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Characterization of monoclonal antibodies against a type SAT 2 foot-and-mouth disease virus

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SUMMARY

This paper is the first to describe characterization of monoclonal antibodies (MAbs) against a South African Territories 2 (SAT 2) foot-and-mouth disease virus (isolate Rho 1/48). Twelve MAbs which neutralized homologous virus were characterized in indirect and sandwich ELISA using purified Rho 1/48 virus particles, subunits, trypsin-treated, and chemically denatured virus. All the Mabs inhibited haemagglutination by parental virus. Binding of the MAbs to 73 SAT 2 field isolates was measured in a sandwich ELISA and defined four distinct antigenic regions. Preliminary characterization of escape mutants selected with some of the MAbs using virus neutralization tests, ELISA, and amino acid sequencing is included. MAbs 2, 25, 40, 48 and 64, reacted with a linear epitope on the VP1 loop region. An amino acid change at position 149 (valine to glutamic acid) was detected in mutants selected by MAb 2 and 40 and this eliminated binding and neutralization by all the other MAb. This epitope was conformation-dependent and was conserved in all 73 isolates of SAT 2 examined. Escape mutants isolated with MAb 41 and 44, had changes at positions 156 (glycine to aspartic acid), or 158 (serine to leucine) respectively. These MAbs bound with Rho 1/48 only out of 73 field strain viruses studies and the reactions of MAbs from the other groups was unaltered. MAb 27, 28 and 37 reacted with a conformation-dependent epitope on VP1 which was not conserved in field isolates. All mutants selected by these MAbs had a single amino acid substitution at position 149 (valine to alanine). The same change was always found in field isolates which did not bind MAbs from this group. MAb 11 reacted with a linear epitope associated with amino acids 147 or 148 on VP1 and showed similar binding characteristics to a conformation dependent MAb 7, no amino acid residue changes were found within VP1 for monoclonal antibody 7 mutants.

INTRODUCTION

Monoclonal antibodies (MAbs) against foot-and-mouth disease (FMD) virus have been produced by many laboratories [1–6]. The majority have been produced against serotypes O, A and C, especially European strains. There have been no reports on the production of MAbs against types SAT (South African Territories)1, SAT 2 and SAT 3, and this paper describes the characterization of MAbs against

a type SAT 2 FMD virus (isolate Rhodesia 1/48) using serological and biochemical methods. MAbs against FMD viruses are ideal reagents for determining and understanding antigenic differences for epidemiological studies such as field strain identification, the identification of epitopes important in protection, examination of vaccine and challenge strains, the production of neutralizing antibody escape mutants for characterization of epitopes at the amino acid level, and the analysis of variation of FMD following cell and animal passage. Studies on FMD viruses have indicated that there are at least four independent antigenic sites on the capsid involved in virus neutralization [7–13]. Such studies identified critical amino acid substitutions of escape mutants. Antigenic sites were proposed which had similar elements to those of poliovirus and human rhinovirus. For the O viruses studied, antigenic site 1 was associated with substitutions on VP1 at amino acids 144, 148 and 154, the critical one being at 144; the site was non-conformational and trypsin-sensitive. Site 2 identified substitutions at amino acids 70, 71, 73 and 31 of VP2, the site was conformational and trypsin-resistant. Site 3 involved residues 43 and 44 of VP1 and was conformational. Site 4 involved residues at 58 on VP3 and was conformationally dependent. Recently a fifth site associated with type O has been identified in this laboratory (submitted for publication) which is conformationally dependent and associated with a residue at 149 on VP1. Use of quintuple MAb escape mutants showed that producing changes at all five sites eliminated the ability of polyclonal cattle, guinea-pig, and mouse sera to neutralize that virus. This paper presents preliminary amino acid sequence data on MAb escape mutants isolated using some of the anti-SAT 2 MAbs characterized in serological assays in an attempt to understand the antigenic nature of SAT 2 as compared to the other serotypes.

MATERIALS AND METHODS

Viruses

Rho 1/48 is the designation of a virus received in 1948 by the IAH, Pirbright from what was formerly Northern Rhodesia. The outbreak from which the samples were obtained was only the second occurrence of type SAT 2 reported since the initial samples defining the serotype were first isolated in 1931 (World Reference Laboratory, Pirbright, unpublished records). The virus has been passaged in primary bovine thyroid cells (BTY) once, and seven times in a continuous cell line of Baby Hamster Kidney (BHK-21) cells and samples have been stored at −70 °C.

