ANTIGENIC DIFFERENTIATION OF AVIAN BRONCHITIS VIRUS VARIANT STRAINS EMPLOYING MONOCLONAL ANTIBODIES

G. Koch, L. Hartog, A. Kant, D. van Roozelaar and G.F. de Boer
Central Veterinary Institute, Dept. of Virology,
Postbox 365, 8200 AJ Lelystad, The Netherlands.

ABSTRACT

Monoclonal antibodies (McAbs) to Infectious Bronchitis Virus (IBV) were prepared and tested for reactivity to different IBV variant strains by an indirect enzyme-immunoassay.

With the exception of two McAbs specific for the nucleoprotein, which did not recognize the SE-17 strain, all McAbs to the matrix and nucleoprotein reacted with all strains tested. McAbs to the matrix and nucleoprotein, therefore, are suitable for diagnostic purposes.

On basis of the reactivity pattern to variant strains of a panel of McAbs specific for the peplomer protein, it was possible to differentiate Dutch variant strains into three groups .

INTRODUCTION

Infectious Bronchitis is a highly contagious, mainly respiratory disease of young chickens. The disease is of economical importance because infection of production flocks and broilers often leads to permanent decrease in egg-laying and growth retardation, respectively. The causative agent is a virus, Infectious Bronchitis Virus (IBV), which belongs to the family of the Coronaviridae.

Although there have been much disagreement on the actual number of IBV structural proteins in the past, it is now generally accepted that IBV contains three structural proteins only (Cavanagh, 1981; Stern et al. 1982; Stern and Sefton 1982). The three structural proteins are a non-glycosylated nucleoprotein (N), and two glycosylated envelope proteins. The nucleoprotein is a phosphorylated protein (Lomniczi and Morser, 1981) with a molecular weight of 50 to 55 kdalton. In the virus particle, N is complexed to a genomic RNA molecule. Together they form the nucleocapsid of the virus. The matrix of the virus consists mainly of a trans-membrane protein (M) embedded in a lipid layer. M is an heterogenous glycoprotein with a molecular weight ranging from 23 to 36 kdalton depending on the extent of glycosylation. The surface protein, which in the electron microscope has a morphology of a peplomer, contains two non-covalently linked subunits S1 and S2. S1 and S2 have molecular weights of 92 and 84 kdalton, respectively. Based on experimental evidence of Cavanagh (1983a, 1983b), it is concluded that S2 subunits form the stick, whereas S1 subunits form the bulb of the peplomer.

J. B. McFerran et al. (eds.), *Acute Virus Infections of Poultry*
© ECSC, EEC, EAEC, Brussels-Luxembourg 1986

The control of IB still is difficult and tedious with outbreaks of the disease occurring both in vaccinated and unvaccinated flocks. Vaccination breaks are attributed, in part, to new antigenically distinct strains. Since the start of the vaccination programme in the Netherlands two decades ago, a large number of new isolates have been obtained and characterized. These isolates are classified into four antigenically distinct groups by serum neutralization (SN) and haem-agglutination inhibition (HI) tests (Locker et al., 1983; Cook, 1984; Davelaar et al. 1984). Both tests, however, show different levels of cross-reactivity patterns of individual sera to heterologous variant strains. Furthermore, in the SN test different results are often obtained when different indicator systems for virus infectivity are used (Cowen and Hitchner, 1975). As a consequence, tests performed at a different time or place often result in different antigenic groupings. New IBV isolates are initially difficult to propagate in the usual in vitro cell culture systems (Sturman and Holmes, 1983; Hofstad, 1984) and the serological characterization by SN and HI tests needs the preparation of homologous antisera. Furthermore, the characterization is hampered by the numerous, but ill defined, antigenic groupings proposed. Therefore, diagnosis and characterization of new field strains is time consuming.

Monoclonal antibodies (McAbs) are continuously produced by immortal hybrid cell lines obtained by the deliberate fusion of myeloma cells and B lymphocytes. Since cloned hybrid cell lines are derived from single B lymphocytes, these cell lines provide us with a source of highly specific antibodies in unlimited quantities. In this way antibodies with specificity for distinct epitopes of different proteins can be obtained.

McAbs to structural proteins of IBV could provide us with techniques for rapid diagnosis and antigenic characterization of IBV isolates in a more simple fashion. For this reason, and to map the antigenic relationships of IBV variant strains at the epitope level, which could provide valuable information useful for the formulation of new vaccines, we prepared McAbs to the three structural proteins of IBV. This report gives an overview of the results obtained up to now.

