−	−	−	−	−	−	−	−
	III	R23	++	++	++	−	−	−	−	−	++	++	++	++
	IV	2-1	++	++	++	++	++	++	++	++	++	++	++	++
H	I	50	++	++	++	−	−	−	−	−	−	−	−	−
	II	S-1	++	++	+	−	−	−	−	−	−	−	−	−
	II	30	++	++	−	−	−	−	−	−	−	−	−	−
	III	29	++	++	−	−	−	−	−	−	−	−	−	−
	III & IV	59	++	++	++	−	−	−	−	−	−	−	−	−
	III & IV	1d	++	++	−	−	−	−	−	−	−	−	−	−
	IV	47, 20	++	++	++	−	−	−	−	−	−	−	−	−
	IV	19, 53	++	++	−	−	−	−	−	−	−	−	−	−
	V	41, B-1	++	++	++	++	++	++	++	++	++	++	++	++
	VI	71, 31	++	++	−	−	−	−	−	−	−	−	−	−
	VII	32, 39, E-1	++	+	−	−	−	−	−	−	−	−	−	−
	VII	61, 80	++	−	−	−	−	−	−	−	−	−	−	−
F	I	7-1	++	++	++	−	−	−	−	−	++	++	++	−
	I	122	++	++	++	−	−	−	−	−	++	++	++	++
	II	2a	++	++	++	−	−	−	+	+	++	++	−	−
	III	135	++	++	−	−	−	−	−	−	−	−	−	−
	ND¶	100	++	++	−	−	−	−	++	++	−	−	−	−

* Dilution endpoint: ++, ≧1:1000; +, 1:1000>~≧1:100; −, <1:100.
† SH, Snyder Hill
‡ TY, Toyoshima; EBI, Ebihara; EDM, Edmonston; MAN, Mantooth.
¶ ND, Not determined.

Table 10. Characterization of the antigenic sites on each protein of RPV

Antigenic site	Biological property*				Effect of drug-treatment†			Reactivity in IFA‡				
								RPV			CDV	MV
	VN	CRN	FI	HI	TM	2ME	SDS	L	LA	RBOK		
NP-I	−	−	−	−	NT	−	−	+	+	+	+	+
II	−	−	−	−	NT	−	+	+	+	+	+	+
III	−	−	−	−	NT	−	−	+	+	+	+	+
IV	−	−	−	−	NT	+	+	+	+	+	+	+
V	−	−	−	−	NT	−	+	+	+	+	−	−
P-I	−	−	−	−	NT	−	−	+	+	+	−	−
II	−	−	−	−	NT	−	−	+	+	+	−	−
III	−	−	−	−	NT	−	−	+	+	+	−	+
IV	−	−	−	−	NT	−	+	+	+	+	+	+
H-I	−	+	+	−	−	+	+	+	+	+	−	−
II	+	+	+	−	−	−	+	+	+	±	−	−
III	+	+	+	−	−	+	+	+	+	−	−	−
IV	+	+	+	−	−	+	+	+	+	±	−	−
V	+	+	+	+	−	+	+	+	+	+	+	+
VI	+	+	+	−	−	−	+	+	+	−	−	−
VII	+	+	+	−	−	+	+	+	+	−	−	−
F-I	−	±	+	−	NT	−	+	+	+	+	−	+
II	−	+	−	−	NT	−	+	+	+	+	+	±
III	−	−	−	−	NT	−	+	+	±	−	−	−

* +, ≧100; −, <100; ±, different activities among epitopes.
† +, affected; −, not affected.
‡ +, ≧100; −, <100; ±, different reactivities among strains.

LEGENDS TO FIGURES

Fig. 1. mIFA staining of Vero cells infected with the L strain of RPV by MAbs against the envelope proteins. (a) anti-H MAb 1d; (b) anti-F MAb 2a.

Fig. 2. IFA staining of Vero cells infected with RVV-H by MAbs 11d against RPV-H.

Fig. 3. SDS-PAGE analysis of RPV proteins from RPV-infected NA cell lysates precipitated by antibodies. Lane 1, RPV-infected rabbit serum; lane 2, MAb S-1; lane 3, MAb 032; lane 4, MAb K11; lane 5, MAb 7-1.

Fig. 4. IFA staining of Vero cells infected with the L strain of RPV by MAbs against the inner proteins. (a) anti-NP MAb K11; (b) anti-P MAb 032.

Fig. 5. IFA staining of Vero cells infected with the L strain of RPV by MAbs against the envelope proteins. (a) anti-H MAb 1d; (b) anti-F MAb 2a.

