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Antigenic variation of the bovine ephemeral fever virus glycoprotein

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Summary. Glycoprotein-specific monoclonal antibodies (MAbs) were used to select escape mutants of bovine ephemeral fever (BEF) virus to determine the escape frequency for different epitopes and to construct an epitope map. At least six antigenic sites were detected by this method and escape frequencies between 10^{-2} and 10^{-8} were recorded. One new non-conformational site was defined by a MAb, 5A5, which neutralized Berrimah and Kimberley viruses as well as three BEF virus strains. Batch to batch variation was detected in the BB7721 strain of BEF virus when tested for MAb neutralization. Eighteen strains of BEF virus, isolated from blood and insects from a variety of locations in Australia over a period of 33 years, were examined using MAbs and at least one epitope could not be detected in strains isolated since 1975. Implications for vaccine development are discussed.

Introduction

Bovine ephemeral fever (BEF) virus is an arthropod-borne rhabdovirus provisionally placed in the genus *Lyssavirus* [1] and causing a febrile disease in cattle and water buffalo. Five structural proteins of BEF virus have been described recently [21] although monoclonal antibody (MAb) studies of the prototype virus indicate that only the N-glycosylated G protein stimulates the production of neutralizing antibodies [5]. Competition binding assay using MAbs have provided evidence for at least three distinct neutralizing antigenic sites on the BEF virus G protein (BB7721 strain) including one non-conformational site [5]. MAb analysis of rabies virus (the prototype of the genus *Lyssavirus*) isolated from various animal species and from different geographic locations has revealed considerable antigenic diversity [7] and this has important consequences for vaccine development.

In this study MAb-resistant variants of BEF virus were selected and used

to determine neutralization escape frequencies for different epitopes and to produce a map of neutralizing antigenic sites. MAbs from three antigenic sites were then used to examine antigenic variation in different batches of virus and in a number of strains of BEF virus which were isolated from blood and insects from a variety of locations in Australia over a period of 33 years.

Materials and methods

Viruses and cells

Viruses were propagated in baby hamster kidney (BHK21) cells and plaque assays were conducted in Vero cells.

The BB7721 prototype strain of BEF virus [8] was used to produce MAbs and MAb-resistant variants. Three batches of BEF virus were investigated for antigenic variation. Batch 1 had received six passages in suckling mouse brain, 34 passages in BHK21 cells, two passages in Vero cells and was plaque cloned three times in Vero cells. Batch 2 received the same number of passages as batch 1 but was plaque cloned separately. Batch 3 received six passages in mouse brain, 25 passages in BHK21 cells and was cloned three times in Vero cells by limiting dilution. It was not plaque cloned. The 17 other strains of BEF virus investigated were obtained from the virus collection of the CSIRO Long Pocket Laboratories, Indooroopilly, Australia. The details of each virus are shown in Table 1. All viruses

Table 1. Bovine ephemeral fever virus isolates listed by strain, passage level, origin, place and year of collection

Virus strain	Passage level[a]	Origin	Place of collection[b]	Year of collection
V 1956	BHK4.SVP4	Cattle	Sydney, N.S.W.	1956
BB 7721	SMB6.BHK25.V3	Cattle	Charters Towers, Q.	1968
CSIRO 1865	SMB3	Cattle	Charters Towers, Q.	1968
CSIRO 659	SMB5	Cattle	Etna Creek, Q.	1970
CSIRO 1818	SMB3.C1.BHK2	Cattle	Upper Barron, Q.	1970
CSIRO 42	SMB4	*An. bancrofti*	Beatrice Hill, N.T.	1975
CSIRO 1866	SMB3	Cattle	Kairi, Q.	1975
CSIRO 1820	SMB3.C1.BHK2	Cattle	Peachester, Q.	1976
CSIRO 366	BHK5	Cattle	Peachester, Q.	1980
DPP 54	BHK3	Cattle	Adelaide River, N.T.	1981
CSIRO 967	SMB4	Cattle	Kairi, Q.	1981
CSIRO 1180	C3.BHK2	Cattle	Peachester, Q.	1982
CSIRO 1619	C3.BHK2	Cattle	Peachester, Q.	1984
CSIRO 1622	BHK2	*An. bancrofti*	Peachester, Q.	1984
CSIRO 1647	AEG1.BHK3	*C. brevitarsis*	Peachester, Q.	1984
CSIRO 1907	C1	Cattle	Samford, Q.	1989
CSIRO 1913	C1	Cattle	Oakey, Q.	1989
CSIRO 1922	C1	Cattle	Cunnamulla, Q.	1989

[a] *BHK* Baby hamster kidney 21 cells, *SMB* suckling mouse brain, *C Aedes albopictus* C6/36 cells, *SVP* super Vero porcine, *AEG Aedes aegyptii, V* Vero

[b] *Q* Queensland, *N.S.W.* New South Wales, *N.T.* Northern Territory

were passaged once in *Aedes albopictus* C6/36 mosquito cell line after the passage level shown in Table 1 and used as C6/36 cell culture supernatant in all assays.