MATERIALS AND METHODS

Viruses

The IBV variant strains were originally obtained from Laboratory Dr. de Zeeuw, De Bilt, The Netherlands (0728, B801, U101, G901-20 and L502) and from the Poultry Health Service, Doorn, The Netherlands (D207, D274, D3128, D3896,

D212 and D1466). The virus strains were received at different embryonic passages. For the adaptation of the virus to grow in chicken embryo kidney (CEK) cells, the viruses were serially passaged in CEKC monolayers until a cytopathological effect could be observed.

Virus growth and purification

Virus was grown on 11-day-old embryonated eggs. Approximately 10^4 median egg infectious dose of virus was injected into the allantoic cavity. After incubation for 24 hr at 38°C, the eggs were chilled at 4°C and subsequently the allantoic fluid was harvested. To remove cellular debris the allantoic fluid was centrifuged at 800 g for 30 minutes. Virus was concentrated from the allantoic fluid by centrifugation for about 24 hr at 10,000 g in a Beckman J-21 centrifuge. The virus pellet was resuspended in TSE buffer (0.01 M Tris-hydrochloride, 0.1 M sodium chloride and 1 mM EDTA). Subsequently, the virus was purified by velocity sedimentation overnight at 4°C through a linear 26 to 65% (w/v) sucrose gradient using a Beckman SW-27 rotor (Cavanagh, 1981). The protein concentration of the virus preparation was determined using bovine serum albumine (BSA) as a standard (Lowry et al., 1951). The virus preparations were stored at -70°C until use.

Preparation of monoclonal antibodies

BALB/c mice, 10-14 weeks of age, were primed by an intraperitoneal inoculation of 50 ug purified IBV emulsified in CFA. After 6 to 8 weeks, the mice were boosted intravenously with the same dose of purified IBV. The fusion of immune spleen cells, harvested 3 days after the booster immunization, and the non-secretor plasmacytoma cells, P3-X-63Ag8.653 (Kearny et al., 1979), was performed according to the technique originally described by Kohler and Milstein (1975) with some slight modifications (Van Zaane and IJzerman, 1984). Peritoneal macrophages were used as feeder cells (Fazekas de St. Groth and Scheidegger, 1980).

Screening of the culture fluids for antiviral antibodies was performed with an indirect enzyme-immunoassay (EIA). As control the fluids were tested for reactivity to proteins present in allantoic fluids of uninfected eggs (AF) and to New castle Disease Virus (NCD). Cells, lines which scored positive in the EIA for IBV and negative for AF and NCD, were cloned by limiting dilution combined with visual inspection. Clones were expanded after the third cloning procedure. Ascites fluid was subsequently produced in pristane treated BALB/c mice (Brodeur et al., 1984).

Enzyme-immunoassay

The enzyme-immunoassay (EIA) was performed on antigen-coated polystyrene microtiter plates (Dynatech M129 A, Cooke). The plates were coated overnight at 4°C with either 0.5-1 µg purified IBV, the same amount of NCD or 12 µg AF diluted in sodium bicarbonate buffer pH 9.6 per well. The plates were stored at 4°C in the same buffer until use. In the antibody binding assay, culture fluid or ascites fluid was serially diluted in EIA diluent (phosphate buffered saline supplemented with 51 gr of sodium chloride per liter, 0.05% Tween 20 and 4% normal horse serum (NHS)) and incubated for 90 minutes at 37°C, which was the incubation temperature at all subsequent steps as well. Subsequently, the plates were incubated with horse-radish peroxidase conjugated to rabbit anti-mouse immunoglobulin (RAM/Ig-PO) (Nordic Lab., Tilburg, The Netherlands) and, finally, stained in a solution of 1 mg/ml 5-amino-2-benzoic acid (5-aminosalicylic acid) (Merck Chemicals, Darmstadt) in phosphate buffer pH 5.95 to which, prior to use, freshly-prepared H_2O_2 was added to a final concentration of 0.05 per cent. The peroxidase activity was quantified by measuring the optical density at 450 nm in a Titertek Multiscan (Flow).

