ANTIGENIC AND POLYPEPTIDE STRUCTURE OF BOVINE ENTERIC CORONAVIRUS AS DEFINED BY MONOCLONAL ANTIBODIES

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INTRODUCTION

Bovine enteric coronavirus (BECV), characterized by Stair *et al* (1972), is now recognized as one of the viral agents that cause acute diarrhoea in young calves (Stair *et al*, 1972). Virions are large and pleomorphic (100 to 150 nm in diameter) and possess a fringe of characteristically club-shaped peplomers, 20 nm long (Sharpee *et al*, 1976). Polypeptide analysis revealed 3 major proteins including glycoproteins of high (GP 65/125 - 65,000 and 125,000 daltons in molecular weight) and low (GP25) molecular weight and a phosphorylated protein (VP 50) (King and Brian, 1982; Laporte and Bobulesco, 1981). Two minor glycoproteins have also been described, GP 105 and GP 100 (King and Brian, 1982 ; J. Laporte, personal communication). Detergent treatment and limited proteolysis of purified virions enabled to localize the structural proteins (King and Brian, 1982 ; Bobulesco, 1983). The peplomers are mainly constituted of GP 125, a glycoprotein which is reduced to a GP 65 component by 2-Mercaptoethanol (2-ME), and of GP 105 and GP 100. On these outer projections are located the structural sites responsible for haemagglutination and virus to cell interactions. GP 25 is more deeply embedded in the virion envelope and interacts with the internal nucleoprotein (Bobulesco, 1983). VP 50 is an internal phosphoprotein, closely associated with the viral genome (King and Brian, 1982).

Current evidence indicates that the coronaviruses which affect mammals can be classified in two antigenically distinct groups (Pedersen *et al*, 1978 ; Gerna *et al*, 1982) a) OC_{43} group to which

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belong BECV, haemagglutinating encephalitis virus of swine (HEV), murine hepatitis virus (MHV), diarrhoea virus of infant mice (DVIM), rat sialodacryoadenitis virus (SDAV), human enteric coronavirus (HECV) b) 229 E group comprising the viruses of transmissible gastroenteritis of swine (TGEV), feline infectious peritonitis (FIPV) and canine enteric coronavirus (CCV). Common antigenic determinants were described on the three structural polypeptides of TGEV, FIPV and CCV (Horzinek *et al*, 1982), but little is known on the precise antigenic relationships at the molecular level between the coronaviruses of the OC43 group.

This report describes the characterization of monoclonal antibodies to BECV, 8 of which reacted with GP 105. These monoclonal antibodies helped to define two biological activities associated with GP 105, haemagglutination and virus to cell interaction. Antigenic relationships between BECV and other coronaviruses, as well as within BECV isolates, were studied using monoclonal antibodies.

MATERIALS AND METHODS

Cell-lines and viruses

HRT 18 cell-line (a human rectal tumor cell-line isolated by Tompkins *et al*, 1974) was grown as previously described (Laporte *et al*, 1981) except that tylosine (10 µg/ml) and lincomycine (200 µg/ml) were added to the medium instead of penicillin and steptomycin. BECV G110 (Laporte *et al*, 1979 ; L'Haridon *et al*, 1981), F_{15} (Laporte *et al*, 1979 ; Laporte and Bobulesco, 1981), NCDCV (Mebus *et al*, 1973), danish and british isolates (Bridger *et al*, 1978), as well as HEV, OC_{43} (Weiss, 1983), and a bovine respiratory coronavirus (Thomas *et al*, 1982) were serially passaged on HRT 18. For biochemical purposes, the viruses were plaque-purified, cultivated on HRT 18 and purified according to a method described previously (Laporte et Bobulesco, 1981).

Primary foetal bovine kidney cells (PFBK) were cultivated in Eagle's minimum essential medium (Eurobio, Paris) supplemented with 10 % foetal calf serum (FCS) (Flow Laboratories, France), 100 IU/ml penicillin and 100 µg/ml streptomycin. These PFBK were used to propagate BECV G110 (L'Haridon *et al*, 1982) and NCDCV (Mebus *et al*, 1973).