Fig. 6. Competitive binding assays of MAbs against the NP. Biotinylated MAbs: (a), B11; (b), M22. Unlabelled MAbs: ○, B11; ●, D12; □, G11; ■, M22; ▲, N21; △, Y13.

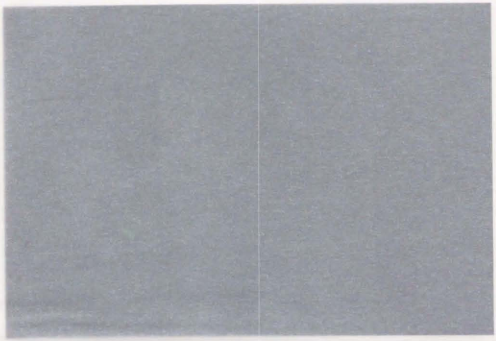
Fig. 7. Effect of impairment of glycosylation by TM on the antigenicity of RPV-H. RIPA of mock (lanes 1 and 2) and RPV-

infected (lanes 3 to 10) NA cell lysates with RPV-infected rabbit serum (lanes 1 to 4) and anti-H MAbs (lanes 5 to 10). These cells were radiolabelled with [^{35}S] methionine (a) or [^{3}H] glucosamine (b) in the absence (lanes 1, 3, 5, 7 and 9) or the presence (lanes 2, 4, 6, 8 and 10) of TM. Lanes 5 and 6, MAb 50; lanes 7 and 8, MAb S-1; lanes 9 and 10, MAb 29.

Fig. 8. Effect of impairment of glycosylation on the antigenicity of RPV-F. RIPA of RPV-infected NA cell lysates with RPV-infected rabbit serum (lanes 1 and 2) and anti-F MAbs (lanes 3 to 10). These cells were radiolabelled with [^{35}S] methionine in the absence (lanes 1, 3, 5, 7 and 9) or the presence (lanes 2, 4, 6, 8 and 10) of TM. Lanes 3 and 4, MAb 7-1; lanes 5 and 6, MAb 100; lanes 7 and 8, MAb 2a; lanes 9 and 10, MAb 135.

Fig. 1.

(a)



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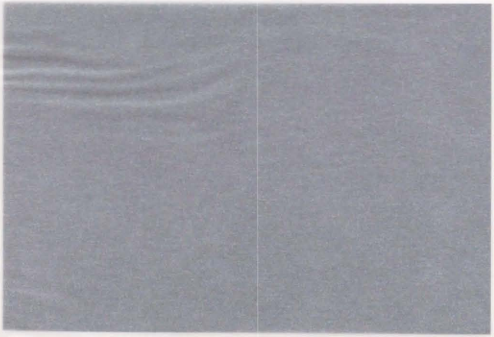
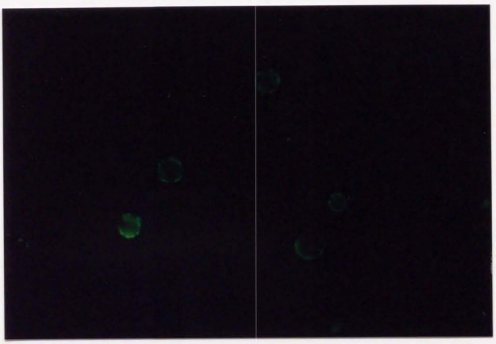


Fig. 1.

(a)



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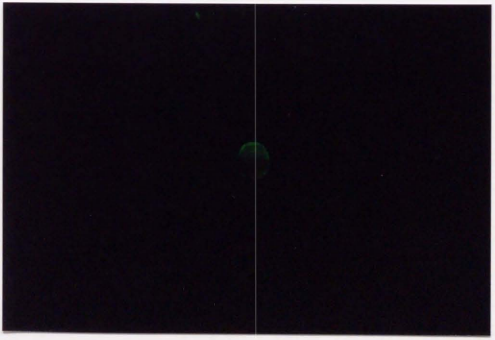


Fig. 2.

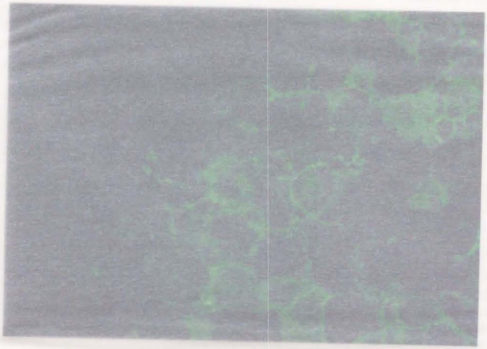


Fig. 2.