Microneutralization test

The SN test was performed essentially as described previously (Blore and Skeeles, 1981). In short, a constant amount of virus (between 100-250 median tissue culture infectious dose ($TCID_{50}$) was mixed with two-fold dilutions of antisera and incubated for 1 hr at room temperature. Subsequently, the virus-serum mixtures were transferred to wells of microtiter plates which contained monolayers of primary CEKC. The plates were placed in a CO_2 incubator at 38-39°C for 48 hrs. To score for IBV-infected cultures, the monolayers were fixed with paraformaldehyde solution and stained by incubation with homologous IBV antiserum and subsequently a goat anti-chick Ig peroxidase conjugate, essentially as described before (Saunders, 1977).

RESULTS

A large number of clones producing McAbs to IBV were obtained. All selected clones produced McAbs which reacted positive in the IBV EIA and negative in the EIA of NCD and AF. The viral protein specificity of the McAbs was characterized by immunoblotting. The results, which will be published in greater detail elsewhere, are summarized below. According to their specificity, McAbs fell into three different groups: Those reacting to M, those reacting to N and those reacting to S. McAbs to S were specific for the S2 subunit which was concluded from the

comparison of the staining pattern of McAbs to S with the staining pattern of polyclonal homologous chicken antisera to IBV. McAbs which did not bind to any protein in the IBV blots were discarded for the time being.

Neutralizing activity of the McAbs

The neutralizing activity of the McAbs was determined in the SN test on CEKC monolayers. Only McAbs mentioned in Table I possessed neutralizing activity. All these McAbs were specific for the S2 subunit of the peplomer protein. The titers never exceeded 1 in 640, rather low considering the antibody concentration in the ascites fluids. Increased but also decreased titers were observed when mixtures of distinct McAbs were prepared. Particularly, increased

TABLE I. **Neutralizing activity of monoclonal antibodies and mixtures thereof.**

Monoclonal		in combination with monoclonal antibody							
antibody	–	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.8
31.1	40[a]								
31.2	0	0							
31.3	80	80	40						
31.4	0	0	0	20					
31.5	80	0	0	1280	0				
31.6	320	640	20	1280	320	80			
31.7	320	80	320	2560	40	40	80		
31.8	320	320	n.d.[b]	320	n.d.	n.d.	640	40	
31.5+31.6	80	40	80	640	n.d.	n.d.	n.d.	1280	160
26.1	≤640	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30.6	≤640	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

(a) Reciproke of the neutralizing titer of McAb in an *in vitro* micro neutralization test. Twofold serial dilutions of ascites fluid, heat inactivated for 30 min at 56°C, were incubated with a constant amount of IBV (100-250 $TCID_{50}$). Subsequently, the mixtures were plated on CEKC monolayers.
(b) n.d., not done.

titers were observed when McAb 31.3 was mixed with other McAbs (Table 1, first compared to fourth column). The virus was always completely neutralized.

Cross-reactivity of McAbs to IBV variant strains

The cross-reactivity of the McAbs to IBV was tested in an indirect EIA using microtiter plates coated with several different variant strains. The variant strains used in this test are claimed to belong to four Dutch (Locher et al. 1983; Cook, 1984; Davelaar et al. 1984) and seven American serotypes (Cowen, 1971; Hopkins, 1974; Cowen and Hitchner, 1975; Johnson and Marquardt, 1975; Darbyshire et al., 1979; Hofstad, 1981). The protein composition of all virus preparations used were evaluated by immunoblotting of the IBV variant strains with homologous polyvalent antisera to ascertain that no negative reactions would be scored by the EIA, because proteins were lacking in any of the preparations.

TABLE II. **Reactivity pattern of panel of monoclonal antibodies to IBV**

Monoclonal antibody	Specificity	Virus strain													
		H120	B222	M41	D207	D274	D3896	D212	D1466	Clarck	Ark	SE-17	Holte	I97	I609
25.1	M[a]	+++[b]	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
26.2	N	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	+++	+++	+++
26.3	N	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	+++	+++	+++
26.1	S_2	+++	+++	+++	+++	+++	+++	+++	+++	–	++	+++	+	+	–
30.6		+++	+++	+++	+++	+++	++	+	++	–	++	+++	+	+	+
31.3		+++	+++	+++	++	+++	++	+	+++	–	+	++	+	+	+
32.3		+++	+++	+++	+++	+++	++	–	–	+++	++	+++	+	++	+
31.7		+++	+++	+++	+++	+++	+	–	++	–	+	++	–	–	+
31.1		+++	+++	+++	+++	+++	++	–	++	–	++	++	–	–	–
31.5		++	++++	–	–	–	–	–	–	–	–	–	–	–	–
31.4		++	++++	+++	–	–	–	+	++	–	–	–	–	–	–
30.7		–	++	–	+++	++	++	–	–	–	–	–	–	–	–
32.1		–	+++	–	++	+++	+	–	–	–	–	–	–	–	–
32.2		–	–	–	++	+++	+	–	–	–	–	–	–	–	–
32.5		–	–	–	+++	+++	+	–	–	–	–	–	–	–	–
30.2		–	–	–	+++	+++	++	–	–	++	–	–	–	–	–

(a) The specificity of monoclonal antibodies (McAb) was tested with the immuno-electroblotting technique. M = matrix protein, N = nucleoprotein, S_2 = subunit of peplomer protein S.