RPd cells, a pig kidney cell-line, were cultivated as previously described (Laude, 1978) and used to grow HEV and TGEV.

Sac^- cells, a permanent rhabdosarcoma cell-line from mice, was grown as previously described (Wege *et al*, 1979) and used to cultivate MHV_4 - JHM and MHV_3.

Purification of the coronaviruses, immunization schedules, fusion procedures and partial characterization of monoclonal antibodies have been described previously (Roseto *et al*, 1982).

Radiolabelling and immuno-precipitation

Polypeptide specificities of monoclonal antibodies were determined by immunoprecipitation of ^{14}C-labelled viral polypeptides from HRT 18 cells infected with BECV G110 (Laporte *et al*, 1980). Confluent monolayers were infected by G110 at a m.o.i. of 10 PFU/cell At 16 h post-infection, the supernatant was discarded and replaced by fresh RPMI 1640 (Eurobio, France) containing 2 % FCS and 5µCi/ml of a ^{14}C-labelled amino-acid mixture (45 mCi/at. C - CEA, France). After a further 6 hours incubation, the medium was discarded, monolayers were washed twice with cold phosphate buffer saline pH = 7.2 (PBS) and the cells were solubilized in lysis buffer (Collins *et al*, 1982). The cytosol extract was centrifuged at 10,000 x g during 30 minutes to remove nuclear debris and aliquots were stored at - 80°C. Before immunoprecipitation, cytosol extracts were preabsorbed with *Staphylococcus aureus* to avoid non-specific binding. Briefly, cytosol extracts were mixed with a suspension of heat-killed formalin-fixed *Staphylococcus aureus* (Pansorbin, Calbiochem) to give a final concentration of 6 % in *Staphylococcus aureus*. After one hour at room temperature, bacteria were pelleted by centrifugation at 11,000 x g for 2 min. and the supernatant was used for immune precipitation. Fifty µl of cytosol extract, containing 150,000 TCA precipitable CPM, were mixed either with 10 µl of polyclonal antiserum or mouse ascitic fluid (MAF), or with 50 µl of undiluted hybridoma supernatant. The mixture was incubated for 1 hour at 37°C and 50 µl of sheep anti-mouse IgG (SAMIg) (Institut Pasteur, Paris) diluted 1/200 in TEN-OVA buffer (Tris 50 mM, pH = 7.2, 150 mM NaCl, 5 mM EDTA containing 2 % Aprotinin and 1 mg/ml ovalbumine), were added. After a further 1 hour incubation at 37°C, 100 µl of a 10 % suspension of heat-killed formalin-fixed *Staphylococcus aureus* were added to the mixture. Incubation was continued for an additional 45 min. at room temperature. Bacteria were pelleted by a 2 min. centrifugation at 11,000 x g, and washed 4 times in TEN-OVA. The final pellet was resuspended in sample preparation buffer containing 3 % sodium dodecyl sulfate (SDS) and heated at 56°C for 5 min. Reduction of viral polypeptides was achieved by incorporating 5 % 2-M.E. in sample buffer before heating. Bacteria were removed by centrifugation and samples were analyzed by electrophoresis on 10 % polyacrylamide slab-gels (Laemmli, 1970). Gels were stained by coomassie brilliant blue and processed for fluorography (Bonner and Laskey, 1974). Radioactivity was revealed by autoradiography of the slab gels on KODAK XAR-5 films. Controls included the precipitation of cytosol extracts from non-infected HRT 18 cells and precipitation of BECV polypeptides by monoclonal antibodies to an unrelated virus (Rotavirus).

