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Epitopes on the Peplomer Protein of Infectious Bronchitis Virus Strain M41 as Defined by Monoclonal Antibodies

HUBERT G. M. NIESTERS,*,[1] NANCY M. C. BLEUMINK-PLUYM,*,[2] ALBERT D. M. E. OSTERHAUS,† MARIAN C. HORZINEK,* AND BERNARD A. M. VAN DER ZEIJST*,[3]

*Institute of Virology, Veterinary Faculty, State University, P.O. Box 80.164, 3508 TD Utrecht, and †National Institute of Public Health and Environmental Hygiene, P.O. Box 1, 3720 BA Bilthoven, The Netherlands

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Sixteen monoclonal antibodies (Mcabs) were prepared against infectious bronchitis virus strain M41, all of them reacting with the peplomer protein. One of them, Mcab 13, was able to neutralize the virus and to inhibit hemagglutination. Competition binding assays allowed the definition of five epitopes, designated as A, B, C, D, and E, of which epitopes A and B are overlapping. Furthermore, the binding of Mcab 13 (epitope E) could be enhanced by the addition of Mcabs from group B, C, and D. A dot immunoblot assay was used to analyze the effect of denaturation on antibody recognition of the epitopes. Only the binding of Mcab 13 was affected, indicating that the epitope involved in neutralization and hemagglutination is conformation dependent. The epitopes A to D were highly conserved among IBV strains, while epitope E was specific for strains M41 and D3896. In this last strain, however, this epitope was not involved in neutralization. © 1987 Academic Press, Inc.

INTRODUCTION

Avian infectious bronchitis virus (IBV) is the etiological agent of a highly contagious respiratory disease of young chickens. The virus also causes a marked and rapid decline of egg production and quality in laying hens (Darbyshire, 1981).

IBV belongs to the coronaviruses, a group of positive-stranded enveloped RNA viruses. The virion contains three major structural proteins: the peplomer or spike (S), nucleocapsid (N), and matrix (M) proteins (Cavanagh, 1981; Stern and Sefton, 1982). The M protein is present as polypeptide p23 and the glycosylated forms gp28, gp31, and gp36. The N protein has an apparent mol wt of 51 kDa (Macnaughton and Madge, 1977).

The characteristic surface projections of the virus are made up by the peplomer protein; the peplomer is composed of two molecules of each of the glycopolypeptides S1 (gp90) and S2 (gp84). Both contain high-mannose, N-linked oligosaccharides. The S2 protein is anchored in the membrane while S1 is attached to S2 via a loose noncovalent association (Cavanagh, 1983a, b).

[1] Present address: California Institute of Technology, Division of Biology 156-29, Pasadena, CA 91125.

[2] Present address: Department of Bacteriology, Veterinary Faculty, State University, P.O. Box 80.171, 3508 TD Utrecht, The Netherlands.

[3] To whom requests for reprints should be addressed at: Department of Bacteriology, State University of Utrecht, P.O. Box 80.171, Yalelaan 1, 3508 TD Utrecht, The Netherlands.

The peplomer protein is believed to be responsible for the induction of neutralizing antibodies and a protective immune response (Mockett *et al.*, 1984; Cavanagh *et al.*, 1986).

In this study we describe the production of monoclonal antibodies (Mcabs) directed against the peplomer protein of IBV strain M41. To map the epitopes we performed reciprocal competitive binding assays. The Mcabs were used in a dot immunoblotting assay to analyze the sensitivity to denaturation of the epitopes responsible for antibody attachment. Furthermore, the variability of these epitopes in different IBV strains was analyzed.

MATERIALS AND METHODS

Virus growth and purification

All IBV strains were obtained from the Poultry Health Institute, Doorn, The Netherlands. Purified stocks were prepared from cloned virus (Niesters *et al.*, 1986).

Immunization of mice

Gradient-purified M41 virus was disrupted in 1% Triton X-100 and diluted to 400 μg/ml in TESV buffer (0.02 *M* Tris–HCl, pH 7.4, 1 m*M* EDTA, 0.1 *M* NaCl). Balb/c mice (from CPB, TNO, Zeist, The Netherlands) were primed intramuscularly with 100 μg virus in 0.5 ml complete Freund's adjuvant and boosted twice at weekly intervals with the same amount of antigen, in

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incomplete Freund's adjuvant. At least 6 weeks after the initial immunization and 3 days before the mice were sacrificed for the preparation of hybridomas, they were boosted intravenously with 10 μg purified intact virus (0.1 ml in TESV).

Production of mouse hybridoma cell lines

Hybridoma cell lines were prepared and cloned as previously described (Osterhaus *et al.*, 1981). Immune ascitic fluid was made in Balb/c mice primed with pristane (2,6,10,14-tetramethylpentadecane; Janssen Chimica, Belgium; Goding, 1980).

Serological screening methods

Hybridoma culture media and mouse body fluids were screened for antibodies in an ELISA. Microtiter plates (Inotech) were coated (3 hr at 37° followed by overnight incubation at 4°) with 0.5 μg virus in 100 μl 0.15 *M* NaCl per well. Plates were washed four times with washing fluid (0.15 *M* NaCl, 0.05% Tween 20 (v/v)). All subsequent steps were performed at 37°. Fifty microliters of V-buffer (0.05 *M* Tris–HCl, pH 7.4, 0.15 *M* NaCl, 1 m*M* EDTA, 0.1% BSA (w/v), 0.05% Tween 20 (v/v), 10% newborn calf serum) and 50 μl hybridoma supernatant or dilutions of ascitic fluid in V buffer were added to each well and incubated for 2 hr. After washing four times with washing fluid, peroxidase-conjugated rabbit anti-mouse IgG (H + L) (Nordic Immunological Laboratories) was added and incubated for 1 hr, followed by four washes with washing fluid. Peroxidase binding was visualized by incubation with 300 μl per well of a freshly prepared solution containing 50 m*M* citric acid (pH 4.0), 2.3 m*M* H_2O_2, 40 m*M* 2-2′-azinobis-3-ethylbenzthiazoline sulfonic acid (ABTS, Sigma). Absorption was measured at 405 nm in a multichannel photometer (Flow Laboratories).