(b) Reactivity of McAb to IBV. The optical density (O.D.) obtained after binding of McAb to heterologous IBV is expressed as a percentage of the maximal O.D. found after binding to homologous virus. +++ > 50%, ++ 25-50%, + twice background-25%, – less than twice background.

The McAbs specific for M (25.1) and N (26.2, 26.3) reacted to all variant strains in the test to the same extent with the exception of the SE-17 strain which was not recognized by both McAbs to N (Table II). McAbs to M and N produced by 20 respectively 3 uncloned hybrid cell lines likewise demonstrated a broad spectrum of reactivity (data not shown).

McAbs directed to S2 showed much less cross-reactivity. Roughly, on basis of the cross-reactivity, the McAbs to S2 could be divided into four groups: Those reacting to most Dutch and American strains, those (31.5) reacting to Massachusetts serotype only, those (31.4) reacting to the Mass. and one Dutch serotype (D212 and D1466 are claimed to belong to the same serotype), and those reacting to more than one Dutch serotype only.

By comparison of the reactivity patterns of McAbs belonging to the first group, differentiation between a number of American variant strains could be made. For instance, comparing the reactivity pattern of McAb 32.3 to the patterns of McAbs 30.6 and 31.3, a distinction between the Clark strain and the rest of the American strains, i.e. Ark, SE-17, Holte, I97 and I609, could be made. Comparison of the reactivity patterns of McAb 26.1 and 30.6 differentiated the I609 strain from the other strains and so on.

Using a panel of four McAbs, each representative of a group, it was possible to differentiate Dutch variant strains into three groups according to the reaction with the panel. The first group consists of the B222 and L502 strains which are recognized by three McAbs of the panel, but not by McAb 30.2. The second group, consisting of the strains D207, D274, D3896, D3128, 0728, U201, L536 and G901-20, is recognized by comparing the reactivity patterns of McAbs 32.3 and 31.4. The third group includes the strains D212, D1466, U101, U121 and B801 which reacted with McAb 31.4 but not with any of the other McAbs of the panel (Table III).

TABLE III. **Reactivity pattern of panel of monoclonal antibodies to the S_2 protein[(a)] of IBV**

Monoclonal antibody	Virus strain													
	B222	L502	D207	D274	D3128	D3896	O728	U102	L536	G901-20	D212	D1466	U101	U121	B801
32.3	+++[(b)]	+++	+++	+++	+++	++	+++	+++	+++	+++	–	–	–	–	–
31.4	+++	+++	–	–	–	–	–	–	–	–	+++	+++	+++	+++	+++
31.5	+++	+++	–	–	–	–	–	–	–	–	–	–	–	–	–
30.2	–	+	+++	+++	+++	++	+++	+++	+++	+++	–	–	–	–	–

[(a)] S_2 = subunit of peplomer protein S.
[(b)] See legend (b) table I.

DISCUSSION

In an attempt to differentiate IBV variant strains, we prepared a panel of McAbs. McAbs to all structural proteins of IBV, except the S1 subunit, were obtained. The specificity of the McAbs could be easily determined in the immuno-

electroblotting technique. Non-specific staining was not observed.

Evaluation of the cross-reactivity to IBV variant strains of the McAbs revealed that the McAbs specific for M and for N are directed to epitopes which are comon to almost all IBV strains. To the contrary, McAbs specific for S2 are directed to epitopes which are variably distributed among the variant strains. It is highly unlikely that the cross-reactivity of McAbs to M and to N should be explained because the variant strains are in fact identical, since the strains used in the test are claimed to belong to eleven distinct serotypes (Cowen, 1971; Hopkins, 1974; Cowen and Hitchner, 1975; Johnson and Marquardt, 1975; Darbyshire et al., 1979; Hofstad, 1981; Locher et al., 1983; Cook, 1984; Davelaar et al., 1984), and since the McAbs to S2 also differentiate a number of the strains. Because of the group-specificity, McAbs 25.1 to M and 26.2 to N are suitable for diagnostic purposes. Actually, we are using both McAbs for the detection of IBV-infected cells in an in vitro micro-neutralization test and in thin sections of organs of IBV-infected chickens.