Virus neutralization

Virus neutralizing activity of monoclonal antibodies was quantitated by a plaque reduction assay. Monoclonal antibodies in MAF or TCF were first precipitated by ammonium sulfate at a final concentration of 50 %, resuspended in PBS and dialyzed during 24 h against a 100 volume of the same buffer. The virus was diluted to contain 100 PFU/0.1 ml and 0.2 ml were mixed with an equal volume of diluted antibody. Virus-antibody mixtures were incubated during 1 hour at 37°C and plated onto HRT 18 cell monolayers in 6 wells costar plates (COSTAR, Cambridge, Mass.). After an adsorption of one hour at 37°C, the supernatant was discarded, monolayers were washed once in RPMI 1640 medium and an agarose overlay was applied as previously described (Vautherot, 1981). Forty to 48 h after infection, plaques were revealed by adsorption of rat red-blood cells (RRBC) (Vautherot, 1981). Neutralization titres were expressed as the reciprocal of the highest dilution giving a 50 % reduction in plaque number (PRD_{50}). Studies on the kinetics of neutralization were performed according to a technique described previously (Volk *et al*, 1982). All MAF were adjusted at an IgG concentration of 0.1 mg/ml assuming an absortivity (1 % 1cm) for mouse Ig of 14 at 280 nm. In some experiments sheep anti mouse IgG, diluted 1/250, was added to the virus antibody mixture after 30 min. at 37°C. This mixture was further incubated during 30 min. at 37°C and then plated on cells as described above.

Haemagglutination inhibition (HAI)

At first, purified BECV was titrated by an haemagglutination assay (HA) using RRBC (Laporte *et al*, 1980). The HA titre of the virus was expressed as the reciprocal of the last dilution which gave a fully agglutination of RRBC. HAI was performed using TCF of MAF preadsorbed with packed RRBC. Monoclonal antibody preparations were diluted in PBS containing 2 mg/ml bovine serum albumin (BSA) and 25 µl of each dilution were mixed with an equal volume of virus suspension containing 4 HA units, in round-bottom microtitration plates. Plates were incubated for 1 hour at 37°C and 50 µl of a 0.8 % RRBC suspension were added in each well. Plates were kept at room temperature for an additional hour. HAI titres were expressed as the reciprocal of the last dilution inhibiting the haemagglutination.

ELISA

Enzyme-linked immunosorbent assays (ELISA) were performed using a modification of a method previously described (Vautherot *et al*, 1981). Wells of microtitration plates (Microelisa, Dynatech) were coated with 0.5 µg of purified BECV in 100 µl of 50 mM Pipes buffer pH = 6.4, 150 mM NaCl. Plates were incubated overnight at 37°C.

Excess viral antigen was removed by 4 washes with PBS containing 0.05 % Tween 20 (PBS-Tween). Unbound sites of the solid phase were saturated with 100 µl/well of a 1 % solution of Gelatin (Biomerieux, France) in 10 mM Tris buffer pH = 7.4 containing 150 mM NaCl. After 1 hour at 37°C, plates were washed 4 times with PBS-Tween and 100 µl of duplicate dilutions of monoclonal antibodies in PBS Tween were added to the wells. Plates were then incubated at 37°C for 2 hours and unbound antibodies were removed by 4 washes in PBS Tween. One hundred microliters of anti-mouse IgG serum conjugated to Horseradish peroxidase were added to each well. Plates were incubated for 2 hours at 37°C and washed 4 times in PBS Tween. Bound conjugated antibodies were detected by the addition of 100 µl of substrate solution, (10 mg of orthophenylene-diamin in 25 ml of 0.035M citrate, 0.066M phosphate buffer pH = 5.0 containing 0.012 % hydrogen peroxide). The colouring reaction was stopped after 12 min. at room temperature by adding 50 µl/well of 5 N H_2SO_4. Optical densities were read in a multichannel photometer (Flow laboratories SA, France).