Other BEF related viruses used were the DPP 63 strain of Berrimah (BER), the CSIRO 368 strain of Kimberely (KIM) and the DPP 61 strain of Adelaide River (AR) viruses and these have been described previously [2].

Monoclonal antibodies

Thirteen of the neutralizing MAbs against the BEF virus glycoprotein, produced by inoculating mice with BEF virus, have been described previously [5]. One additional MAb against the BEF G protein, 5A5, was produced as a by-product of inoculating mice with purified BEF virus nucleocapsid protein for the purpose of producing MAbs to the N protein as described by Cybinski et al. [5]. This antigen was obtained by removing the bulk of the G protein from purified BEF virus antigen as described by Walker et al. [21]. Fusion, selection of MAbs and the production, purification, concentration and biotinylation of ascites fluid, was carried out as described by Cybinski et al. [5]. Immunoblotting was conducted as described by Walker et al. [21].

Selection of MAb resistant variants

Thirteen monoclonal antibodies capable of neutralizing the BB7721 strain of BEF virus were used for selection of variants by a previously described method [12]. Serial 10-fold dilutions of the BB7721 strain of BEF virus were prepared, mixed with ascitic fluid containing MAb and diluted to approximately 10 times the dilution required to neutralize 100 50% tissue culture infective doses ($TCID_{50}$) of virus. Ascitic fluids with titres less than 100 were diluted 1 in 10. After 1 h incubation at room temperature (RT), 0.2 ml of virus/antibody mixture was added to monolayers of Vero cells grown in 55 × 12 mm petri dishes (Disposable Products, Australia). Following adsorption for 1 h at 37 °C, 5 ml of Leibovitz 15 (L15) (Flow Laboratories, USA), nutrient agar overlay containing the same concentration of MAb as used previously was added. Plates were incubated at 37 °C until plaques were clearly visible and could be counted (4 to 5 days). Well separated plaques, representing possible escape mutants, were picked from the highest dilution showing plaques and were transferred to monolayers of BHK21 cells in T25 (Falcon Plastic, USA) tissue culture flasks in 5 ml of growth medium. When cytopathic effect was evident, the supernatants were stored in liquid nitrogen vapour and tested for neutralization by MAbs. Variants which escaped neutralization were identified numerically according to the MAb used for selection and then alphabetically if more than one reactivity pattern was observed.

The approximate escape frequency was calculated by the following formula:

$$\frac{\text{Plaque forming units per ml in the presence of MAb}}{\text{Plaque forming units per ml in the absence of MAb}}.$$

Virus neutralization tests

Neutralization tests on the BEF virus (BB7721) escape mutants were conducted essentially as described previously [4] using two-fold dilutions of ascitic fluid in microtitre plates and adding an equal volume of diluent containing 100 $TCID_{50}$ of test virus, incubating for 1 h at RT, then adding Vero cells and covering with paraffin oil. Neutralizing titres of BEF variants were read after 4 to 5 days incubation at 37 °C and compared with the titres obtained for the parent virus.

Neutralization tests on other strains of BEF virus, some of which did not produce cytopathic effect in tissue culture, were conducted by a modified neutralization/immunofluorescence technique. MAb or polyclonal antiserum was diluted 1/4 and dispensed in

duplicate into 96 well tissue culture plates. An equal volume of diluent containing 10 $TCID_{50}$ of virus was added. Control wells containing virus only were included for each strain. After incubation at RT for 1 h, medium containing 0.5×10^6 C6/36 cells was added to each well. The plates were sealed and incubated at 28 °C for 6 days. Presence or absence of neutralization was detected by an indirect immunofluorescence assay as described by Cybinski and Zakrzewski [6]. The optimal dilution for the antiserum and conjugate was predetermined by titration against BEF virus (BB7721)-infected C6/36 cell culture supernatant.

ELISA tests

Competitive binding assay were carried out as described by Cybinski et al. [5]. The rationale for the capture ELISA (cELISA) was to capture virus with a MAb directed against a specific antigenic site and then to detect captured virus with a biotinylated MAb specific for a different antigenic site. MicroELISA plates (Dynatech, Federal Republic of Germany) were coated with purified MAb immunoglobulin in carbonate buffer pH 9.6 for 2 h at RT. Virus supernatant diluted at 1/4 was added and incubated overnight at 4 °C. Biotinylated MAb was then added and incubated for 1 h at RT, followed by biotinylated streptavidin horseradish peroxidase complex (Amersham, UK) for 1 h at RT. Between each incubation the wells were washed three times with PBS containing 0.05% Tween 20. Colour was developed by adding 5-aminosalicyclic acid [9] containing 0.02% hydrogen peroxide. After 30 min incubation at RT, the absorbance was measured in an ELISA reader (Titertek Multiskan II) using a 492 nm filter. The cELISA values are expressed as Optical Density at 492 nm ($\times 10^3$). The optimal concentration of purified MAb, biotinylated MAb and complex was predetermined against BEF virus (BB7721)-infected BHK21 cell culture supernatant.