Isotype determination

The immunoglobulin class of the hybridoma antibodies present in the culture media of the clones was determined by ELISA with rabbit antisera to mouse IgM (Miles), IgG1, IgG2, and IgG3 (Nordic) and goat anti-rabbit IgG serum conjugated with horseradish peroxidase (Nordic). Independent radioimmunoprecipitation tests with isotype-specific second antibody were carried out.

Dot-immunobinding assay

Antigen consisted of purified virus, isolated as described above, or allantoic fluid from uninfected eggs. "Native antigen" was prepared by dilution in TESV buffer or after treatment at 37° for 20 min with a final solution of 10 m*M* Tris–HCl, pH 7.4, 0.2 *M* NaCl, 0.5 *M* KCl, 2 m*M* EDTA, 1 m*M* phenylmethylsulfonyl fluoride (PMSF), and 2% (v/v) NP-40. "Denaturated antigen" was boiled for 2 min in a solution containing 2% SDS and 5% 2-mercaptoethanol (Talbot *et al.*, 1984).

Native and denaturated viral antigens or proteins from allantoic fluid (1 μg in 2.5 μl) were spotted onto nitrocellulose strips (Schleicher & Schuell, 0.45 μm pore size). The strips were dried under a heat lamp, saturated in gelatin buffer (500 m*M* NaCl, 10 m*M* sodium phosphate, pH 7.3, 10 m*M* EDTA, 0.25% gelatin, 0.5% Triton X-100), and probed for 2 hr with Mcab solution. After three washes with gelatin buffer, peroxidase-conjugated rabbit anti-mouse IgG (H + L) was added and incubated for another 2 hr. The strips were washed with phosphate-buffered saline (PBS, pH 7.4). Binding was visualized by incubation with a freshly prepared substrate solution containing PBS, 0.5 mg/ml 3-3′-diaminobenzidinetetrahydrochloride (DAB, Serva), and 0.3% H_2O_2. The reaction was stopped by washing the strips with PBS (Roos *et al.*, 1982).

Radioiodination of viral surface glycoproteins

Gradient-purified virus was labeled using Iodogen (Pierce) to a specific activity of 1.9 μCi/μg (Markwell and Fox, 1978).

Immunoprecipitation, virus neutralization, and hemagglutination inhibition

Approximately 100,000 cpm ^{125}I-labeled M41 virion proteins in 25 μl were immunoprecipitated with 2 μl ascitic fluid (Van der Zeijst *et al.*, 1983). Immunoprecipitates were analyzed by electrophoresis in 15% SDS–polyacrylamide gels (Rottier *et al.*, 1981).

Virus neutralization was carried out according to Kusters *et al.* (1987). Hemagglutination inhibition tests were performed with 8 hemagglutinating units in microtiter plates as described by Alexander *et al.* (1976).

Coupling of horseradish peroxidase (HRPO) to monoclonal antibodies

Immunoglobulin fractions were isolated from ascites fluids by precipitation at 4° for 2 hr with an equal volume of saturated ammonium sulfate (pH 7.0). After overnight dialysis against 0.01 *M* sodium carbonate buffer (pH 9.5), antibodies were coupled to horseradish peroxidase (HRPO; Boehringer-Mannheim) by the periodate method (Wilson and Nakane, 1978) and stored at −70°.

Antibody blocking assay

Before carrying out the actual competition binding assays, the amount of binding in the ELISA was deter-

mined for all Mcabs (peroxidase conjugated and nonconjugated). A series of twofold dilutions was reacted with antigen-coated plates. The resulting dose–response curve yielded the level of maximum binding (a measure for the relative avidity) and the Mcab concentration at which 50% binding occurred.

For the competition assays, virus-coated plates were incubated for 1 hr at 37° with 200 μl per well of PBS containing 0.2% Tween 20 and 10% newborn calf serum to saturate all binding sites on the plastic. The solution was removed and the plates were washed four times with washing fluid. A mixture of the peroxidase-conjugated Mcab (at twice the concentration giving half maximal binding) and the unconjugated, competing antibody at various concentrations from the linear part of the dose–response curve, were added simultaneously. Dilutions were made in PBS containing 1% newborn calf serum. The mixture was incubated for 1 hr at 37°. The plates were then washed four times with washing fluid and binding of the peroxidase-conjugated Mcab was visualized as described above. Nonspecific binding to plates without antigen was taken to represent background. Binding of each conjugate to viral antigen in the absence of competitor represented 100% binding (0% inhibition), while the binding of the conjugate in the presence of excess of the same unlabeled Mcab represented 0% binding (100% competition).

To define epitope groups, competition was considered to be positive only if it occurred symmetrically, i.e., when similar results were obtained when the antibody was used as competitor and as peroxidase-conjugated antibody. The percentage of competition usually reached a plateau. Competition was rated as strong if it was more than 60%, and significant if it was more than 35%.

RESULTS

Production and characterization of monoclonal antibodies

Our purpose was to generate and subsequently analyze Mcabs directed against the surface glycoproteins of IBV strain M41. To obtain predominantly Mcabs to surface proteins, immunized mice were boosted with intact sucrose-gradient-purified virions by intravenous injection a few days before the fusion. Spleen cells from two hyperimmunized mice were fused in independent experiments. The hybridoma cell lines secreting antiviral Mcabs were screened with the aid of an ELISA. The amount of viral antigen which provided maximum antibody binding and minimum background with polyvalent rabbit anti-M41 sera had been determined previously (0.5 μg sucrose-purified intact virions per well). These conditions were used throughout. Sixteen Mcabs directed against the peplomer protein were selected for further analysis (Table 1). Their polypeptide specificities had been characterized by immunoprecipitation of ^{125}I-radiolabeled purified virions; typical results are shown in Fig. 1. No cross-reactivity was detected with allantoic fluid from uninfected eggs, both by ELISA and immunoprecipitation of ^{125}I-radiolabeled allantoic fluid. Cellular proteins, such as actin, transferrin, and ovalbumin, sometimes coprecipitated with the viral polypeptides (results not shown). These proteins may associate nonspecifically with the virion (Cavanagh, 1981; Lomniczi and Morser, 1981; Wadey and Westaway, 1981).

Characterization of the isotypes of the Mcabs showed that most antibodies belonged to the IgG3 (eight) or IgG1 (six) subclass, whereas only two (Mcab 6 and 13) were from the IgG2 subclass. Of all the Mcabs only one, Mcab 13, was able to neutralize virus infectivity *in ovo* as well as to inhibit hemagglutination significantly . This Mcab did not neutralize the other IBV strains listed in Table 2.