RESULTS

Characterization of monoclonal antibodies

Twelve hybridomas secreting monoclonal antibodies to BECV strain G110 were established from 271 hybrid colonies (Roseto *et al*, 1982). Polypeptide specificity of the monoclonal antibodies was assayed by two methods a) immunoprecipitation of ^{14}C-labelled viral polypeptides and b) immunoperoxidase staining (IPS) of viral structural polypeptides separated by PAGE (Laporte et Bobulesco, 1981) and transferred on nitrocellulose sheets by transverse electrophoresis (Towbin *et al*, 1979). Five monoclonal antibodies (A_9, A_{20}, B_5, I_7 and J18 - table 1) precipitated GP 105 together with a polypeptide of 150.000 daltons in mol. wt. (Fig. 1). B_5, I_7 and J_{18} also stained GP 105 by IPS whereas A_9 and A_{20} failed to react with SDS denatured structural polypeptides. The three remaining anti GP 105 (C_{13}, F_7 and I_{16} - table) only reacted in IPS and failed to precipitate GP 105 although they precipitated faintly the 150.000 daltons polypeptide (Fig. 1). GP 105, both in the cytosol extract and in the whole virus was found to differ from the major outer glycoprotein GP 125, in that the latter was reduced to GP 65 when 2-M.E. was incorporated in the sample preparation buffer.

Monoclonal antibodies E_5, H_7, H_{19} and I_{12} neither precipitated any of the radiolabelled polypeptides, nor reacted with transferred viral proteins in IPS. Work is in progress to further characterize thes monoclonal antibodies.

In order to correlate biological functions with polypeptides, monoclonal antibodies were studied for their ability to neutralize the virus and to inhibit haemagglutination with BECV G110 (Table 1).

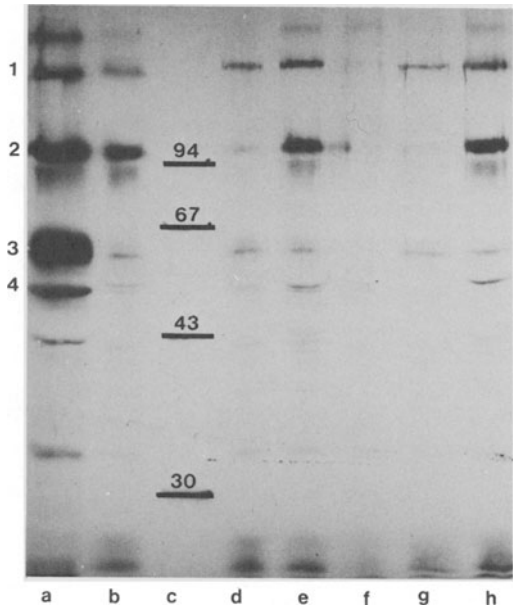

Fig. 1 :
Immunoprecipitation of viral polypeptides with:
a) polyclonal hyperimmune serum precipitating 150 K (1), GP 105 (2), GP 65 (3) and VP 50 (4).
b and h) A_9, d) F_7, e) J_{18}, f) I_{12}, g) I_{16}.

Table 1 : Characterization of monoclonal antibodies to BECV G 110.

Monoclonal antibodies	Isotype	Specificity		Reciprocal titre		
		Immunoprecip. (a)	Immunostaining (b)	IIF	Neutralization	HAI
A_9	IgG_2b	GP105 & 150K	N.R. (c)	32	90	256
A_{20}	IgG_2a	GP105 & 150K	N.R.	128	4×10^3	0
B_5	IgG_2a	GP105 & 150K	GP 105	128	3.2×10^3	0
I_7	IgG_2a	GP105 & 150K	GP 105	32	1×10^3	0
J_{18}	IgG_2a	GP105 & 150K	GP 105	256	5.5×10^3	0
C_{13}	IgG_1	150K	GP 105	64	1×10^2	32
F_7	IgG_1	150K	GP 105	64	< 10	0
I_{16}	IgG_1	150K	GP 105	256	< 10	0
E_5	IgG_{2a}	N.R.	N.R.	128	< 10	0
H_7	IgG_{2a}	N.R.	N.R.	256	< 10	0
H_{19}	IgG_{2a}	N.R.	N.R.	64	< 10	0
I_{12}	IgG_{2a}	N.R.	N.R.	128	< 10	0

(a) *Immunoprecipitation of radiolabelled polypeptides from BECV infected cells.*

(b) *Immunoperoxidase staining of viral structural polypeptides, separated by electrophoresis on polyacrylamide gel and transferred on nitrocellulose sheets by transverse electrophoresis.*