FMD viruses were grown in BHK 21 cells in the absence of bovine serum. Infected tissue culture was clarified by low speed centrifugation and the supernatant stored at −20 °C after the addition of an equal volume of sterile glycerol containing 2% normal bovine serum. Purification of some strains was made on 15–45% sucrose density gradients [14]. The concentration of purified virus (146S) was established by examination of the RNA adsorption at 259 nm. Collected samples were stored at −70 °C without further additions.

Preparation of 12S subunit particles. One ml of purified isolate Rho 1/48 containing 160 μg virus was acidified by the addition of 2 ml of 0·1 M-NaH_2PO_4. Phenol red indicator solution (0·05 ml) was added and the mixture was left at

room temperature for 10 min, after which the pH was adjusted to 7·4 by the addition of 0·1 M-NaOH.

Preparation of trypsin-treated virus. To 160 μ of purified virus Rho 1.48 in 1·0 ml of sucrose was added 50 μl of trypsin solution (2 mg/ml in 0·1 M phosphate buffer, pH 7·4). The mixture was incubated at 37 °C for 15 min. The virus was then diluted to the assay concentration in the relevant buffer with no further treatment.

Preparation of denatured virus. To 1 ml of purified virus Rho 1/48 containing 160 μg was added 10 mg of sodium dodecyl sulphate (SDS) (giving final concentration 1%) and 20 μl of mercaptoethanol (to a final concentration 2%). The mixture was heated for 3 min in boiling water.

Monoclonal antibodies. These were prepared as described by Butcher and colleagues [15]. Mice were primed by subcutaneous injection of 10 μg of Rho 1/48 (purified and inactivated with binary ethylenimine, (BEI)), diluted to 50% in Freund's complete adjuvant (FCA). After 1 month the mice received the same treatment and at 2 months the mice received 50 μg of virus diluted in PBS at multiple sites. After a further 3 days the spleens were taken and fusions made using NSO cells. Positive clones were initially selected using the capture ELISA with Rho 1/48 purified whole virions as antigen, as described below. All MAbs used in this study were re-cloned and tested for virus neutralizing activity. The isotype specificity of the MAbs was determined from tissue culture preparations using a kit from 'The Binding Site', (Birmingham University, UK). Ascites fluids were prepared by intraperitoneal inoculation of the hybridoma cells into mice previously sensitised with FCA. Ascites fluids were clarified by centrifugation and stored at −20 °C.

ELISA characterization

Indirect ELISA. Microtitre ELISA plates (Nunc, Maxisorb) were coated by adding 50 μl of various antigen preparations at 2 μg/ml, diluted in 0·05 M carbonate/bicarbonate buffer, pH 9·6, by incubation overnight at 4 °C. Plates were washed four times in PBS and then blotted almost dry. MAbs (50 μl) were then added in triplicate in threefold dilutions in blocking buffer (PBS containing final concentrations of 5% skimmed-milk powder (Marvel), 0·1% Tween 20). Control antigen-coated wells received three-fold dilutions of polyclonal mouse or guinea pig antiserum prepared against Rho 1/48, beginning at 1.50. The plates were incubated at 37 °C for 1 h while being rotated (Rotatest, Luckhams). The plates were then washed as above and 50 μl of anti-mouse IgG conjugated to horseradish peroxidase (Dako) at optimal dilution was added per well diluted in blocking buffer. Anti-guinea pig IgG (Dako) labelled with horseradish peroxidase was added to appropriate wells. The plates were incubated at 37 °C for 1 h as above and washed. Substrate solution (OPD/H_2O_2) was added to each well (50 μl) and the test stopped by the addition of 50 μl per well of 1 M-H_2SO_4 after 10 min incubation at room temperature. The optical density (OD) for each well was read using a multichannel spectrophotometer at 492 nm.

Examination of denatured virus. Denatured purified Rho 1/48 was diluted to 8 μg/ml in carbonate/bicarbonate buffer or PBS. Twofold dilutions were made (50 μl per well) in triplicate across eight wells of a microtitre plate. The plates were

incubated at 4 °C overnight. After washing, MAbs (50 μl in blocking buffer) were added at a concentration found to be in excess. A dilution of MAb in blocking buffer was added which was four times that of the last dilution shown to be at the maximum OD observed for each MAb. This was determined by reference to the indirect ELISA titrations of the MAb against the Rho 1/48. Plates were incubated at 37 °C for 1 h with rotation. The additions of anti-mouse conjugate and substrate to detect bound MAb was as described above.