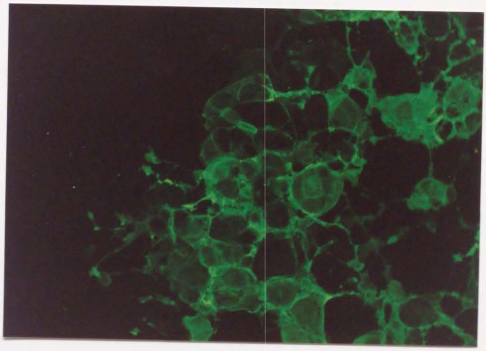


Fig. 3.

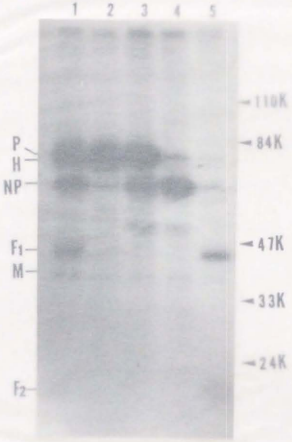


Fig. 3.

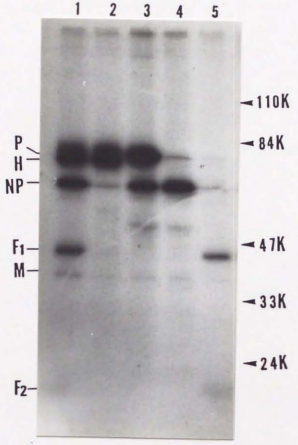
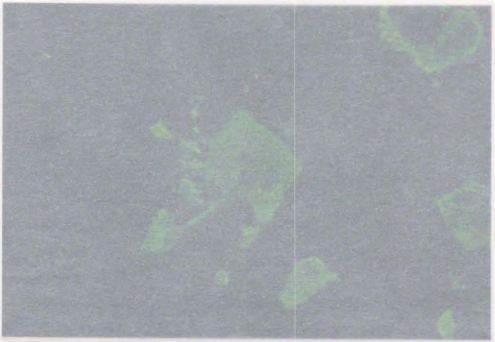


Fig. 4.

(a)



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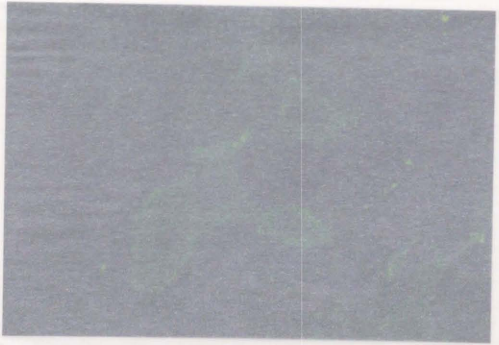
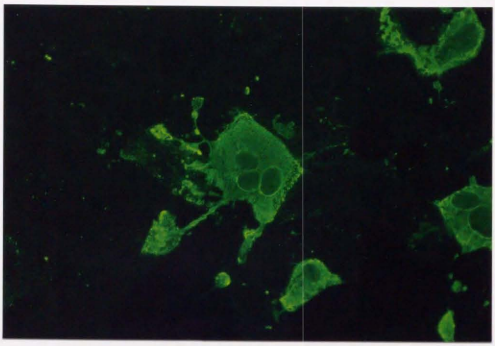


Fig. 4.

(a)



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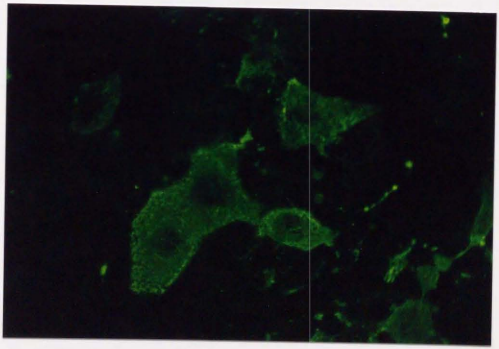
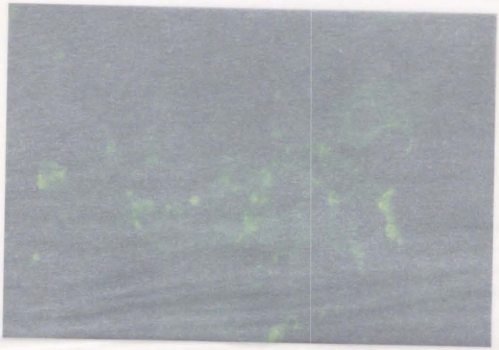


Fig. 5.