The results suggest a difference in antigenic drift of the IBV structural proteins. No antigenic drift of M was observed thus far, as is apparent from the reactivity patterns of one cloned and 20 uncloned McAbs specific for M to the IBV variant strains (Table II and data not shown). The results suggest that N is also highly conserved. Two cloned and three uncloned McAbs to N reacted to all the variant strains with the exception of McAbs 26.2 and 26.3 which did not react to the SE-17 strain (Table II and data not shown). The number of McAbs with specificity for N, however, is to small to draw definitive conclusions. Furthermore, it should be stated that these conclusions are valid for one antigenic determinant of M and of N only, because formally it has not been proven, for instance by competition assays, that the 21 McAbs to M and 5 McAbs to N are directed to different epitopes. However, it is unlikely that all McAbs to M and to N have the same epitope specificity, in light of the heterogeneity of the antibody response.

Differences in optical densities of the reactions of McAbs directed to S2 were observed in the EIA, which could indicate differences in affinity of these McAbs for the strains. However, in the EIA the extent of the antibody-antigen reaction is dependent on antibody affinity, antibody concentration and density of the relevant antigen determinant (Steward and Lew, 1985). Particularly, the density of S determinants is difficult to control, because the amount of S varies between different isolates and even between different preparations of the same strain (Brown et al., 1984). The concentration of the S1 and S2 subunits could not be determined from the immunoblots, since the technique allows for qualitative

interpretations only. Thus, the observed differences in optical densities can still be caused by differences in antigen concentration or in the affinity of the McAb for IBV variant strains. Therefore, only qualitative differences in the EIA are considered. Nevertheless, comparison of the reactivity patterns of different McAbs to the variant strains indicates that the affinity of some McAbs for different variant strains is variable. Considering these facts, the results permit the differentiation of variant strains. The grouping obtained by comparing the reactivity patterns of four McAbs to the Dutch variant strains is less discriminatory than the grouping according to results of the SN and HI tests. Up to now four serotypes have been proposed of which D207, D3128, D3896 and D212 represent the prototypes (Locher et al., 1983; Cook, 1984; Davelaar et al., 1984). Results obtained in the cross SN-test using reconvalescent sera showed close relationship between 0728, D274 and D207 and relationship to a varying extent between D212, D1466, U101 and B801 (Koch et al., submitted for publication). The McAbs did not make a distinction between the strains D207, D3128 and D3896. Possibly, these results indicate that the serotypes D207, D3128 and D3896 are more closely related to each other than to strains of the D212 serotype.

Some of the McAbs to S2 possessed in vitro neutralizing activity of virus infectivity. However, this activity was only observed with high concentrations of antibody present in ascites fluid. Furthermore, these McAbs neutralized other strains besides the homologous variant strain. Therefore we think that the observed neutralization is a consequence of the steric hindrance of virus infection by a high amount of antibody molecules bound to the virus and not by the binding of antibody to a region involved in neutralization. From this view our results support the observations of Mockett et al. (1984) and Cavanagh et al. (1984), which indicate the importance of the spike protein and in particular the S1 subunit in virus replication.

Recently, we obtained McAbs which showed high titres in the SN test. The cross-neutralization pattern of these McAbs permitted for the discrimination of more variant strains. Currently, we are determining the structural protein specificity of these McAbs.

ACKNOWLEGDEMENT

We thank Mrs. R. de Kok-Heuckeroth and J.C. Hoogeveen-Hilhorst for typing of the manuscript.