Results

Characterisation of a novel neutralizing MAb

A neutralizing MAb, designated 5A5, was obtained from a fusion using purified, Triton X-100 treated BEF virus as inoculum. This MAb was shown to neutralize three strains of BEF virus as well as the BEF related viruses, BER and KIM. Neutralizing titres and IFA reactions are shown in Table 2. The MAb 5A5 reacted with the BEF G protein in immunoblot assays in the presence of β-mercapto-ethanol, indicating that this was a non-conformational site. The immunoglobulin heavy chain and light chain were IgG1 and κ respectively. Binding of biotinylated 5A5 to BEF antigen was not inhibited by any of the other 13 MAbs in a competitive binding ELISA (Fig. 1). Conversely, the 13 biotinylated MAbs were not inhibited by 5A5 in reciprocal assays (data not shown).

Escape frequency

Between one and 10 plaques were picked from the plate containing the fewest plaques (i.e. the highest dilution showing plaques) and these were tested for

Fig. 1. Competitive binding assay using a constant amount of biotinylated antibody and varying concentrations of competing antibodies. **A** MAbs 5A5 (○), 12A5 (●), 11D1 (□), 8B6 (■), 16A6 (△) and DB5 (▲) competing with biotinylated 5A5. **B** MAbs 5A5 (○), 13A3 (●), 17B1 (□), 15B5 (■) and 3D6 (△) competing with biotinylated 5A5. **C** MAbs 5A5 (○), 13C6 (●), 9C5 (□), 1C6 (■) and 8D3 (△) competing with biotinylated 5A5

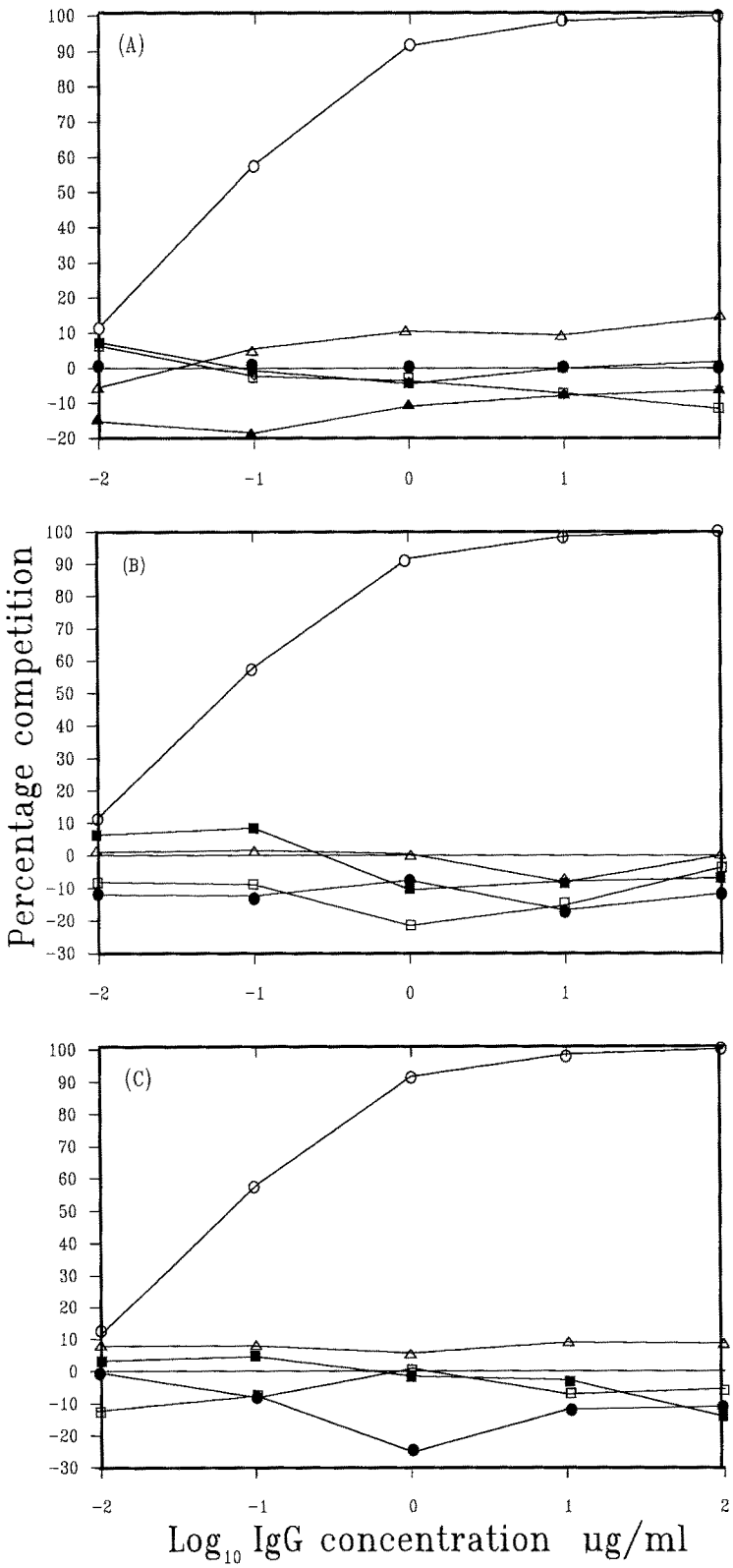
(A)
(B)
(C)
Percentage competition
Log10 IgG concentration ug/ml