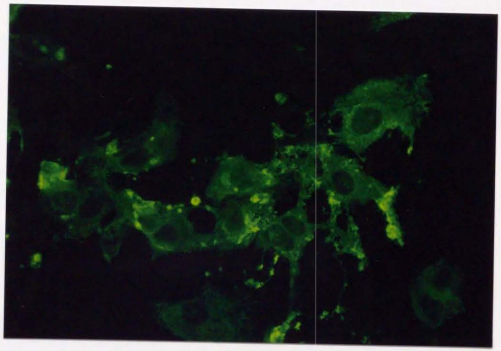
(a)



(b)

Fig. 5.

(a)



(b)

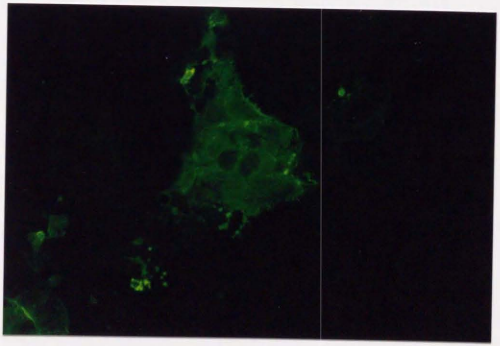
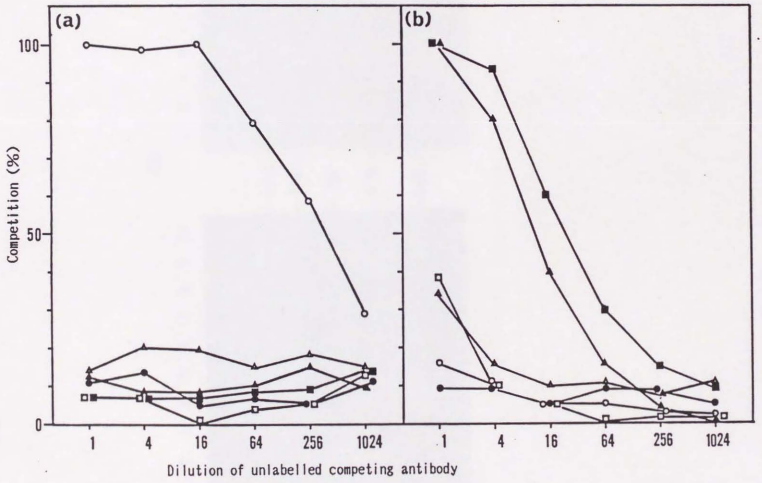


Fig. 6.


(a)
(b)
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0
Competition (%)
1
4
16
64
256
1024
Dilution of unlabelled competing antibody

Fig. 7.

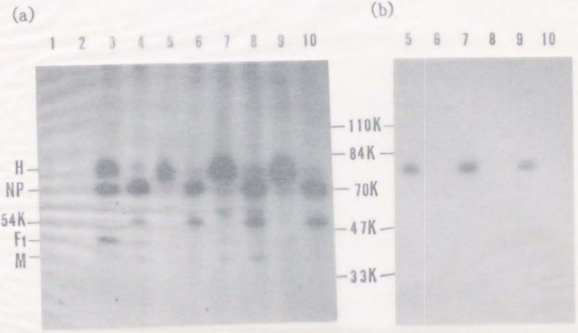


Fig. 7.

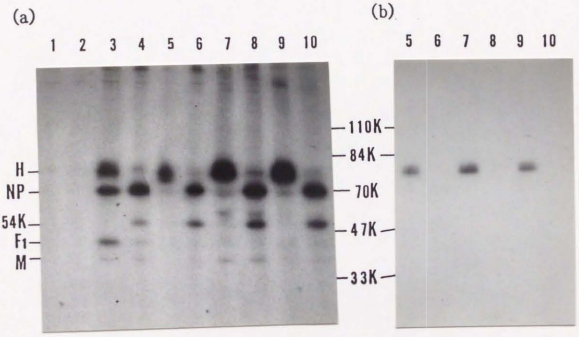


Fig. 8.

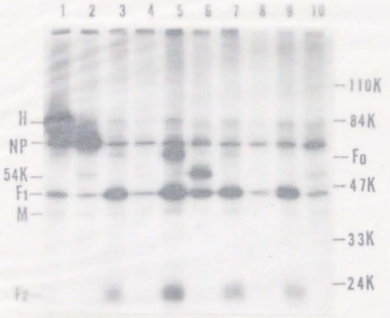
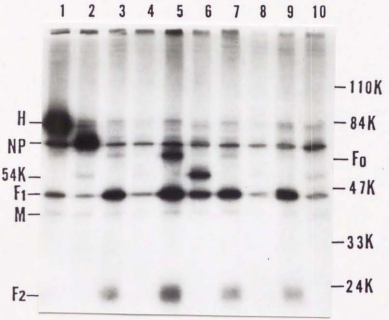


Fig. 8.



