A COMPARISON OF BOVINE CORONAVIRUS STRAINS USING MONOCLONAL ANTIBODIES

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ABSTRACT

Eight monoclonal antibodies (MAbs) were raised against a bovine coronavirus (B.C.V.) which had been isolated in Scotland and was designated S2. The MAbs were divided into two groups on the basis of their reactions with S2 virus in indirect immunofluorescence (I.F.), neutralisation and haemagglutination inhibition (H.A.I.) tests. Five of the MAbs were positive by all three tests but failed to bind to proteins in Western immunoblotting experiments. The remaining three MAbs were positive in I.F. tests only, two of which were shown to bind to the 52K nucleocapsid protein by Western immunoblotting. Different patterns of antigen distribution within infected cells were demonstrated when the MAbs were used in the I.F. test. However only minor strain variations were detected by I.F. and H.A.I. tests when the MAbs were tested against each of five cell culture adapted strains of B.C.V. Twenty-nine isolates of B.C.V. have been grown in neonatal calf tracheal organ cultures: attempts are being made to further characterise these isolates.

INTRODUCTION

The structural proteins of B.C.V. have been well defined.^{1, 2} Four proteins are described: the internal nucleocapsid protein N, the matrix glycoproteins E1, the peplomer glycoproteins E2 and the haemagglutinin glycoprotein E3. Monoclonal antibodies to both E2 and E3 have been found to neutralise the virus in vitro³ and in vivo⁴. The E3 protein is responsible for the ability of B.C.V. to haemagglutinate red blood cells (R.B.C.'s).^{5, 6}

Only minor strain variations have been found between different isolates of B.C.V. using both polyclonal antisera and MAbs.^{7, 8, 9} The number of isolates available for such studies is limited because of the difficulties involved in the adaptation of B.C.V. to growth in cell culture systems. However organ cultures of foetal and neonatal calf tracheas have been used successfully as an alternative method for the primary isolation and growth of B.C.V.^{10, 11, 12}

This report describes the characterisation of eight MAbs raised to the Scottish S2 isolate of B.C.V. and the use of these MAbs in the

identification of variations between five cell culture adapted isolates of B.C.V. A further four MAbs raised against a different isolate of B.C.V. were kindly supplied by the Central Veterinary Laboratory (C.V.L.) at Weybridge.

Twenty-nine isolates of B.C.V. from faecal samples were grown in neonatal calf tracheal organ cultures.

MATERIALS and METHODS

Viruses

Human rectal tumour cells, HRT-18, were used to culture B.C.V. The Scottish isolates of B.C.V., S1 and S2, had been isolated in tracheal organ culture and adapted to grow in HRT-18 cells.¹³ Three reference strains i.e. M,¹⁴ PQ¹⁵ and CK¹⁶ were also used.

Production of the monoclonal antibodies

MAbs to B.C.V. S2 isolate were produced from Balb-C mice essentially following a procedure described by Deregts et al.² Hybridoma cells were screened for antibody production by indirect I.F., neutralisation and H.A.I. tests. Antibody producing cells were terminally diluted three times before inoculation into the peritoneal cavity of pristane (2, 6, 10, 14-tetramethylpentadecane) primed mice. Ascitic fluid was collected, pooled, aliquoted and stored at -70°C.

Western immunoblotting

S2 viral proteins were separated by P.A.G.E. employing a vertical discontinuous gel system with a 3% stacking and a 10% resolving gel. The proteins were electroblotted overnight on to nitrocellulose membranes. The membranes were cut into strips and incubated for one hour at room temperature with a blocking solution of PBS/0.5% Tween 20. Bound viral proteins were detected by incubation with ascitic fluid diluted at 1:40 in PBS/T followed by Protein A labelled with I¹²⁵. The strips were then processed for autoradiography and incubated for 9 days at -70°C before developing.

Tracheal organ culture/Faecal Samples

Tracheal organ cultures were derived from neonatal Jersey bull calves.¹⁷ The organ cultures were set up as described by Thomas and Howard,¹⁷ except that each tracheal ring was placed in an individual petri dish.

Faecal samples from diarrhoeic calves were prepared as 10% suspensions in PBS and tested for B.C.V. in an indirect ELISA. BCV positive samples were inoculated on to tracheal organ cultures using a method similar to that described by Stott et al.¹⁰. Media was harvested twice a week and monitored for H.A. activity.

RESULTS

Production of hybridomas

Eight hybridoma cell lines secreting antibodies to B.C.V. detectable by at least one of the 3 screening tests were successfully cloned from two fusions.

The MAbs could be divided into two groups based on the results of these tests. In one group the MAbs (S2/1, S2/2, S2/3, S2/4 and S2/7) were positive by all 3 tests. In the other group the remaining 3 MAbs (S2/5, S2/6 and S2/8) were positive by I.F. only (Table 1).