(c) *N.R. = not reacting.*

Five monoclonal antibodies which precipitated GP 105 and 150K (A_9, A_{20}, B_5, I_7 and J_{18}) together with C_{13} (anti GP 105 as revealed by IPS) neutralized the virus. However differences could be observed within this group regarding the persistence of a non-neutralized fraction. Monoclonal I_7 neutralized G110 without leaving any non-neutralized fraction, whereas a 3 % to 10 % non-neutralized fraction was always observed with the other neutralizing monoclonal antibodies. These data were confirmed by studies on the kinetics of neutralization (Fig. 2) and were independent of the protein concentration. The remaining two anti GP 105 monoclonal antibodies (I_{16} and F_7) never displayed any neutralizing activity even when MAF were used instead of TCF. In order to detect a complement dependent neutralization (Collins *et al*, 1982) and to ascertain that the absence of neutralization was not due to low affinity binding of monoclonal antibodies, neutralization assays were performed in the presence of complement and/or sheep anti-mouse IgG (Table 2). The addition of complement had no visible effect on the neutralization by monoclonal antibodies except for A_9 for which a two-fold increase in the neutralization titre was observed. The addition of sheep anti mouse IgG induced a 15-fold increase in the neutralizing titre of monoclonal antibody A_9, together with a reduction from 20 to 5 % of the non-neutralized fraction (Fig. 3).

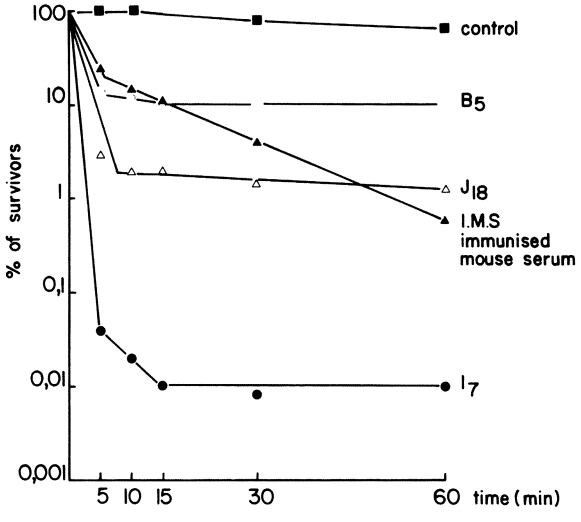

Fig. 2 : Kinetics of neutralization of BECV G 110 by immunized mouse serum (IMS), and monoclonal antibodies B_5, J_{18} and I_7.

Table 2 : Neutralization of BECV G110 by monoclonal antibodies in the presence of anti-mouse IgG and/or complement.

Monoclonal antibodies	Neutralization titre (PRD_{50})			
	Control	Complement[a]	AMIσ [b]	AMIg + complement
A_9	8×10^2	1.8×10^3	1.25×10^4	1.25×10^4
A_{20}	1.5×10^5	1.5×10^5	1.5×10^5	1.5×10^5
B_5	1.3×10^6	1.3×10^6	1.3×10^6	1.3×10^6
I_7	5.5×10^3	N.T.	2.3×10^3	N.T.
J_{18}	2.2×10^5	N.T.	2.2×10^5	N.T.
C_{13}	1.1×10^4	1.1×10^4	1.1×10^4	1.1×10^4
F_7	< 100	N.T.	< 100	N.T.
I_{16}	< 100	< 100	< 100	< 100
E_5	< 100	N.T.	< 100	N.T.
H_7	< 100	N.T.	< 100	N.T.
H_{19}	< 100	N.T.	< 100	N.T.
I_{12}	< 100	< 100	< 100	< 100

(a) Guinea-pig complement

(b) Sheep anti-Mouse IgG (H + L)