A direct relationship exists between the avidity and the maximal amount of Mcab bound to a given amount of antigen (Frankel and Gerhard, 1979; Bruck *et al.*, 1982; Van Drunen Littel-van den Hurk *et al.*, 1985). The Mcabs were titrated in an indirect ELISA (Fig. 2.)

TABLE 1

PROPERTIES OF MONOCLONAL ANTIBODIES DIRECTED AGAINST THE PEPLOMER PROTEIN OF IBV—M41

Hybridoma cell line	Isotype specificity	Neutralization titer[a]	Hemagglutination inhibition titer[b]
1	IgG3	—	—
2	IgG3	—	—
3	IgG1	—	—
4	IgG1	—	—
5	IgG3	—	—
6	IgG2	—	—
7	IgG3	—	—
8	IgG3	—	—
9	IgG1	—	—
10	IgG3	—	—
11	IgG3	—	—
12	IgG3	—	—
13	IgG2	630,000	2560
14	IgG1	—	—
15	IgG1	—	—
16	IgG1	—	—

[a] Neutralization assays were performed with 100 EID_{50} per egg and serial 10-fold dilutions of the ascitic fluid. Only significant neutralization (>100) is indicated.

[b] Inhibition was considered to be positive if the titer was 160 or more.

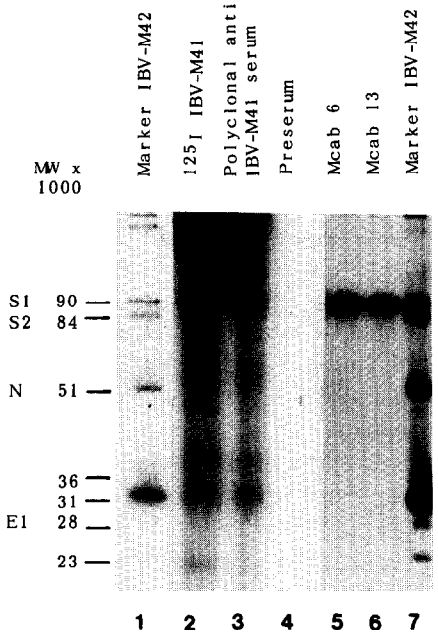


FIG. 1. Specificity of IBV-M41 monoclonal antibodies for the peplomer protein. ^{125}I-radiolabeled M41 virions were disintegrated and the proteins were immunoprecipitated with ascitic fluids of the monoclonal antibodies. The immunoprecipitates were electrophoresed. Migration positions of the viral structural proteins are shown on the left. Markers: lanes 1 and 7, [^{35}S]methionine-labeled IBV-M42; lane 2, ^{126}I-iodine-labeled M41. Immunoprecipitates: lane 3, rabbit anti-M41 hyperimmune serum; lane 4, rabbit preserum; lane 5, Mcab 6; lane 6, Mcab 13.

and avidities were estimated using the plateau level absorbance values; they varied between 0.45 and 2.0. Mcabs 14, 15, and 16 did not reach a plateau level within the range studied. Mcab 13 behaved in a remarkable way: at concentrations up to 0.5 μg protein per well, this Mcab was able to saturate those viral antigen coated, while at concentrations higher than 2 μg per well, more antibodies could be bound (Fig. 2B).

Mapping of epitopes

Experiments were made to determine whether the Mcabs can be divided into clusters recognizing the same immunogenic region of the peplomer protein. All preparations except Mcabs 14, 15, and 16 retained their binding capacity after coupling to horseradish peroxidase. The avidity of the latter Mcabs apparently is too low in the ELISA. The remaining 13 Mcabs were used in the antibody competition assay. Dose–response curves for the peroxidase-conjugated (not shown) and the nonconjugated antibodies (Fig. 2) were plotted to determine the proper concentrations for the blocking assay. The relative avidity as well as the kinetics of self-competition were determined. Each antibody was used both as a competitor and as a peroxidase-conjugated preparation. Mcabs were considered to recognize the same epitope if use of reciprocal antibody preparations prevented binding. The percentage of competition was normalized to 100% in the homologous reaction. Five epitopes were found, which were designated A, B, C, D, and E. Epitopes A and B overlap, while a one-way competition was observed between Mcabs from group C and those of group D and between Mcab 1 (group A) and those of groups B and C (Fig. 3, Table 3).

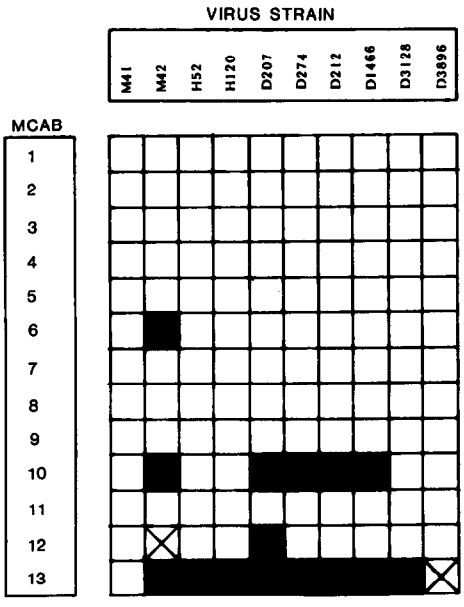
Besides competition, enhancement was also observed. The binding of peroxidase-conjugated Mcab 13 could be enhanced by the binding of Mcabs 4, 8, 9, 10, and 12 (Table 3, Fig. 4). Furthermore, the binding of peroxidase-conjugated Mcab 5 could be enhanced by Mcabs 9 and 12; the binding of peroxidase-conjugated Mcab 6 was enhanced by Mcab 12 (Table 3). Enhancement was not observed in the reciprocal assays. Pairs of Mcabs which induced more than 50% enhancement are designated by arrows in Table 3.

Cross-reactivity of Mcabs among IBV strains

The Mcabs were tested for their cross-reactivity with other IBV strains in an indirect ELISA (Table 2). All

TABLE 2

CROSS-REACTIVITY OF MONOCLONAL ANTIBODIES TO IBV-M41 WITH OTHER IBV VIRUS ISOLATES



Note. Twice the amount of monoclonal antibody giving maximal binding to IBV-M41 was used. Binding to the heterologous virus is expressed as percentage of the A_{405} obtained with IBV-M41. Empty squares: 50 to 100%; crossed squares: 25 to 50%; full squares less than 25%.