Table 2. Neutralizing titres and immunofluorescence reactions of viruses with monoclonal antibody 5A5

Virus	Neutralizing titre[a]	Immunofluorescence reaction
BEF (BB7721)	512	+
BEF (CSIRO 42)	32	+
BEF (CSIRO 53)	128	+
BER (DPP 63)	16	+
KIM (CSIRO 368)	4096	+
AR (DPP 61)	<2	−

BEF Bovine ephemeral fever virus, *KIM* Kimberley virus, *AR* Adelaide River virus, *BER* Berrimah virus

[a] Titres expressed as the reciprocal of the dilution required to neutralize 100 $TCID_{50}$ of virus

Table 3. Frequency with which bovine ephemeral fever virus (BB7721) escaped neutralization by monoclonal antibodies

Selecting MAb	Escape frequency
DB5	10^{-4}
13A3	5×10^{-5}
13C6	6×10^{-3}
17B1	2×10^{-5}
9C5	4×10^{-5}
1C6	3×10^{-5}
15B5	10^{-4}
12A5	5×10^{-5}
16A6	5×10^{-7}
8D3	10^{-5}
3D6	$2 \times 10^{-3}/10^{-5}$ [a]
8B6	5×10^{-8}
5A5	10^{-3}

[a] Small plaques/large plaques

resistance to neutralization by the selecting MAb. The average excape frequency calculated for each MAb is shown in Table 3. The values were mostly around 10^{-5} or less, with the exception of 13C6, 3D6 and 5A5, for which higher frequencies were obtained. In contrast, escape frequencies for 16A6 and 8B6 were lower than average. The MAb 3D6 selected two distinct plaque types (small and large), which showed two different escape frequencies (10^{-3} and 10^{-5} respectively).

BEF virus batch variation

Neutralizing titres for the 14 MAbs used in this study against the three batches of BEF virus (BB7721) are listed in Table 4. Most MAbs gave the same titre with each batch of virus. However, batch 1 contained a proportion of virus which was partially resistant to the MAb 13C6 as indicated by unexpected virus breakthrough at low dilutions. Batches 2 and 3 were partially resistant to the MAb 5A5, while batch 2 was also partially resistant to the MAb 3D6.

Epitope mapping

A total of 43 variants of BEF virus (BB7721), which escaped neutralization by the selecting MAb, were obtained and these were tested against the other 13 MAbs. To determine whether a variant was resistant or not, neutralizing titres were compared against those of the parent virus. A virus was considered to be resistant to neutralization only if the neutralizing titre was < 2. Variants showing only partial resistance to the selecting MAb were excluded from the study. However, in three cases, there was a greater than 10-fold reduction in titre of a variant by MAbs not used for their selection and this was recorded as partial resistance.

Patterns of neutralization and resistance to neutralization, shown in Fig. 2,

Table 4. Neutralization of bovine ephemeral fever virus batches with monoclonal antibodies

Monoclonal antibody	Neutralizing titre		
	BEF virus batch number		
	1	2	3
DB5	256[a]	256	256
13A3	1024	1024	1024
13C6	16	4096	4096
17B1	>8192	>8192	>8192
9C5	256	256	256
1C6	256	256	256
15B5	256	256	256
12A5	1024	1024	1024
11D1	8	8	8
16A6	128	128	128
8D3	256	256	256
3D6	512	16	512
8B6	64	64	64
5A5	256	16	16

[a] Titres expressed as the reciprocal of the dilution required to neutralize 100 $TCID_{50}$ of virus