Sandwich ELISA. This was essentially as described for the indirect ELISA except that plates were first coated with an optimal dilution of broadly-reactive polyclonal anti-type SAT 2 rabbit serum in 50 μl per well of carbonate/bicarbonate buffer and incubated overnight at 4 °C. The plates were washed and 50 μl per well of the various Rho 1/48 FMD virus antigens diluted to 2 μg/ml in blocking buffer were added, as described above. Addition of MAbs and other reagent was as described previously.

Epitope characterization by MAbs. This was performed and analysed as described elsewhere (16). Briefly, microtitre plate wells were coated by overnight incubation with 50 μl of a pre-titrated polyclonal rabbit antiserum made against type SAT 2 as described above. Type SAT 2 (tissue culture with glycerol) virus isolates, diluted 1/3 in blocking buffer, were then added to washed plates in duplicate rows from row 2 row 7 across 12 wells of the plates. The first row received homologous Rho 1/48 glycerinated virus. Row 8 received blocking buffer only. The plates were rotated for 1 h at 37 °C, then washed and 50 μl of anti-SAT 2 MAbs were added at a single dilution (in a four-fold excess as described above). The eight wells of duplicate columns 1–10 received MAbs, columns 11 and 12 received a dilution of polyclonal guinea-pig antiserum against SAT 2 at a saturating level. The plates were rotated at 37 °C for 1 h. Washed plates then received 50 μl per well of anti-mouse or anti-guinea-pig horse radish peroxidase conjugate, after which they were incubated for 1 h at 37 °C. Addition of substrate and stopping solution was as described previously. The relative binding of the MAbs to the different isolates compared to Rho 1/48 was calculated. The OD value from wells containing MAb but no virus was subtracted from all the results in that column. The mean OD in the wells where MAb interacted with the viruses was then expressed as a percentage of the mean OD value for the polyclonal guinea-pig serum obtained for these viruses. These values were then expressed as a percentage of those obtained for Rho 1/48, thereby comparing the binding of each MAb to test strains with binding for the virus which was used to prepare the MAbs. The initial calculation of the MAb binding as a percentage of the polyclonal reaction with each isolate was made to adjust for variation in the amounts of each of the viruses bound in the test plates.

Haemagglutination-inhibition assay

Viruses were titrated for haemagglutinating (HA) activity. Purified viruses were diluted in 50 μl PBS containing Mg^{++} (0·005% MgCl) and 0·1% bovine serum albumin (BSA) in round-bottomed microtitre plates. A 0·4% suspension of guinea-pig red blood cells (collected in Alsever's solution and washed four times in PBS before use), was added to each well (50 μl). The plates were incubated at 37 °C for 1 hr and then left at room temperature. The HA patterns were read and the last well showing 50% HA was taken as the end-point. Haemagglutination-

Inhibition assays (HAI) were made by adding 4 HA units of each virus in 50 μl of PBS/BSA to two-fold dilutions of MAb in the same buffer (50 μl). Control wells contained only virus and dilution buffer. The degree of HAI was expressed as the reciprocal of the antibody dilution showing 50% HA.

Selection of escape mutants

Dilutions of Rho 1/48 (50 μl) were mixed with 50 μl of BHK-21 cells (5×10^5 ml) in Eagle's medium containing 8% normal bovine serum in microtitre plate wells. The plates were covered, incubated for 2 days at 37 °C (CO_2 atmosphere) and then examined microscopically for CPE. The titre of the virus was estimated using the method of Karber [17]. One ml of virus containing 10^6 TCID50 was added to 50 μl of each of the MAb ascites. After mixing and incubation at 37 °C for 20 min, the mixture was pipetted onto a monolayer of washed (PBS) BHK-21 cells (25 ml flask). Ten ml of Eagle's medium was then added containing each of the ascites diluted 1/50, the cells were then incubated at 37 °C. After 24 h the infected cell debris was removed by low speed centrifugation and 1 ml of the supernatant was processed as above by the addition of MAb ascites to both virus and growth medium. One ml was also processed in the same way except that the growth medium contained no MAb. A further one ml was taken and processed as above without addition of MAb at either stage. The virus contained in the clarified supernatant fluids was examined in the sandwich ELISA as described above to determine the reaction of all the MAbs with the viruses growing after the various passages. Mutants were detected where the binding of the selecting MAb was eliminated and these were passaged in BHK-21 cells in the absence of further MAb pressure. Mutants which maintained the same antigenic profile with respect to the selecting MAbs were tested in VN tests against all MAbs. Infectious virus samples were stored at −70 °C in an equal volume of sterile glycerol.