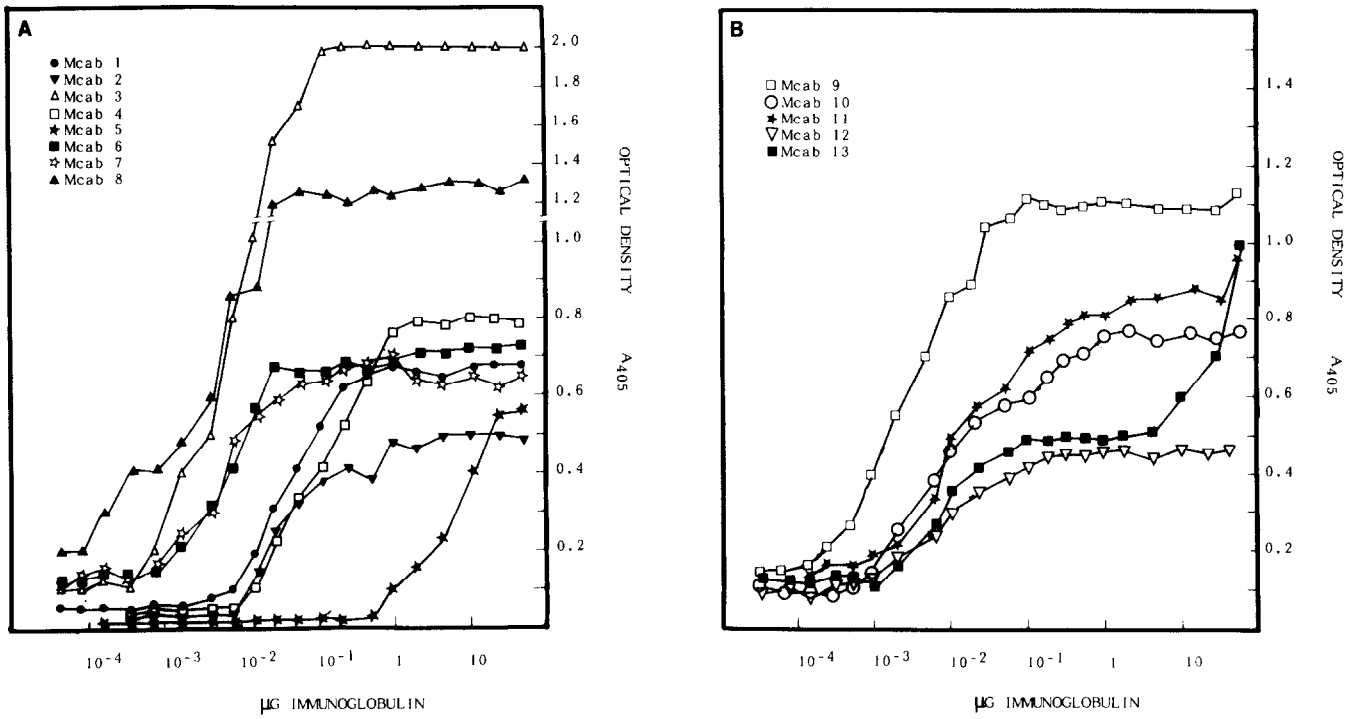


FIG. 2. Avidity of the monoclonal antibodies to the IBV-M41 peplomer protein. Serial twofold dilutions of antibody were incubated in the wells of microtiter plates containing absorbed purified IBV-M41 virions. Binding to the viral antigen was detected by the addition of peroxidase-labeled rabbit anti-mouse IgG and the resulting A_{405} was read and used to rate Mcabs for avidity. The other properties of the monoclonal antibodies are summarized in Table 1.

strains belong to Dutch neutralization serotypes. The origin and serological relationships of the strains has been described before (Davelaar *et al.*, 1984; Kusters *et al.*, 1987). The M42 (Beaudette) strain is a laboratory strain of the same serotype as M41. The Mcabs 1–12 of the epitopes A to D recognized most strains. An exception is Mcab 10 (epitope D) which failed to react with the M42 strain as well with D207, D212, D274, and D1466, while Mcab 6 and Mcab 12 did not recognize strains M42 and D207, respectively. Epitope E was only present in strains M41 and D3896; however, Mcab 13 did not neutralize D3896 (results not shown).

Effect of denaturation of viral protein on Mcab recognition

In order to study the need for a native structure of the peplomer protein for antibody attachment, we performed a dot immunoblot analysis on native and denaturated virus. All Mcabs recognized the viral antigen in its native structure, i.e., in buffer with or without 2% NP-40. After denaturation by boiling in SDS and 2-mercaptoethanol, the Mcabs recognizing the epitopes A to D still bound to the protein. The reactivity of Mcab 13, however, was fully abolished by denaturation. Representative results for each epitope are shown in Fig. 5.

DISCUSSION

In this paper we describe properties of Mcabs directed against the peplomer protein of IBV strain M41. Probably due to the immunization protocol in which mice were boosted intravenously with intact purified virions a few days before the fusion, only anti-peplomer protein, Mcabs were obtained. Similar observations have been made with influenza virus (Gerhard *et al.*, 1980), vesicular stomatitis virus (Lefrancois and Lyles, 1982a, b), measles virus (Giraudon and Wild, 1981), and Newcastle disease virus (Iorio and Bratt, 1983).

Although the Mcabs do precipitate the peplomer protein S1, we cannot exclude that this is the result of its association with S2, although the latter protein could never be precipitated as a clear band (Fig. 1). The Mcabs did not recognize (glyco)proteins of the allantoic fluid in the ELISA, the dot blot analysis (see Fig. 5), or after immunoprecipitation of radiolabeled allantoic fluid. This indicates that the Mcabs are not directed to oligosaccharide side chains of the peplomer protein (Jackson *et al.*, 1981).