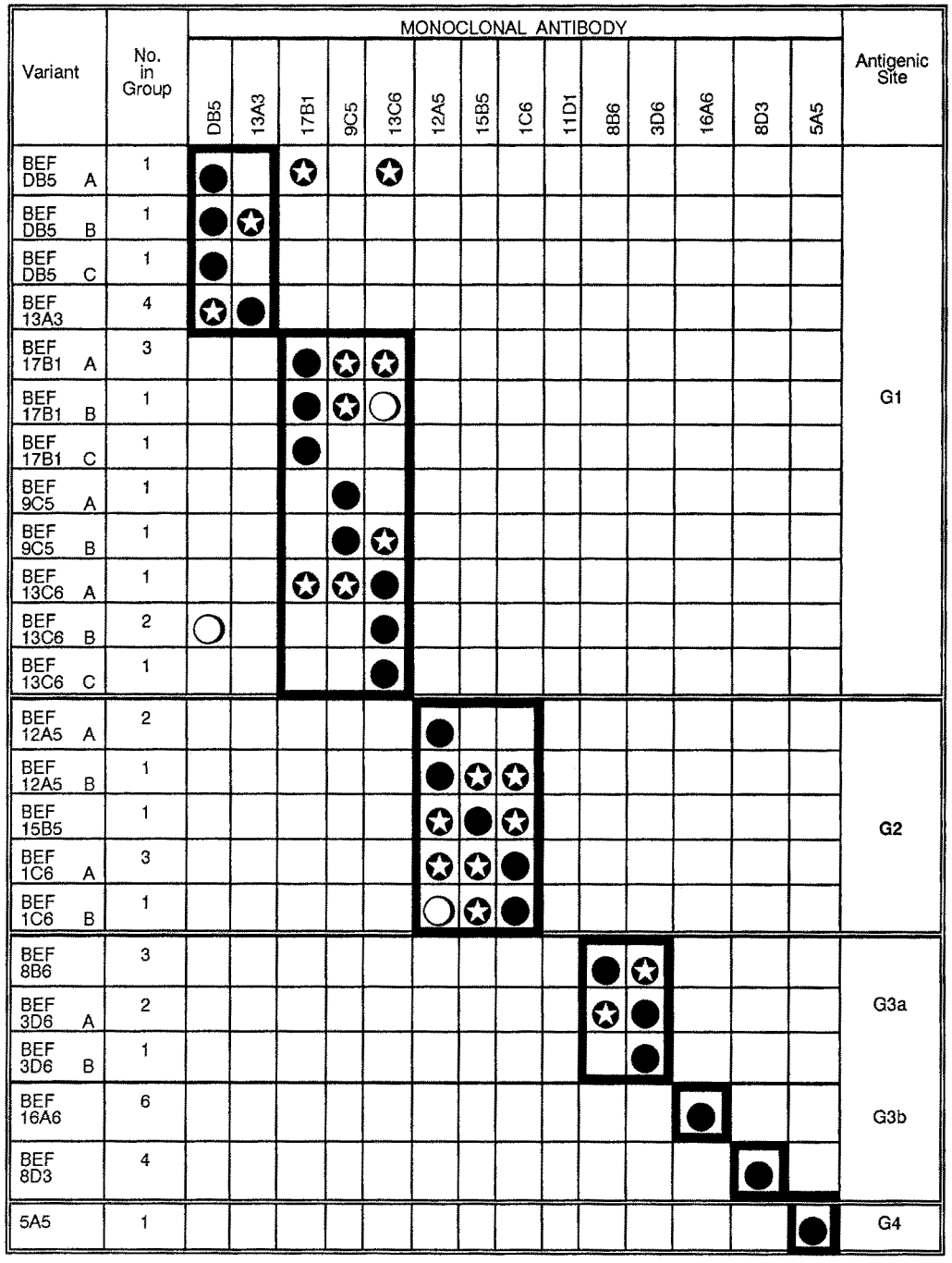

Fig. 2. Antigenic map of the BB7721 strain of bovine ephemeral fever virus. Neutralization resistant variants were selected using monoclonal antibodies (MAbs), then tested for susceptibility (□) or resistance to neutralization by the MAb used in selection of the variant (●) and by another MAb in the panel (✪). ○ Partial resistance. Labelling of antigenic sites as G1, G2, G3a, G3b is taken from Cybinski et al. [5]. Antigenic site G4 is a new site detected by competitive binding assay and by MAb resistance

allowed the variants to be grouped into at least six clusters representing viruses with mutations on the same antigenic site. No attempt was made to produce variants with the MAb 11D1, as the neutralizing titre was too low and this MAb may represent an additonal site.