Virus neutralization test

Parental Rho 1/48, field isolates and mutant viruses were titrated in microtitre plates as described above to determine the $TCID_{50}$. Each MAb or polyclonal serum was diluted two-fold (starting dilution 1/100) in quadruplicate in 50 μl Eagle's medium across 10 wells of a microtitre plate. Virus (50 $TCID_{50}$ diluted in 50 μl of Eagle's medium) was added to each well containing antibody dilutions and to wells containing only Eagle's medium, across 12 wells of the microplate. The mixture was incubated at 37 °C for 20 min in an atmosphere of 5% CO_2. BHK-21 cells were then added to each well (100 μl diluted in Eagle's medium containing 8% adult bovine serum at a concentration of 5×10^5/ml). Microplates were covered and incubated at 37 °C for 2 days before being examined microscopically for CPE. The virus neutralization (VN) titres were calculated using the method of Karber as described above.

Sequencing of escape mutants

The nucleotide and amino acid sequence of Rho 1/48 VP1, VP2 and VP3, has been determined in this laboratory by Rowe (unpublished data). Sequence data is also available for the entire genome of SAT 2 Ken 3/57 and various regions of the VP1 of 35 SAT 2 field isolates. RNA from mutant viruses was reverse-transcribed and the region of the genome coding for VP1 was amplified by the polymerase

Table 1. *Isotype of monoclonal antibodies prepared against Rho 1/48 foot-and-mouth disease virus*

MAb	Isotype
2	IgG2b
7	IgG2b
11	IgG2b
25	IgG2b
27	IgG2b
28	IgG2a
37	IgG2b
40	IgA
41	IgM
44	IgG2a
48	IgG2a
64	IgG2b

chain reaction (PCR) using primers derived from the Rho 1/48 genome sequencing study. The PCR products were cloned and then sequenced using the Multiwell microtitre plate sequencing-T7 DNA polymerase system, (Amersham, UK).

RESULTS

Isotyping

Table 1 shows the isotypes of the MAbs. Seven of the MAbs were IgG2b, three were IgG2a, one was IgM and one IgA.

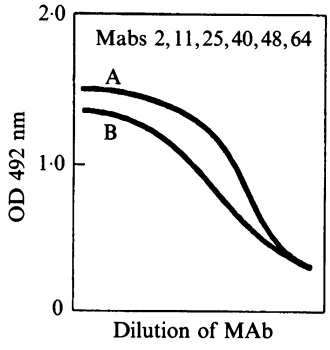
ELISA

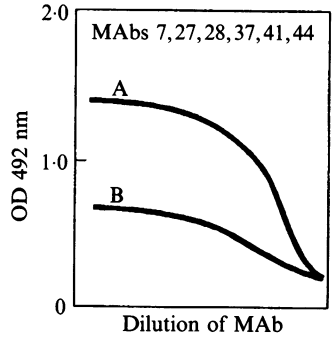
The reactions of MAbs in the sandwich and indirect ELISA against various antigen preparations are summarized in Figure 1. These data divided the MAbs into two groups. MAbs 2, 11, 25, 40, 48 and 64 reacted well with 146S (purified virus) in the sandwich and indirect assays with plateau height (maximum constant colour) of approximately 1·5 OD and high end-points (last dilution where colour was detectable above background). Although their reaction with 12S subunits was lower, the relationship between the results for the two assays was similar to the 146S titrations. This group of MAbs reacted with denatured virus. The low reaction where the denatured antigen was most concentrated (1/20–1/80) was due to the effect of the residual SDS detergent action preventing optimum antigen coating of the plates. The other group, MAbs 7, 27, 28, 37, 41 and 44 reacted well with 146S in the sandwich assay but poorly in the indirect assay. This difference was pronounced when the MAbs were titrated against 12S where the reaction in the sandwich assay was similar to the first group but the reaction in the indirect assay was greatly reduced. None of the MAbs in this group reacted with denatured virus. MAbs from both groups did not react with trypsin-treated virus.