All 13 antibodies were used in a blocking assay to identify the number of epitopes on the peplomer protein. The definition of epitopes by this assay is based on the assumption that a Mcab binding to a specific

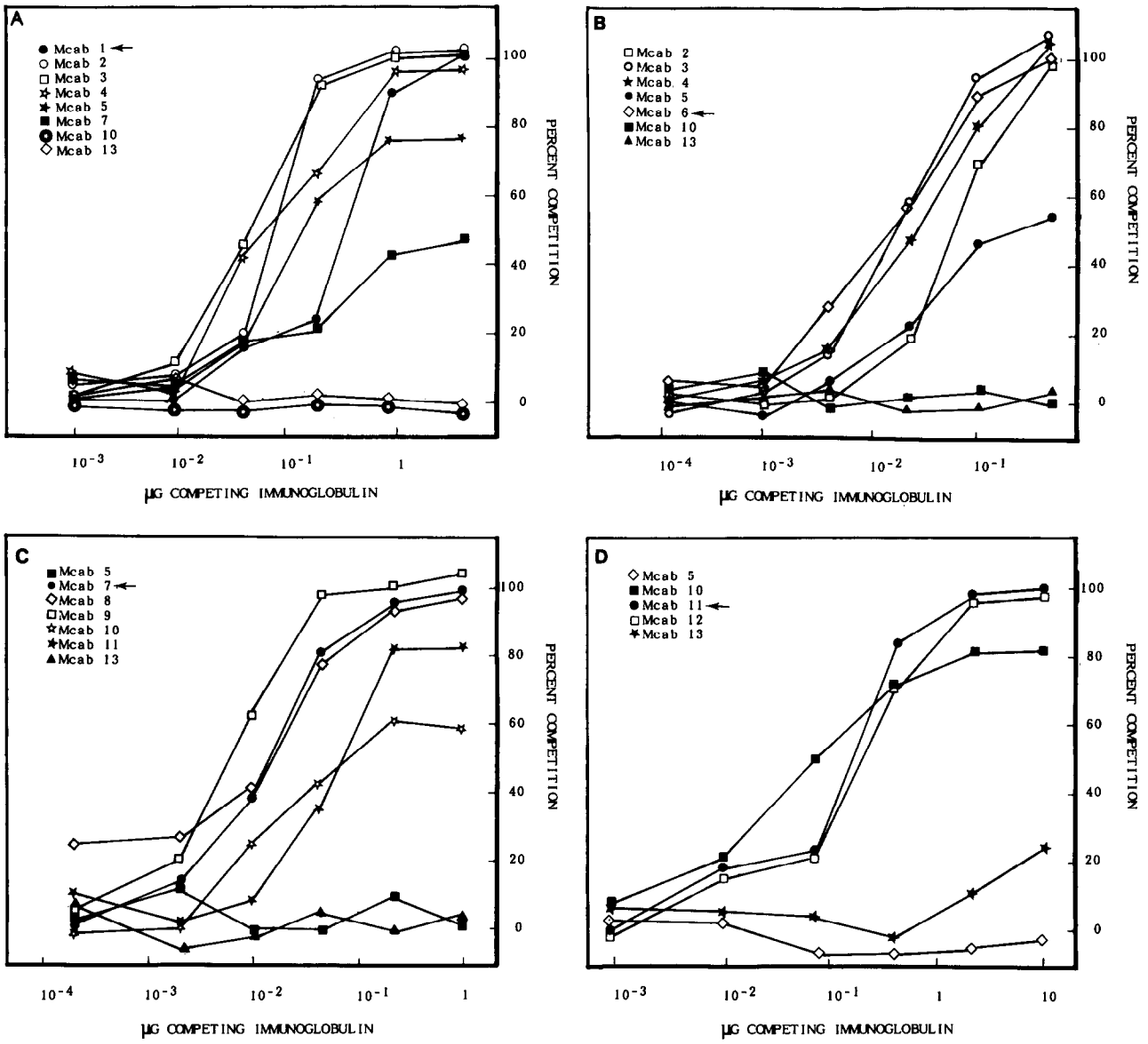


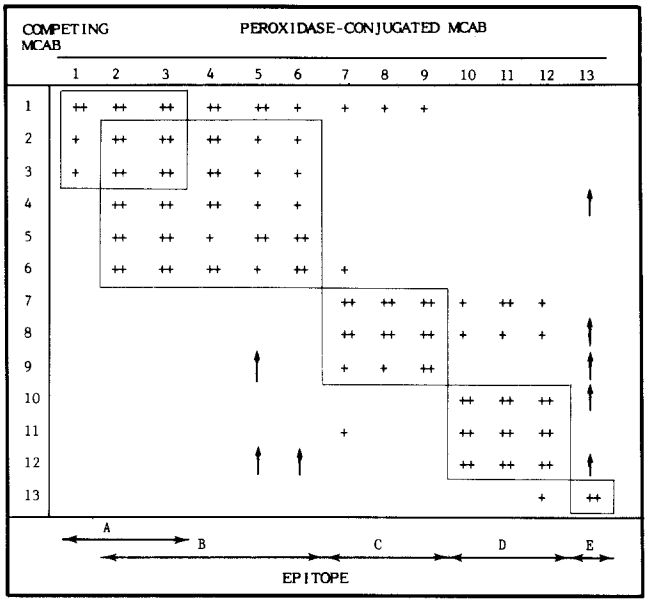
FIG. 3. Antibody blocking assays. Microtiter plates were coated with purified virus and incubated with peroxidase-labeled Mcabs to which various dilutions of the competing antibodies had been added. The percentages of competition calculated are plotted against the concentration of competitor and are normalized to 100% competition in the homologous reaction. In each panel the competing, unlabeled antibody (marked with an arrow) is constant and the curves represent competition with various peroxidase-labeled Mcabs.

site hinders the attachment of another antibody to the same or a proximal site (Stone and Nowinski, 1980). However, two antibodies directed to the same site will compete in a manner related to their relative avidities and concentrations. In practice, a spectrum of relative interference efficiencies will be obtained (Stone and Nowinski, 1980; Lefrancois and Lyles, 1982a, b.) Two-way competition is significant for the identification of Mcabs recognizing the same epitope, even when the degree of competition is low. Five epitopes (A to E) could be delineated on the peplomer protein, epitopes A and B overlapped. Furthermore there was extensive one-way competition between epitopes C and D and to a lesser extent between epitopes A and C. This does not necessarily indicate overlap of the epitopes, but may also be the result of conformational changes in the peplomeric protein induced by a Mcab against one epitope, leading to a reduced binding of a Mcab to another epitope.