Table 1. Characterisation of the MAbs

<u>MAB</u>	<u>Indirect I.F.*¹</u>	<u>Neutralisation*¹</u>	<u>H.A.I.*¹</u>	<u>Protein specificity*²</u>
S2/1	+	+	+	N.R.
S2/2	+	+	+	N.R.
S2/3	+	+	+	N.R.
S2/4	+	+	+	N.R.
S2/5	+	-	-	52K protein
S2/6	+	-	-	N.R.
S2/7	+	+	+	N.R.
S2/8	+	-	-	52K protein

*¹as determined with S2 virus

*²protein specificity determined by Western immunoblotting

N.R. signifies there was no reaction of the MAb with nitrocellulose bound viral proteins

Patterns of immunofluorescence

Different patterns of fluorescence were observed when the MAbs were reacted with S2 virus infected HRT-18 cells at 20 hrs post infection. The MAbs positive by all 3 of the screening tests gave a mainly perinuclear pattern of I.F. The MAbs positive by I.F. only gave a more even distribution of fluorescence throughout the cell cytoplasm. Distinctive large granules were seen with MAb S2/4. On membrane fluorescence tests, a clear bright membrane fluorescence was observed when the MAbs positive by all 3 of the screening tests were used. The MAbs positive by I.F. only were negative.

Isotype/Western immunoblotting

The immunoglobulins secreted by the hybridoma cell lines all belonged to the IgG class of antibody. Only 2 of the MAbs bound to blotted proteins (Table 1). These were MAbs S2/5 and S2/8 which both bound to a protein with a molecular weight of about 52 K (Fig. 1). This value agrees closely with that reported by other workers for the nucleocapsid N protein.^{1, 2}

Comparison of different strains of B.C.V.

Using the 8 MAbs directed against S2 virus, differences were shown by I.F. using S2/5 MAb only and in H.A.I. by S2/2 MAb (Table 2). No differences were demonstrated with the 4 MAbs supplied by C.V.L.

Isolation of B.C.V. from faecal samples in tracheal organ culture

H.A. titres obtained from consecutive harvests from a series of tracheal organ cultures are shown in Table 3.

Table 2. Differences detected by indirect I.F. and H.A.I.

<u>Virus</u>	TEST	
	<u>Indirect I.F.*¹</u> <u>S2/5 MAb</u>	<u>H.A.I.*²</u> <u>S2/2 MAb</u>
S1	<100	1280
S2	9051	320
CK	<100	<20
M	<100	<20
PQ	<100	<20

*¹ Titres given are the reciprocal of the highest dilution of MAb ascitic fluid giving clear fluorescence with virus infected cells

*² Titres given are the reciprocal of the highest dilution of ascitic fluid causing complete inhibition of haemagglutination by 8 H.A. units of virus.

Fig. 1. Autoradiograph of Western immunoblot.

The reaction of MAbs S2/5 and S2/8 with a 52K molecular weight protein may be seen in lanes 5, 19 and 8, 20 respectively.

Table 3. H.A. titres of B.C.V. in tracheal organ culture

<u>Faeces</u>	<u>Harvest No</u>				
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>
L3317	-	-	-	-	-
L3032	16	≥128	≥128	≥128	8
L2759	-	-	-	-	-
N339	≥128	≥128	32	32	16
K2472	-	-	-	-	-
N164	-	-	-	-	-
L1096	-	64	≥128	≥128	32
L1121	16	≥128	64	64	16
Negative control	-	-	-	-	-

H.A. titres are the reciprocal dilutions

Out of a total of 60 faecal samples inoculated onto tracheal organ cultures, 29 have grown to give H.A. titres of at least 32. All isolates reacted positively when tested in an indirect ELISA for the detection of B.C.V. antigen.

DISCUSSION

Eight MABs were raised against the S2 isolate of B.C.V. These were divided into two groups on the basis of their reactions with S2 virus in I.F., neutralisation and H.A.I. tests. Five of the MABs gave positive reactions in all 3 of these tests whilst the remaining 3 were positive on I.F. tests only. Since the E3 protein has been confirmed as the viral haemagglutinin^{5, 6} it is reasonable to suggest that the MABs positive in H.A.I. tests are directed against this protein. Confirmation of this has yet to be obtained.

It was demonstrated in Western immunoblotting that 2 of the MABs which were I.F. positive only were directed against the nucleocapsid N protein. This is consistent with the negative results obtained in neutralisation and H.A.I. tests with these MABs.

Using MABs in immunofluorescence tests distinct patterns of B.C.V. antigen distribution within infected HRT-18 cells were defined. The putative anti E3 MABs gave a mainly perinuclear pattern of fluorescence and showed positive membrane fluorescence. The anti-N MABs gave a more even distribution of cytoplasmic fluorescence and were membrane fluorescence negative. Similar patterns have been described for murine hepatitis virus (M.H.V.)¹⁸, transmissible gastroenteritis virus of swine (T.G.E.V.)¹⁹ and turkey enteric coronavirus (T.C.V.).²⁰

Only minor variations between the 5 cell culture adapted isolates of B.C.V. were demonstrated using MABs. In order to gain a more complete understanding of the possible antigenic variations between B.C.V. strains it is desirable to test a larger number of isolates. The use of neonatal calf tracheal organ cultures has proved a useful method for the primary isolation of virus from faecal samples and should provide a sufficient number of isolates to allow a more thorough investigation of antigenic variation to be made.

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