Haemagglutination inhibition

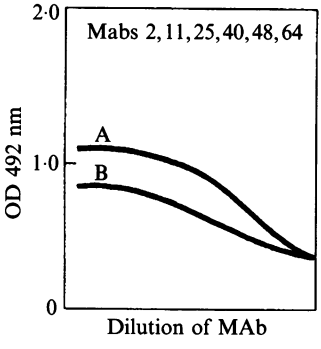
Table 2 shows the results comparing the ability of the MAbs to inhibit HA of purified field isolates of SAT 2 viruses compared to Rho 1/48. MAbs 2, 25, 48 and 64 inhibited all viruses. MAbs 27, 28 and 37 shared a HAI pattern by showing no

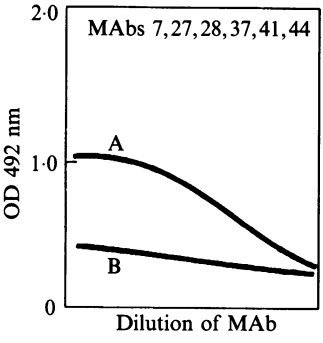
BINDING TO PURIFIED 146S RHO 1/48



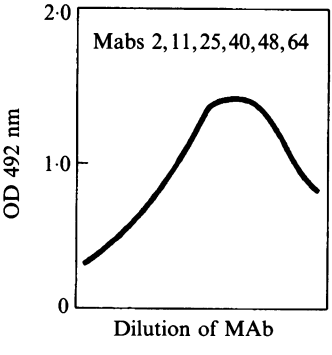


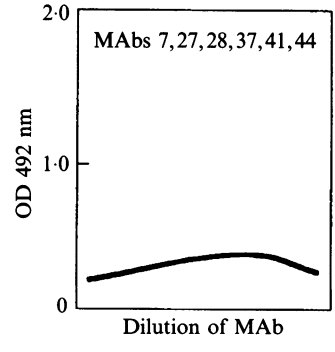
BINDING TO 12S SUBUNIT PARTICLES RHO 1/48





BINDING TO DENATURED PURIFIED RHO 1/48





A = antigen in sandwich ELISA
B = antigen in indirect ELISA
Denatured antigen added as dilution range vs constant MAb
MAbs giving reaction pattern are shown in boxes

Fig. 1. Reaction profiles of monoclonal antibodies in sandwich ELISA against different antigens.

Table 2. *Analysis of purified SAT 2 viruses by HAI, using MAbs prepared against Rho 1/48*

Virus	Monoclonal antibody										
	2	25	48	64	7	11	27	28	37	40	41	44
Rho 1/48	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	320	5120	2560
BB 170/40	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	1280	0	0
Zam 5/75	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	320	0	0
Ken 183/74	> 5120	> 5120	> 5120	> 5120	5120	> 5120	5120	2560	5120	320	160	160
Moz 1/70	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	2560	2560	> 5120	80	0	0
Tan 5/68	> 5120	> 5120	> 5120	> 5120	> 5120	> 5120	2560	1280	5120	160	0	0
Bot 23/78	> 5120	> 5120	> 5120	> 5120	160	160	0	0	0	0	0	0
Bot 6/78	> 5120	> 5120	> 5120	> 5120	0	480	0	0	0	0	0	0
Bot 3/77	> 5120	5120	> 5120	> 5120	0	0	0	0	0	0	0	0

Table 3. *Analysis of monoclonal binding to purified SAT 2 viruses by sandwich ELISA*

	Monoclonal antibody											
Virus	2	25	48	64	7	11	27	28	37	40	41	44
Rho 1/48	100	100	100	100	100	100	100	100	100	100	100	100
BB 170/74	100	100	100	100	100	100	100	100	100	100	0	0
Zam 5/75	100	100	100	100	100	100	100	100	100	60	0	0
Ken 183/74	100	100	100	100	70	100	70	60	80	100	35	30
Moz 1/70	100	100	100	100	100	100	60	50	60	40	0	0
Tan 5/68	100	100	100	100	100	100	70	70	80	50	0	0
Bot 23/78	100	100	100	100	30	40	0	0	0	0	0	0
Bot 6/78	100	100	100	100	0	50	0	0	0	0	0	0
Bot 3/77	100	80	100	100	0	0	0	0	0	0	0	0

Number = % reaction as compared to binding of MAbs with Rho 1/48.