Cooperative effects among antibodies directed against different epitopes were observed. Binding of Mcab 13 in the ELISA was enhanced by Mcabs 4, 8, 9, 10, and 12. These Mcabs, however, did not enhance neutralization or hemagglutination inhibition (HI). But

TABLE 3

SUMMARY OF COMPETITION IMMUNOASSAYS BETWEEN MONOCLONAL ANTIBODIES TO IBV-M41 S PROTEIN



Note. Competition more than 60%, ++; competition between 35 and 60%, +; enhancement more than 50%, ↑.

neutralization and HI assays are not accurate enough to detect a twofold increase in titer. The binding of Mcab 5 and 6 was enhanced by Mcabs 9 and 12 and by Mcab 12, respectively. Enhancement of binding has been reported for Mcabs against VSV (Lefrancois and Lyles, 1982a, b), yellow fever virus (Schlesinger *et al.*, 1984), Semliki Forest virus (Boere *et al.*, 1984), and recently with another coronavirus, TGEV (Delmas *et al.*, 1986). It might be related to a more advantageous conformation of the epitope involved, thereby increasing the binding of the antibody. Enhancement was always a one-way process.

Only epitope E is involved in both neutralization and hemagglutination inhibition. The corresponding Mcab 13 did not neutralize other IBV strains, but in the ELISA it reacted with one other IBV strain, D3896 (see Table 2). Two neutralizing Mcabs directed to S1 of IBV-M41 from Mockett *et al.* (1984) were also strain specific. Apparently, neutralization epitopes are not shared by IBV strains. In contrast, Jimenez *et al.* (1986) found that the critical epitopes involved in neutralization of TGEV were highly conserved.

The epitopes A to D were not involved in any known biological function. From Tables 2 and 3 we can deduce that the epitopes A–C are conserved. Only four Mcabs 6, 10, 12, and 13 differentiated between IBV strains. Conservation of epitopes is not unexpected in view of the conserved amino acid sequences of IBV strains (Niesters *et al.*, 1986; J. G. Kusters *et al.*, manuscript in preparation).

With the use of the dot immunoblotting assay, we were able to characterize the role of the conformation of the protein in antibody attachment. When the tertiary structure of the protein is disrupted by SDS and 2-mercaptoethanol, attachment of only Mcab 13 is abolished, indicating that Mcab 13 requires the native structure of the protein for recognition, whereas epitopes A to D are conformation independent (Fig. 5). Epitope E was also sensitive to SDS or NP-40/2-mercaptoethanol alone (data not shown). Talbot *et al.* (1984) found that two out of three neutralization relevant epitopes in MHV-JHM were completely sensitive to SDS denaturation, and one was partially sensitive. In the case of TGEV all six critical determinants were

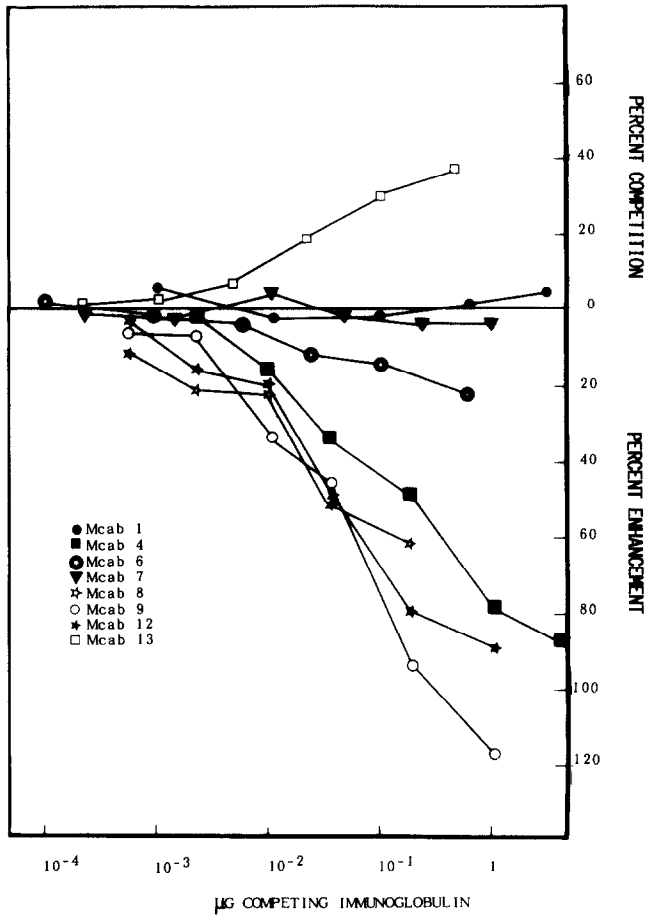


FIG. 4. Competition and enhancement of unlabeled Mcabs to the binding in an ELISA of peroxidase-labeded Mcabs 13 (epitope E). The assay was carried out as a normal competition assay. Unlike in Fig. 3, the data have not been normalized to 100% competition in the homologous system. Instead, the direct percentage of A_{405} in the control assay has been plotted.

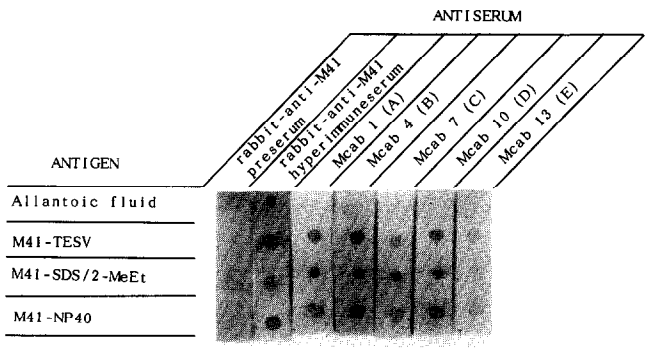


FIG. 5. Dot immunoblotting analysis of binding of monoclonal antibodies to IBV-M41 virions in their native (TESV, NP-40) or denatureated state (SDS and 2-mercaptoethanol; SDS/2-MeEt). Approximately 1 μg purified virions were applied as a dot on nitrocellulose strips and probed with an amount of monoclonal antibody giving twice maximal binding. Rabbit anti-M41 pre- and hyperimmune serum were used as negative and positive controls, respectively. The binding was visualized with a peroxidase-labeled second antiserum as described under Materials and Methods. As negative control 2 μg allantoic fluid of uninfected eggs was applied. The monoclonals and the competition groups they belong to are indicated.

sensitive to denaturation by SDS and 2-mercaptoethanol (Jimenez *et al.*, 1986).