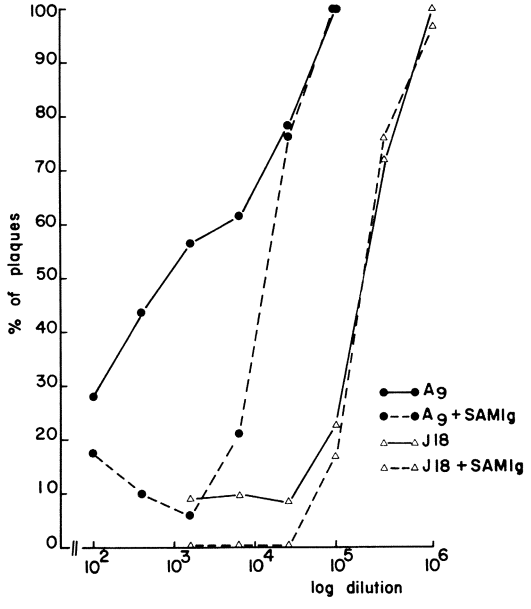

Fig. 3 : Comparative neutralization of BECV G110 by monoclonal antibodies A_9 and J_{18} in the presence or absence of sheep anti-mouse IgG serum (SAMIg).

This reduction of non-neutralized fraction from 10 % to 0 % was a common feature of monoclonal antibodies A_9, A_{20}, B_5, C_{13} and J_{18} when SAMIg was added. However monoclonal I_7, the one which neutralized without leaving any non-neutralized fraction, was never affected in its neutralizing activity by the addition of SAMIg. The addition of anti-immunoglobulins had no effect on non-neutralizing monoclonal antibodies. Together with the results on the kinetics of neutralization, these data suggest that the unneutralized fraction results from an equilibrium between bound and free antibody. Two monoclonal antibodies inhibited the agglutination of RRBC by BECV G110, A_9 and C_{13}, both of which neutralized the virus and were anti-GP 105. Absence of HAI by the other monoclonal antibodies was not due to an inability of the antibodies to bind to intact virions as binding was observed in ELISA assays (data not shown).

Monoclonal antibodies directed against GP 105 have been shown to inhibit both viral multiplication *in vitro*, and haemagglutination. The existence of monoclonal antibodies that outlined different functional domains on outer glycoproteins has been described for naked virus (Burstin *et al*, 1982) as well as enveloped virus (Kida *et al*, 1982). Whether these differences between monoclonal antibodies can be related to differences in affinity remains to be studied.

Cross-reactivity of monoclonal antibodies with coronaviruses of the OC_{43} group and with TGE

BEC, HECV (two isolates of human enteric coronavirus) and a bovine respiratory coronavirus (Thomas *et al*, 1982) could not be distinguished by an indirect immunofluorescence assay (IIF) performed on monolayers of HRT 18 infected with these viruses and stained with monoclonal antibodies (Roseto *et al*, 1982) (table 3). However OC_{43} and HEV, also grown on HRT_{18}, differed from BECV as OC_{43} was recognized by six non-neutralizing monoclonal antibodies (F_7, I_{16}, E_5, H_7, H_{19} and I_{12}) and HEV only by 5 monoclonal antibodies (I_{16}, E_5, H_7, H_{19} and I_{12}) (Table 3). The reactivity of monoclonal antibodies with HEV was not affected when this virus was grown on RPd cells (Laude, 1978). MHV_4-JHM as well as MHV_3 were recognized by 4 monoclonal antibodies (E_5, H_7, H_{19} and I_{12}) in an IIF test performed on acetone fixed Sac^- monolayers (Table 3). Monoclonal antibodies to BECV failed to detect any viral antigen in RPd cells infected with TGE (Purdue strain) (data not shown).

Antigenic comparison of BECV isolates

The reactivity of monoclonal antibodies to G110 was assayed at first by IIF staining of HRT 18 monolayers infected by BECV isolates F_{15} (Laporte *et al*, 1979), NCDCV (Mebus *et al*, 1973), british and danish (Bridger *et al*, 1978). No differences could be detected between G110 and F15 (Table 4). However monoclonal anti-

Table 3 : Cross-reactivity of monoclonal antibodies to BECV with coronaviruses of the OC 43 group.