BEF virus strain variation

The results of neutralization tests on 18 strains of BEF virus are shown in Table 5. All strains were neutralized by polyclonal mouse antiserum, MAb 17B1 and MAb 12A5. MAb 3D6 neutralized only five strains, V1956, BB7721, CSIRO 1865, CSIRO 1818 and CSIRO 42 all of which were isolated in or before 1975.

Capture ELISA

The results of the cELISA are shown in Table 6. The combination of MAbs 17B1/12A5 (capture antibody/detecting antibody) resulted in high absorbance values ranging from 106 to 505 $\times 10^{-3}$ for all 18 strains. The combination of 17B1/3D6 and 1C6/3D6 gave absorbance values from 12 to 264 for six strains,

Table 5. Neutralization results for bovine ephemeral fever virus strains with glycoprotein specific monoclonal antibodies

Virus strain	Detecting antibody				
	17B1 (G1)[a]	12A5 (G2)	3D6 (G3)	polyclonal antibody	control medium
V 1956	+[b]	+	+	+	−
BB 7721	+	+	+	+	−
CSIRO 1865	+	+	+	+	−
CSIRO 659	+	+	−	+	−
CSIRO 1818	+	+	+	+	−
CSIRO 42	+	+	+	+	−
CSIRO 1866	+	+	−	+	−
CSIRO 1820	+	+	−	+	−
CSIRO 366	+	+	−	+	−
DPP 54	+	+	−	+	−
CSIRO 967	+	+	−	+	−
CSIRO 1180	+	+	−	+	−
CSIRO 1619	+	+	−	+	−
CSIRO 1622	+	+	−	+	−
CSIRO 1647	+	+	−	+	−
CSIRO 1907	+	+	−	+	−
CSIRO 1913	+	+	−	+	−
CSIRO 1922	+	+	−	+	−

[a] MAb (antigenic site)
[b] Presence (+) or absence (−) of neutralization

Table 6. Capture ELISA results for bovine ephemeral fever virus strains with glycoprotein specific monoclonal antibodies

Virus strain	17B1 (G1)/3D6 (G3a)[a]	1C6 (G2)/3D6 (G3a)	17B1 (G1)/12A5 (G2)
V 1956	28[b]	63	214
BB 7721	87	264	372
CSIRO 1865	22	37	141
CSIRO 659	6	4	177
CSIRO 1818	26	39	163
CSIRO 42	75	102	240
CSIRO 1866	7	2	188
CSIRO 1820	6	2	156
CSIRO 366	8	4	505
DPP 54	7	2	295
CSIRO 967	8	3	217
CSIRO 1180	9	4	500
CSIRO 1619	8	3	490
CSIRO 1622	8	2	106
CSIRO 1647	9	3	288
CSIRO 1907	7	3	254
CSIRO 1913	8	3	115
CSIRO 1922	13	12	153

[a] Capture antibody (antigenic site)/Detecting antibody (antigenic site)
[b] Optical density$_{492}$ $\times$ 10^3 (absorbance value)

the five strains that were neutralized by MAb 3D6 and strain CSIRO 1922, indicating that MAb 3D6 bound to these six strains. The absorbance values for the other 12 strains ranged from two to nine $\times$ 10^{-3}. The combinations of two MAbs from the same antigenic site gave low absorbance values of < 10 for all 18 strains (data not shown) and this absorbance range was selected as negative and an indication of non binding.

Discussion

Antigenic variation has been observed previously for rabies virus and is thought to be one cause of vaccine failure [22]. The present study shows that BEF virus variants can be selected experimentally from cloned BEF virus grown in the presence of MAb. In addition, batches of virus with different passage history and different BEF virus isolates were not antigenically identical.

Antigenic variants of BEF virus were tested for neutralization by a panel of neutralizing MAbs and it was found that these variants escaped neutralization not only by the MAb used for selection but also by other MAbs. The epitopes defined by these MAbs were therefore assumed to be structurally linked. An antigenic map produced by this method indicated the presence of at least six antigenic sites on the BEF virus G protein including a previously undescribed site defined by the MAb 5A5 (Fig. 2). A previous antigenic map of the BEF

G protein, produced using a competitive binding assay, contained at least three antigenic sites, which have been marked for comparison in Fig. 2. This included one non-conformational site (site Gl) [5]. The MAb 5A5 represents a fourth site obtained by this method and the second non-conformational site. The competitive binding assay relies on antibody binding to a particular site on the G protein, which not only prevents other antibodies binding to the same site, but also interferes with binding at nearby sites by stearic hindrance. It has been shown that escape mutants of rabies virus and vesicular stomatitis virus may differ from the parent virus by a single nucleotide, resulting in a single amino acid substitution in the G protein [13, 14]. Such a modification can have an effect on epitopes containing that amino acid, but will not necessarily have any detectable effect on nearby epitopes unless a conformational change takes place. Thus, the detection of more sites by escape mutants than by competition assays is to be expected.