It is not yet possible to define the number of epitopes involved in neutralization of IBV. Both our Mcab 13 and the Mcabs described by Mockett *et al.* (1984) neutralize no other IBV strains than M41. In contrast, polyclonal chicken antiserum against M41 does neutralize several IBV strains, but this does not imply that more than one epitope on S1 is involved in neutralization, as suggested by Mockett *et al.* (1984). Polyclonal antisera to one epitope contain a number of antibody species which could show cross-neutralization not observed with just one Mcab species. Recently, Koch *et al.* (1986) found that some Mcabs to S2 were able to neutralize IBV. However, this was only observed with high concentrations of antibody and might be a consequence of steric hindrance by the excess of antibody. Additional high-titer neutralizing antibodies aganist IBV strains are needed to answer this question.

Wege *et al.* (1984) identified six antigenic determinants on the peplomeric protein of MHV-JHM. Two overlapping determinants were involved in neutralization. Antibodies to one of these were able to protect mice *in vivo* against infection. Talbot and Buchmeier (1985) identified five epitopes on MHV strain 4 of which three were involved in neutralization. One of these three determinants was conserved among the strains tested. The nonneutralizing antibodies recognized all strains except MHV strain S. This strain was recognized only by one set of nonneutralizing antibodies. Delmas *et al.* (1986) showed that there are four major and three minor antigenic determinants on the TGE virus peplomeric protein, of which two are involved in neutralization.

It will be of interest to map our Mcabs on the peplomeric protein. We have cloned and sequenced the protein (Niesters *et al.*, 1986), so the epitopes can be mapped both by the expression of small fragments of recombinant DNA clones (Stanley and Luzio, 1984) and by the PEPSCAN method (Geysen *et al.*, 1984). Mapping of the epitopes recognized by Mcab 13 by these methods is probably difficult, since the results of the dot immunoblot assay indicate that several distinct parts of the protein form the neutralization epitope. In this respect mapping of the Mcabs which enhance Mcab 13 might give useful information. A direct approach to map the neutralization epitope recognized by Mcab 13 is to isolate mutants escaping neutralization.

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REFERENCES

ALEXANDER, D. J., BRACEWELL, C. D., and GOUGH, G. E. (1976). Preliminary evaluation of the haemagglutination tests for avian infectious bronchitis virus. *Avian Path.* **5,** 125–134.

BOERE, W. A. M., HARMSEN, T., VINJE, J., BENAISSA-TROUW, B. J., KRAAIJEVELD, C. A., and SNIPPE, H. (1984). Identification of distinct antigenic determinants on Semliki Forest virus by using monoclonal antibodies with different antiviral activities. *J. Virol.* **52,** 575–582.

BRUCK, C., PORTETELLE, D., BURNY, A., and ZAVADA, J. (1982). Topographical analysis by monoclonal antibodies of BLV-gp51 epitopes involved in viral functions. *Virology* **122,** 353–362.

CAVANAGH, D. (1981). Structural polypeptides of coronavirus IBV. *J. Gen. Virol.* **53,** 93–103.

CAVANAGH, D. (1983a). Coronavirus IBV: Further evidence that the surface projections are associated with two glycopolypeptides. *J. Gen. Virol.* **64,** 1787–1791.

CAVANAGH, D. (1983b). Coronavirus IBV: Structural characterization of the spike protein. *J. Gen. Virol.* **64,** 2577–2583.

CAVANAGH, D., DAVIS, P. J., DARBYSHIRE, J. H., and PETERS, R. W. (1986). Coronavirus IBV: Virus retaining spike glycopolypeptide S2 but not S1 is unable to induce virus-neutralizing or haemagglutination-inhibiting antibody, or induce chicken tracheal protection. *J. Gen. Virol.* **67,** 1435–1442.

DARBYSHIRE, J. H. (1981). Immunity to avian infectious bronchitis virus. *In* "Avian Immunology" (M. E. Rose, L. N. Payne, and B. M. Freeman, Eds.) British Poultry Science, Ltd., Edinburgh.

DAVELAAR, F. G., KOUWENHOVEN, B., and BURGER, A. G. (1984). Occurrence and significance of infectious bronchitis virus variant strains in egg and broiler production in the Netherlands. *The Vet. Quarterly* **6,** 114–120.

DELMAS, B., GELFI, J., and LAUDE, H. (1986). Antigenic structure of transmissible gastroenteritis virus. II. Domains in the peplomer glycoprotein. *J. Gen. Virol.* **67,** 1405–1418.

FRANKEL, M., and GERHARD, W. (1979). The rapid detection of binding constants for antiviral antibodies by a radioimmunoassay. An analysis of the interaction between hybridoma proteins and influenza virus. *Mol. Immunol.* **16,** 101–106.

GERHARD, W., YEWDELL, J., FRANKEL, M. E., LOPES, A. D., and STAUDT, L. (1980). Monoclonal antibodies against influenza virus. In "Monoclonal Antibody Hybridomas: A New Dimension in Biological Analysis (R. H. Kennett, T. J. McKearn, and K. B. Bechtor, Eds.), pp. 317–333. Plenum, New York.

GEYSEN, H. M., MELOEN, R. H., and BARTELING, S. J. (1984). Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proc. Natl. Acad. Sci. USA* **81,** 3998–4002.

GIRAUDON, P., and WILD, T. F. (1981). Monoclonal antibodies against measles virus. *J. Gen. Virol.* **54,** 325–332.

GODING, J. W. (1980). Antibody production by hybridomas. *J. Immunol. Methods* **39,** 285–308.

IORIO, R. M., and BRATT, M. A. (1983). Monoclonal antibodies to Newcastle disease virus: Delineation of four epitopes on the HN glycoprotein. *J. Virol.* **48,** 440–450.