Monoclonal antibodies	BECV G110	B. RCV (a)	HECV (b)	OC_{43} (c)	HEV (d)	MHV_4 (e)
A_9	+	+	+	-	-	-
A_{20}	+	+	+	-	-	-
B_5	+	+	+	-	-	-
I_7	+	+	+	-	-	-
J_{18}	+	+	+	-	-	-
C_{13}	+	+	+	-	-	-
F_7	+	+			-	-
I_{16}	+	+			+	-
E_5	+	+	+		+	+
H_7	+	+	+	+	+	+
H_{19}	+	+	+	+	+	+
I_{12}	+	+	+	+	+	+

(a) Bovine Respiratory Coronavirus (tested on HRT cells)
(b) Human Enteric Coronavirus (tested on HRT cells)
(c) Human Respiratory Coronavirus (tested on HRT cells)
(d) Hemagglutinating Encephalitis Coronavirus (tested on HRT and RPd cells)
(e) Mouse Hepatitis Virus strain JHM (tested on SAC^- cells)

body A_9 never stained NCDCV infected cells (HRT_{18} or PFBK) ; similarly F_7 failed to react with the third passage on HRT 18 of the british and danish BECV isolates (Table 4).

Neutralization and HAI were used to further investigate the cross-reactivity of monoclonal antibodies with G110, F_{15} and NCDCV. Both assays confirmed the similarities between G110 and F15 (data not shown) and that A_9 never reacted with NCDCV (Table 5). No other difference could be observed between NCDCV and G 110 (Table 5).

The absence of binding of F_7 to the 4th passage of british BECV isolate was also confirmed by an ELISA test (data not shown) using purified virus as antigen. Comparison of the two viruses (british and danish isolates) by neutralization did not reveal any noticeable difference (Table 6). As during the first two passages of the british isolate on HRT 18, monoclonal antibody F_7 stained a few infected cells, the virus was cloned in order to investigate if different viral populations could be isolated from the original inoculum. Plaque purification yielded 7 clones, 2 of which reacted with F_7 whereas the remaining 5 were negative. Work is in progress to investigate the stability of these viral clones.

The absence of reactivity of monoclonal antibody A_9 with NCDCV and F_7 with the british and danish BECV isolates were the most obvious differences detectable among the BECV isolates tested.

Table 4 : Cross-reactivity of monoclonal antibodies with BECV isolates (IIF staining).

Monoclonal antibodies	G110 and F_{15}	NCDCV	B.BECV and D.BECV
A_9	+	-	+
A_{20}	+	+	+
B_5	+	+	+
I_7	+	+	+
J_{18}	+	+	+
C_{13}	+	+	+
F_7	+	+	-
I_{16}	+	+	+
E_5	+	+	+
H_7	+	+	+
H_{19}	+	+	+
I_{12}	+	+	+

Table 5 : Reactivity of monoclonal antibodies with NCDCV.

Monoclonal antibodies	Neutralisation titre (PRD_{50})		HAI		ELISA	
	G110	NCDCV	G110	NCDCV	G110	NCDCV
A_9	90	0	256	0	2100 *4 x 10^5* ⁂	0 *0* ⁂
A_{20}	4×10^3	4×10^3	< 2	< 2	1.4×10^3	1.2×10^3
B_5	3.2×10^3	1×10^4	< 2	< 2	2×10^3	1.5×10^3
I_7	1×10^3	1×10^3	< 2	< 2	3×10^3	3.5×10^3
J_{18}	5.5×10^3	4×10^3	< 2	< 2	1.3×10^4	2×10^4
C_{13}	1×10^2	1×10^2	32	32	1×10^3	2×10^3
F_7	< 10	< 10	< 2	< 2	8×10^2	2.5×10^3
I_{16}	< 10	< 10	< 2	< 2	2.8×10^3	3×10^3
E_5	< 10	< 10	< 2	< 2	8×10^3	9.5×10^3
H_7	< 10	< 10	< 2	< 2	5×10^3	5×10^3
H_{19}	< 10	< 10	< 2	< 2	1.8×10^3	2.5×10^3
I_{12}	< 10	< 10	< 2	< 2	3×10^3	2.5×10^3

⁂ Mouse ascitic fluid.

Table 6 : Neutralization of british and danish BECV isolates by monoclonal antibodies.