REFERENCES

Blore, P.J. and Skeeles, J.K. 1981. Use of a constant-virus diluting-serum microneutralization technique for the detection and quantification of

Infectious Bronchitis virus antibodies. Avian Dis. 25, 801-809.
Brodeur, B.R., Tsang, P. and Larose, Y. 1984. Parameters affecting ascites tumour formation in mice and monoclonal antibody production. J. Immunol. Meth. 71, 265-272.
Brown, T.D.K., Cavanagh, D. and Boursnell, M.E.G. 1984. Confirmation of the presence of avian Infectious Bronchitis virus (IBV) using cloned DNA complementary to the 3' terminus of the IBV genome. Avian Pathol. 13, 109-117.
Cavanagh, D. 1981. Structural polypeptides of coronavirus. 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., Darbyshire, J.H., Davis, P. and Peters, R.W. 1984. Induction of humoral neutralizing and haemagglutination-inhibiting antibody by spike protein of avian bronchitis virus. Avian Pathol. 13, 573-583.
Cook, J.K.A. 1984. The classification of new serotypes of Infectious Bronchitis virus isolated from the poultry flocks in Britain between 1981 and 1983. Avian Pathol. 13, 733-739.
Cowen, B.S., Hitchner, S.B. and Lucio, B. 1971. Avian Dis. 15, 518-526.
Cowen, B.S. and Hitchner, S.B. 1975. Serotyping of avian Infectious Bronchitis viruses by the virus-neutralization test. Avian Dis. 19, 583-595.
Darbyshire, J.H., Rowell, J.G., Cooke, J.K.A. and Peters, R.W. 1979. Taxonomic studies on strains of avian Infectious Bronchitis virus using neutralisation tests in tracheal organ cultures. Arch. Virol. 61, 227-238.
Davelaar, F.G., Kouwenhoven, B. and Burger, G.A. 1984. Occurrence and significance of Infectious Bronchitis virus variant strains in egg and broiler production in The Netherlands. Vet. Quarterly 6, 114-120.
Fazekas de St. Groth, S. and Scheidegger, D. 1980. Production of monoclonal antibodies: strategy and tactics. J. Immunol. Meth. 35, 1.
Hofstad, M.S. 1984. Avian Infectious Bronchitis. In: "Disease of Poultry" (Ed. M.S. Hofstad, B.W. Calnek, H.J. Barnes, W.M. Reid, and H.W. Yoder) (Iowa State Univ. Press, Ames, Iowa, USA). 8th edition. pp. 487-503.
Hofstad, M.S. 1981. Cross-immunity of chickens using seven isolates of avian Infectious Bronchitis virus. Avian Dis. 25, 650.
Hopkins, S.R. 1974. Serological comparison of strains of Infectious Bronchitis virus using plaque-purified isolants. Avian Dis. 18, 231-239.
Johnson, R.B. and Marquardt, W.W. 1975. The neutralizing characteristics of strains of Infectious Bronchitis virus as measured by the constant-virus variable-serum method in chicken tracheal cultures. Avian Dis. 19, 82-90.
Kearny, J.F., Radbuch, A., Liesegang, B. and Rajewsky, K.J. 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. 123, 1548-1550.
Köhler, G. and Milstein, C. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) 256, 495-497.
Locher, M., Lohr, J.E., Kosters, J., Kouwenhoven, B. and Davelaar, F.G. 1983. Vergleichende serologische Untersuchungen von süddeutschen Feldisolaten des Virus der Infektiosen Bronchitis der Hühner und den Höllandischen Variantstammen D207, D3128 und D3896 in Trachealkulturen. Berl. Muench. Tierärtzl. Wschr. 96, 269-274.
Lomniczi, B. and Morser, J. 1981. Polypeptides of Infectious Bronchitis virus. I. Polypeptides of the virion. J. gen. Virol. 55, 155-164.
Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randell, R.J. 1951. Protein

measurements with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.
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 virus coronavirus strain Massachusetts M41. J. gen. Virol. 65, 2281-2286.
Saunders, G.L. 1977. Development and evaluation of an enzyme-labeled antibody test for rapid detection of hog cholera antibodies. Am. J. Vet. Res. 38, 21-25.
Stern, D.F., Burgess, L. and Sefton, B.M. 1982. Structural analysis of virions proteins of the avian coronavirus Infectious Bronchitis virus. J. Virol. 42, 208-219.
Stern, D.F. and Sefton, B.M. 1982. Coronavirus proteins: Structure and function of the oligosaccharides of the avian Infectious Bronchitis virus. J. Virol. 44, 804-812.
Steward, M.W. and Lew, A.M. 1985. The importance of antibody affinity in the performance of immunoassays for antibody. J. Immunol. Meth. 78, 173-190.
Sturman, L.S. and Holmes, K.V. 1983. The molecular biology of coronaviruses. Adv. Virus Research 28, 35-112.
Van Zaane, D. and IJzerman, J. 1984. Monoclonal antibodies against bovine immunoglobulins and their use in isotype-specific ELISAs for rotavirus. J. Immunol. Meth. 72, 427-441.