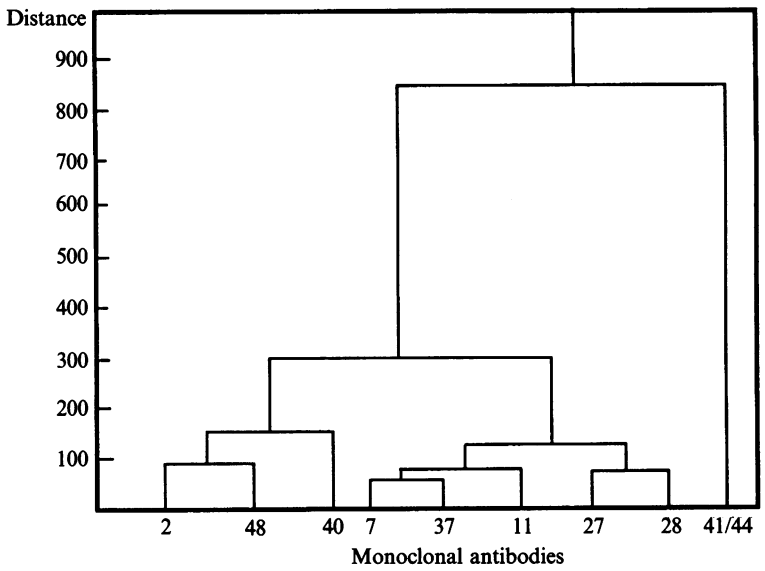


Fig. 2. Relationship of MAbs determined with reference to 73 field isolates in sandwich ELISA. Dendrogram relates MAb binding data from sandwich ELISA. MAbs are grouped according to their similarity of reactions with 73 field strain SAT 2 FMD viruses as analysed by multivariate statistics using hierarchical cluster analysis (Unistat Statistical Package, Unistat Ltd, London). The distance measure has been analysed according to Euclid formula (Distance $(x, y) = \sqrt{\Sigma_i(x_1 - y_1)^2}$) using complete linkage, which selects for the largest distance between pairs in each cluster.

reaction with the same three isolates. MAbs 41 and 44 only showed significant HAI of the homologous Rho 1/48. MAb 7 and 11 differed from the other MAbs and to each other with respect to one virus (Bot 6/78, HAI by 11 and not 7). MAb 40 showed a different pattern to MAbs 2, 25, 48 and 64, since it did not show HAI activity against three of the viruses and was similar to the 27, 28 and 37 MAbs.

Epitope characterization ELISA

The binding patterns of the MAbs with the virus isolates used in the HAI are shown in Table 3. A similar pattern of reaction based on which viruses were bound