More than one reaction pattern was observed for seven of the MAbs (Fig. 2) suggesting that different amino acids in the same epitope were being affected. More than one reaction pattern was also reported for a number of epitopes on the rabies virus G protein [12] and several mutation sites have been identified by sequence analysis [14].

The results shown in Tables 3 and 4 suggest that cultures of BEF virus contain different sub-populations of virus that can be selected simply by passaging, or by growing in the presence of a neutralizing antibody. Variants were selected at frequencies ranging from 10^{-3} to 10^{-8} compared with 10^{-4} to 10^{-5} for rabies virus [12]. Epitopes with a high escape frequency might be more likely to alter on passaging and this was found to be the case for epitopes reacting with the MAbs 3D6, 13C6 and 5A5 (Table 4). The low escape frequency observed for 8B6 and 16A6 may indicate a high proportion of lethal or non-cytopathic mutations in the region of the genome that codes for these epitopes. These epitopes may represent areas on the G protein which have important functions in growth or transmission and need to be conserved. The escape frequency for 8B6 is 10^{-8}, or the square of the average escape frequency for other epitopes, suggesting that two amino acid substitutions may be necessary to escape neutralization by this MAb.

Interestingly, the 8B6 epitope, which has the lowest escape frequency, suggesting some important function, is on the same antigenic site as the 3D6 epitope (by competition assays and by escape mutants) which has been absent from BEF virus since 1975 with no apparent effect.

The discovery of an additional, non-conformational antigenic site, 5A5, is of interest for two reasons. Firstly, this site may be linear and may therefore be a candidate for a future synthetic peptide vaccine. Secondly, a MAb to BEF virus which also neutralizes KIM virus may explain the cross-reactions with KIM virus of sera from cattle which were naturally infected with BEF virus [2].

The BEF virus strains used in this study were selected to give a wide rep-

resentation of strains isolated in Australia. The criteria for selection were, date of collection, host origin and geographical distribution. The strains represent isolates from all of the major epidemics that occurred in Australia from 1956 to 1989 [18] (M. F. Uren, pers. comm.). The host origin includes the three sources of BEF virus isolates in Australia; cattle, mosquitoes and *Culicoides* midges [3, 8, 16]. The geographical distribution includes the majority of the endemic area of eastern and northern Australia. All BEF virus strains were grown in C6/36 mosquito cells to achieve a common source and to eliminate the difficulties found in passaging some BEF virus strains in other tissue or cell types (Zakrzewski, unpubl. data). One MAb was chosen to represent each of the three antigenic sites described by Cybinski et al. [5]. Previous serological studies using polyclonal antibodies have failed to show any significant differences between strains from within Australia or in strains from different countries [11, 15, 17]. The results in Tables 4 and 5 clearly indicate that antigenic variation occurs in the BEF virus G protein and this could result in vaccine failures. There was no correlation between neutralization by 3D6 and the host origin or the geographic distribution. However, the epitope defined by the MAb 3D6 was absent in all strains isolated since 1975. It was also absent in the CSIRO 659 strain isolated in 1970 (Table 5) and the CSIRO 53 strain isolated in 1974 [5]. It has recently been shown, by immuno-blotting, that the 3D6 epitope is absent from the Beijing strain of BEF virus, although 12 other BB7721 epitopes are present (Cybinski and Walker, unpubl. data). The CSIRO 1922 strain, isolated in 1989, was not neutralized by the MAb 3D6 although low level binding was shown by ELISA. The phenomenon of binding without neutralization has been reported previously for BEF virus [5] and for rabies virus [19]. Any change in BEF virus or the viral glycoprotein since the period 1970–1975 has not been reported and there are no reports of any changes in disease characteristics (M. F. Uren and T. D. St. George, pers. comm.). The epidemiology of the disease has changed in eastern Australia from an advancing wave form, moving north to south, last seen in 1975–76 [19], to an irregular pattern of district epidemics. The change shown in CSIRO 1922, is also not reflected in changes in disease expression in naturally or experimentally infected cattle. The virus strains used in this study had a varied passage history, the number of passages since isolation ranged from one to 34 (Table 1). These differences did not appear to account for the presence or absence of the MAb 3D6 epitope. BB7721 and CSIRO 1865 strains were isolated from the same animal but have had 34 and three passages respectively, and both possess the MAb 3D6 epitope. Conversely, strains such as CSIRO 42 and CSIRO 967 have had the same number of passages in suckling mouse brain and have different MAb 3D6 reactivity.

The BB7721 prototype strain of BEF virus, isolated in 1968, has been used in Australia to produce a commercial BEF virus vaccine [20] and it is the strain used in major diagnostic laboratories. The data presented in this report clearly demonstrate that this strain is now different, by MAb assay, from the strains

circulating in the host cattle population. It has also been shown that epitopes on at least three antigenic sites have high mutation frequencies. This information should be taken into account when evaluating diagnostic tests especially those based on one particular MAb, and when considering the development of sub-unit or recombinant vaccines based on the viral glycoprotein.