Monoclonal antibodies	G 110	B.BECV	D.BECV
A_9	1.4×10^4 (a)	7.5×10^3 (a)	5.8×10^3 (a)
A_{20}	8.5×10^2	1.4×10^3	1.8×10^3
B_5	4.8×10^3	2.1×10^3	3.8×10^3
I_7	$> 8 \times 10^5$	$> 8 \times 10^5$	5×10^5
J_{18}	1.7×10^5	1.5×10^5	1.6×10^5
C_{13}	7×10^3 (a)	2.1×10^4 (a)	1.8×10^4 (a)
F_7	< 20	< 20	< 20
I_{16}	< 10	< 10	< 10
E_5	< 10	< 10	< 10
H_7	< 10	< 10	< 10
H_{19}	< 10	< 10	< 10
I_{12}	< 20	< 20	< 10

(a) *Neutralization tests were performed in the presence of sheep anti-mouse IgG (H + L)*

CONCLUSION

Monoclonal antibodies directed against GP 105 also precipitated a 150 Kdaltons polypeptide. Whether this 150 Kpolypeptide is a glycosylated precursor to GP 105, as described for MHV_4 (Siddell, 1982) remains to be shown.

Anti GP 105 monoclonal antibodies displayed neutralizing and haemagglutination inhibition activities. Neutralization by anti GP 105 was comparable to that described for monoclonal antibodies anti MHV_4-GP1 (Collins *et al*, 1982). The presence of a non-neutralized fraction, presumably resulting from an equilibrium between free and bound antibody, was reported for other monoclonal antibodies (Volk *et al*, 1982). The addition of anti mouse IgG to neutralizing monoclonal antibodies to BECV consistently decreased this non - neutralized fraction from 10 % to 0 %.

Detailed results on the biological activity of monoclonal antibodies to the other outer glycoproteins of BECV are not yet available. However this report shows that GP 105, a minor component of the peplomers, is involved in virus to cell interactions as well as in viral-induced haemagglutination. Previous publications described an HA activity of BECV (King and Brian, 1982) and HEV (Pocock, 1978) associated with GP65/125 as shown by bromelain treatment and sensitivity to reducing agents. Two anti-GP 105 displayed HAI activity showing that either GP 105 is closely asso-

ciated with the haemagglutinin, or that GP 105 itself carries a part, if not all, of the viral receptor for RRBC.

Antibodies against GP 105 defined at least two functional domains, one outlined by neutralizing monoclonal antibodies (A_{20}, B_5, I_7, J_{18}) and the other one being recognized by monoclonal antibodies with neutralizing and HAI activity (A_9 and C_{13}).

The reactivity of monoclonal antibodies to BECV with other coronaviruses showed that monoclonal antibodies E_5, H_7, H_{19} and I_{12} outlined antigenic determinant(s) common to MHV, OC_{43}, HEV and BECV. However OC_{43} and HEV were also recognized by monoclonal antibodies I_{16} and F_7 which both are directed against the structural GP 105.

A bovine respiratory coronavirus (Thomas *et al*, 1982) was found to be similar to BECV G110 in its reactivity with monoclonal antibodies. Experimental studies on the compared pathogenicity of this virus and BECV confirmed this similarity (Reynolds, 1982).

HECV, isolated from diarrhoeic babies (Bobulesco, 1983) and from young adults (J. Laporte, unpublished results), did not differ from BECV regarding their reactivity with monoclonal antibodies. Storz *et al* (1981) reported an accidental contamination of man by BECV and Patel *et al*, (1982) succeeded in infecting gnotobiotic calves with an HECV isolate. Whether BECV and HECV are different is not yet resolved but cross contamination was clearly demonstrated. HECV can easily be differentiated from OC_{43} and is more closely related to BECV than to human respiratory coronavirus.

Antigenic analysis of BECV isolates revealed minor changes on GP 105. The US isolate (NCDCV) failed to react with A_9 and the british isolate was found to contain at least two viral populations which could be selected by plaque purification. These antigenic changes were the only ones which could be detected as all the other monoclonal antibodies reacted with the different isolates in a very similar manner.

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