CONCLUSIONS

It is very important for a control of rinderpest and the ecological study of morbilliviruses to understand the antigenic and biological properties of the proteins of rinderpest virus (RPV). For this purpose, I attempted to characterize the structural proteins of RPV antigenically and functionally and analysed natural variations among the proteins of morbilliviruses using a number of monoclonal antibodies (MAbs) against RPV.

A total of 40 MAbs against the four structural proteins of RPV was established for the first time. Of the 40 MAbs, 10 were directed to the nucleoprotein (NP), six to the polymerase (P) protein, 19 to the haemagglutinin (H) protein and five to the fusion (F) protein as determined by radioimmunoprecipitation assay. From a competitive binding assay using the MAbs against each structural protein, at least five, four, seven and three separate antigenic sites were identified on the NP, P, H and F proteins, respectively. These 40 MAbs were used for various tests and the results were summarized in Table 10.

The MAbs against the envelope proteins, the H and F proteins, exhibited some biological activities. None of the MAbs against the inner proteins, the NP and P protein exhibited such activities. Since most of the antigenic sites of the H protein were able to induce virus neutralizing activity, it is suggested that the epitopes relating to neutralization exist on a wide range of the antigenic domain on the H protein of RPV. The complement-required neutralization activity observed in anti-H and anti-F MAbs may be partly relevant to the mechanism of the

successful immunization with recombinant vaccinia viruses expressing the H or F protein of RPV. The antigenic site which was conserved completely among morbilliviruses induced haemagglutination inhibition (HI) activity against measles virus (MV). This finding strongly confirmed the existence of antigenic similarity in the H protein of morbilliviruses which had been previously suggested by cross-HI test using anti-RPV or anti-CDV serum and tween-80-treated MV as an antigen.

The antigenic sites defined in the present study are considered to be independent of N-linked oligosaccharide chains. This result may suggest that a sugar is of no importance for inducing neutralizing activity against RPV.

The antigenic sites on the H protein were more sensitive to 2-mercaptoethanol than those of the other proteins of RPV. All the sites on the envelope proteins were sensitive to SDS, although more than half of those of the inner proteins were resistant to it. These results indicate that the antigenic sites on the envelope proteins of RPV are predominantly dependent on the three-dimensional structure and especially those on the H protein are mostly formed by posttranslational organization.

In general, the NP of morbilliviruses showed high degree of epitopic homology; the P and F proteins showed a partial epitopic homology; the H protein showed a low degree of epitopic homology. Since RPV-MV intertypic epitopes were predominantly found in the P and F proteins, the antigenic relationship between RPV and MV appeared to be closer than that between RPV and CDV. On the other hand, the inner proteins were considered to be mostly

conserved among the RPV strains, but the outer proteins to be greatly different. Therefore, it appears necessary for elucidating the evolutionary relationship among morbilliviruses to analyse in detail the P protein, which may have group-specific epitopes.

This study clarified some of the antigenic and biological properties of RPV, and these basic informations will be useful in developing more effective vaccine or diagnosis methods to control rinderpest and also for the evolutionary studies of morbilliviruses.

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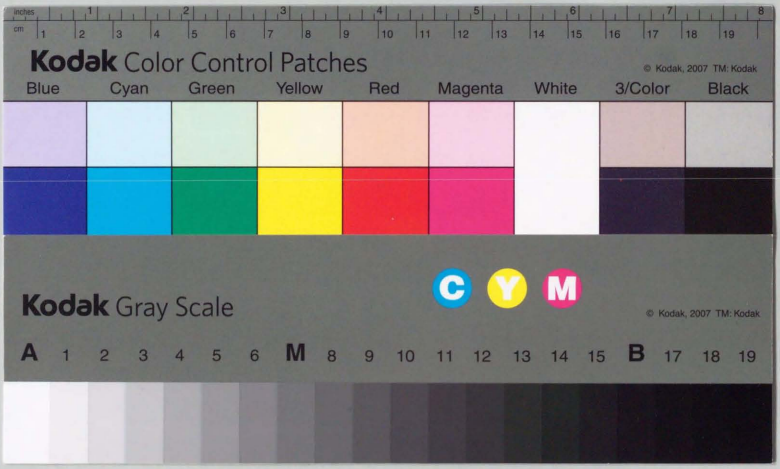
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