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References

1. Calisher CH, Karabatsos N, Zeller H, Digoutte J-P, Tesh RB, Shope RE, Travassos da Rosa APA, St. George TD (1989) Antigenic relationships among rhabdoviruses from vertebrates and hematophagous arthropods. Intervirology 50: 241–257
2. Cybinski DH (1987) Homologous and heterologous antibody reactions in sera from cattle naturally infected with bovine ephemeral fever group viruses. Vet Microbiol 13: 1–9
3. Cybinski DH, Muller MJ (1990) Isolation of Arboviruses from cattle and insects at two sentinel sites in Queensland Australia 1979–1985. Aust J Zool 38: 25–32
4. Cybinski DH, St. George TD, Paull NI (1978) Antibodies to Akabane virus in Australia. Aust Vet J 54: 1–3
5. Cybinski DH, Walker PJ, Byrne KA, Zakrzewski H (1990) Mapping of antigenic sites on the bovine ephemeral fever virus glycoprotein using monoclonal antibodies. J Gen Virol 71: 2065–2072
6. Cybinski DH, Zakrzewski H (1983) The isolation and preliminary characterization of a rhabdovirus in Australia related to bovine ephemeral fever virus. Vet Microbiol 8: 221–235
7. Dietzschold B, Rupprecht CE, Tollis M, Lafon M, Mattei J, Wiktor TJ, Koprowski H (1988) Antigenic diversity of the glcoprotein and nucleocapsid protein of rabies and rabies-related virus: implications for epidemiology and control of rabies. Rev Infect Dis 10: 5785–5798
8. Doherty RL, Standfast HA, Clark IA (1969) Adaptation to mice of the causative agent of ephemeral fever in cattle from an epizootic in Queensland, 1968. Aust J Sci 31: 365–366
9. Ellens DJ, Gielkens ALJ (1980) A simple method for the purification of 5-amino salicylic acid. Application of the product as substrate in enzyme-linked immunosorbent assay (ELISA). J Immunol Methods 27: 325–332
10. Flamand A, Wiktor TJ, Koprowski H (1980) Use of hybridoma monoclonal antibodies in the detection of antigenic differences between rabies and rabies-related virus proteins: II The glycoprotein. J Gen Virol 48: 105–109
11. Inaba Y (1973) Bovine ephemeral fever (three day sickness). Stiff sickness. Bull Off Int Epizootiol 79: 627–673
12. Lafon M, Wiktor TJ, Macfarlan RI (1983) Antigenic sites on the CVS rabies virus glycoprotein: analysis with monoclonal antibodies. J Gen Virol 64: 843–851
13. Luo L, Snyder RM, Wagner RR (1988) Point mutations in glycoprotein gene of vesicular stomatitis virus (New Jersey serotype) selected by resistance to neutralization by epitope-specific monoclonal antibodies. Virology 163: 341–348

14. Prehaud C, Coulon P, Lafay F, Theirs C, Flamand A (1988) Antigenic site II of the rabies virus glycoprotein: structure and role in viral virulence. J Virol 62: 1–7
15. Snowdon WA (1970) Bovine ephemeral fever: the reaction of cattle to different strains of ephemeral fever virus and the antigenic comparison of two strains of virus. Aust Vet J 46: 258–266
16. Standfast HA, St. George TD, Dyce AL (1976) The isolation of ephemeral fever virus from mosquitoes in Australia. Aust Vet J 52: 242
17. Tian F, Jiang C, Zakrzewski H, Davis SS (1987) A comparison of a Chinese and an Australian strain of bovine ephemeral fever virus. Aust Vet J 64: 159–160
18. Uren MF, St. George TD, Kirkland PD, Stranger RS, Murray MD (1987) Epidemiology of bovine ephemeral fever in Australia. Aust J Biol Sci 40: 125–136
19. Uren MF, St. George TD, Stranger RS (1983) Epidemiology of ephemeral fever of cattle in Australia 1975–1981. Aust Biol 36: 91–100
20. Vanselow BA, Abetz I, Trenfield K (1985) A bovine ephemeral fever vaccine incorporating adjuvant Quil A: a comparative study using adjuvants Quil A, aluminium hydroxide gel and dextran sulphate. Vet Rec 117: 37–43
21. Walker PJ, Byrne KA, Cybinski DH, Doolan DL, Wang Y (1991) Proteins of bovine ephemeral fever virus. J Gen Virol 72: 67–74
22. Wiktor TJ, Koprowski H (1980) Antigenic variants of rabies virus. J Exp Med 152: 99–112

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