JACKSON, D. C., BROWN, L. E., and WHITE, D. O. (1981). Antigenic determinants of influenza virus haemagglutinin. VI. Antigenic characterization of the oligosaccharide side chains from HA1 of influenza virus haemagglutinins. *J. Gen. Virol.* **52,** 163–168.

JIMENEZ, G., CORREA, I., MELGOSA, M. P., BULLIDO, M. J., and ENJUANES, L. (1986). Critical epitopes in transmissible gastroenteritis virus neutralization. *J. Virol.* **60,** 131–139.

KOCH, G., HARTOG, L., KANT, A., VAN ROOZELAAR, D., and DE BOER, G. F. (1986). Antigenic differentiation of avian infectious bronchitis variant strains employing monoclonal antibodies. *Isr. J. Vet. Med.* **42,** 89–97.

KUSTERS, J. G., NIESTERS, H. G. M., BLEUMINK-PLUYM, N. M. C., DAVELAAR, F. G., HORZINEK, M. C., and VAN DER ZEIJST, B. A. M. (1987). Molecular epidemiology of infectious bronchitis virus in the Netherlands. *J. Gen. Virol.* **68,** 343–352.

LEFRANCOIS, L., and LYLES, D. S. (1982a). The interaction of antibody with the major surface glycoprotein of vesicular stomatitis virus. 1. Analysis of neutralizing epitopes with monoclonal antibodies. *Virology* **121,** 157–167.

LEFRANCOIS, L., and LYLES, D. S. (1982b). The interaction of antibody with the major surface glycoprotein of vesicular stomatitis virus. 2. Monoclonal antibodies to nonneutralizing and cross-reactive epitopes of Indiana and New Jersey serotypes. *Virology* **121,** 168–174.

LOMNICZI, B., and MORSER, J. (1981). Polypeptides of infectious bronchitis virus. 1. Polypeptides of the virion. *J. Gen. Virol.* **55,** 155–164.

MACNAUGHTON, M. R., and MADGE, M. H. (1977). The polypeptide composition of avian infectious bronchitis virus particles. *Arch. Virol.* **55,** 47–56.

MARKWELL, M. A. K., and FOX, C. F. (1978). Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril. *Biochemistry* **17,** 4807–4817.

MOCKETT, A. P. A., CAVANAGH, D., and BROWN, T. D. K. (1984). Monoclonal antibodies to the S1 spike and membrane proteins of avian infectious bronchitis coronavirus strain Massachusetts M41. *J. Gen. Virol.* **65,** 2281–2286.

NIESTERS, H. G. M., LENSTRA, J. A., SPAAN, W. J. M., ZIJDERVELD, A. J., BLEUMINK-PLUYM, N. M. C., HONG, F., VAN SCHARRENBURG, G. J. M., HORZINEK, M. C., and VAN DER ZEIJST, B. A. M. (1986). The peplomer protein sequence of the M41 strain of coronavirus IBV and its comparison with Beaudette strains. *Virus Res.* **5,** 253–263.

OSTERHAUS, A. D. M. E., VAN WEZEL, A. L., VAN STEENIS, B., DROST, G. A., and HAZENDONK, T. G. (1981). Monoclonal antibodies to polioviruses. Production of specific antibodies to the Sabin vaccine strain. *Intervirology* **16,** 218–224.

ROOS, M. H., MOLLENHAUER, E., DEMANT, P., and RITTNER, C. (1982). A molecular basis for the two locus model of human complement component C4. *Nature (London)* **298,** 854–856.

ROTTIER, P. J. M., SPAAN, W. J. M., HORZINEK, M. C., and VAN DER ZEIJST, B. A. M. (1981). Translation of three mouse hepatitis virus strain A59 subgenomic RNAs in *Xenopus laevis* oocytes. *J. Gen. Virol.* **38,** 20–26.

SCHLESINGER, J. J., WALSH, E. E., and BRANDISS, M. W. (1984). Analysis of 17D yellow fever virus envelope protein epitopes using monoclonal antibodies. *J. Gen. Virol.* **65,** 1637–1644.

STANLEY, K. K., and LUZIO, J. P. (1984). Construction of a new family of high efficiency bacterial expression vectors: Identification of cDNA clones coding for human liver proteins. *EMBO J.* **5,** 1429–1434.

STERN, D. F., and SEFTON, B. M. (1982). Coronavirus proteins: Biogenesis of avian infectious bronchitis virus virion proteins. *J. Virol.* **44,** 794–803.

STONE, M. R., and NOWINSKI, R. C. (1980). Topological mapping of murine leukemia virus proteins by competition binding assays with monoclonal antibodies. *Virology* **100,** 370–381.

TALBOT, P. J., and BUCHMEIER, M. J. (1985). Antigenic variation among murine coronaviruses: Evidence for polymorphism on the peplomer glycoprotein E2. *Virus Res.* **2,** 317–328.

TALBOT, P. J., KNOBLER, R. L., and BUCHMEIER, M. J. (1984). Western and dot immunoblotting analysis of viral antigens and antibodies: Application to murine hepatitis virus. *J. Immunol. Methods* **73,** 177–188.

VAN DER ZEIJST, B. A. M., BLEUMINK, N., CRAWFORD, L. V., SWYRYD, E. A., and STARK, G. R. (1983). Viral proteins and RNAs in BHK cells persistently infected by lymphocytic choromeningitis virus. *J. Virol.* **48,** 262–270.

VAN DRUNEN LITTEL-VAN DEN HURK, S., VAN DEN HURK, J. V., and BABIUK, L. A. (1985). Topographical analysis of bovine herpesvirus type-1 glycoproteins: Use of monoclonal antibodies to identify and characterize functional epitopes. *Virology* **144,** 216–227.

WADEY, C. N., and WESTAWAY, E. G. (1981). Structural proteins and glycoproteins of infectious bronchitis virus particles labelled during growth in chick embryo cells. *Intervirology* **15,** 19–27.

WEGE, H., DORRIES, R., and WEGE, H. (1984). Hybridoma antibodies to the murine coronavirus JHM: Characterization of epitopes on the peplomer protein (E2). *J. Gen. Virol.* **65,** 1931–1942.

WILSON, M. B., and NAKANE, P. K. (1978). Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. *In* "Immunofluorescence and Related Staining Techniques" (W. Knapp, K. Holubar, and G. Wick, Eds.), pp. 215–224. Elsevier/North Holland, New York.