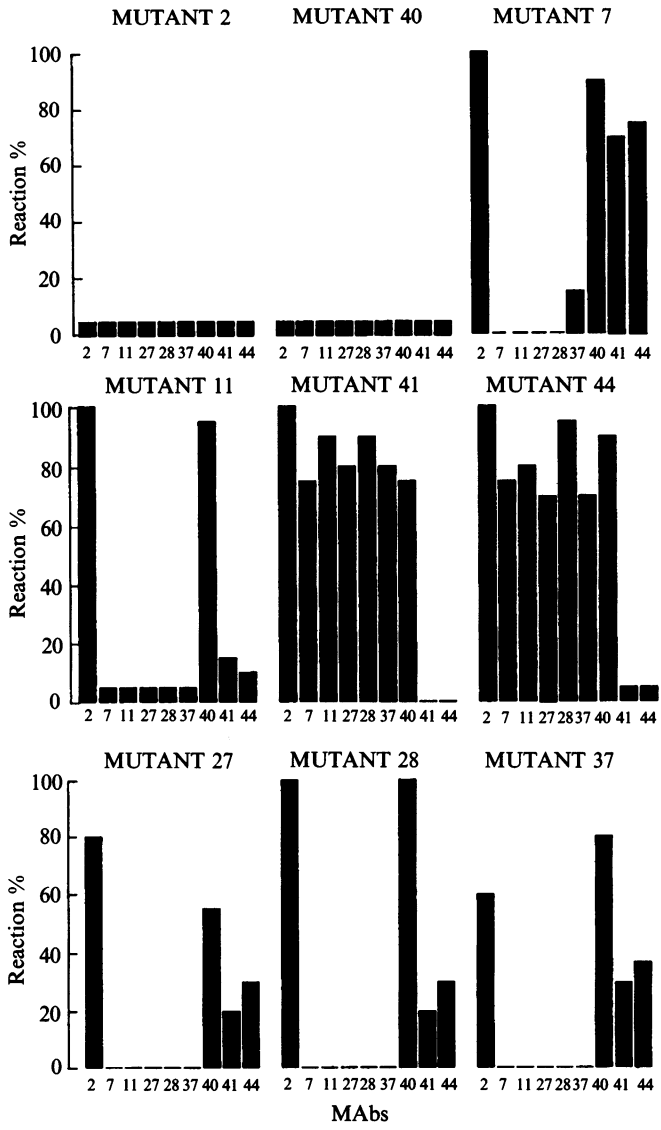


Fig. 3. Reaction of MAbs with escape mutant viruses in sandwich ELISA.
■ = binding of MAb as % of reaction with parental isolate Rho 1/48.

by the MAbs, was obtained. Thus MAb 2, 25, 48 and 64 reacted with all isolates; 7 and 11 differed in their reaction with Bot 6/78; 27, 28 and 37 failed to bind the same three isolates; 41 and 41 only reacted significantly with Rho 1/48; MAb 40 showed a pattern to MAbs 27, 28 and 37 Figure 2 shows the data from examining 73 SAT 2 field isolates. The dendrogram shows how the MAbs are related with reference to their reaction patterns with the viruses. The data was analysed using heirarchical cluster analysis (multivariate statistical package-Unistat IV software, Unistat Ltd, London). This shows that there are strong links between MAbs 2 and 48 which are both related to 40. MAbs 41 and 44 react identically and form a separate cluster.

Table 4. *Characterisation of MAb escape mutants on VP1 of SAT 2 Rho 1/48 by amino acid sequence analysis*

MAb escape mutant	Amino acid position	Amino acid change
2	149	V–E
40	149	V–E
27	149	V–A
37	149	V–A
11	147	R–W
	148	A–S
7	NONE	NONE
41	156	G–D
44	158	S–L

Table 5. *Neutralization of MAb escape mutant viruses by MAbs*

	Virus								
MAb	Rhol/48	2	7	11	27	37	40	41	44
2	> 5120	< 5	> 5120	> 5120	> 5120	> 5120	< 5	> 5120	> 5120
7	5120	< 5	< 5	5	< 5	5	< 5	2560	5120
11	2560	< 5	< 5	< 5	< 5	10	< 5	5120	> 5120
25	> 5120	< 5	1280	> 5120	> 5120	2560	10	> 5120	> 5120
27	640	< 5	< 5	< 5	< 5	5	< 5	80	160
28	5120	< 5	< 5	5	< 5	< 5	< 5	2560	> 5120
37	2560	< 5	5	40	< 5	5	< 5	640	5120
40	2560	< 5	1280	5120	5120	1280	< 5	2560	1280
41	160	10	80	160	80	80	5	< 5	< 5
44	320	10	80	640	160	80	5	< 5	< 5
48	> 5120	10	5120	> 5120	> 5120	5120	5	> 5120	> 5120
64	> 5120	< 5	> 5120	> 5120	> 5120	> 5120	10	> 5120	2560
PC/GP	5120	1280	2560	5120	1280	1280	640	5120	1280

MAbs 27 and 28 are clustered with MAb 37 which is most strongly related to MAb 7. MAb 11 is related to the same cluster.

Analysis of MAb binding to escape mutants by sandwich ELISA

Results for the reaction between the MAbs and MAb escape mutants assessed using the sandwich ELISA are shown in Figure 3. No MAbs reacted with MAb 2 and MAb 40 escape mutants. MAb escape mutants 11, 27, 28 and 37 gave a similar reaction profile where the binding of MAbs 2 and 40 was unaffected and the reaction of MAbs 41 and 44 was reduced. MAb escape mutant 7 showed a similar profile except that the binding of MAbs 41 and 44 were unaffected. All MAbs except 41 and 44 bound with MAb escape mutants 41 and 44.

MAb escape mutants

Preliminary data on escape mutants prepared with MAbs 2, 7, 11, 27, 37, 40, 41 and 44 by amino acid sequence analysis of VP1, are summarized in Table 4. This data indicates that an amino acid residue change in mutants selected by MAb 2 and 40 is valine to glutamic acid (V–E) at position 149 of VP1. These mutants were not neutralized or bound by any of the MAbs from other 'groups' as shown

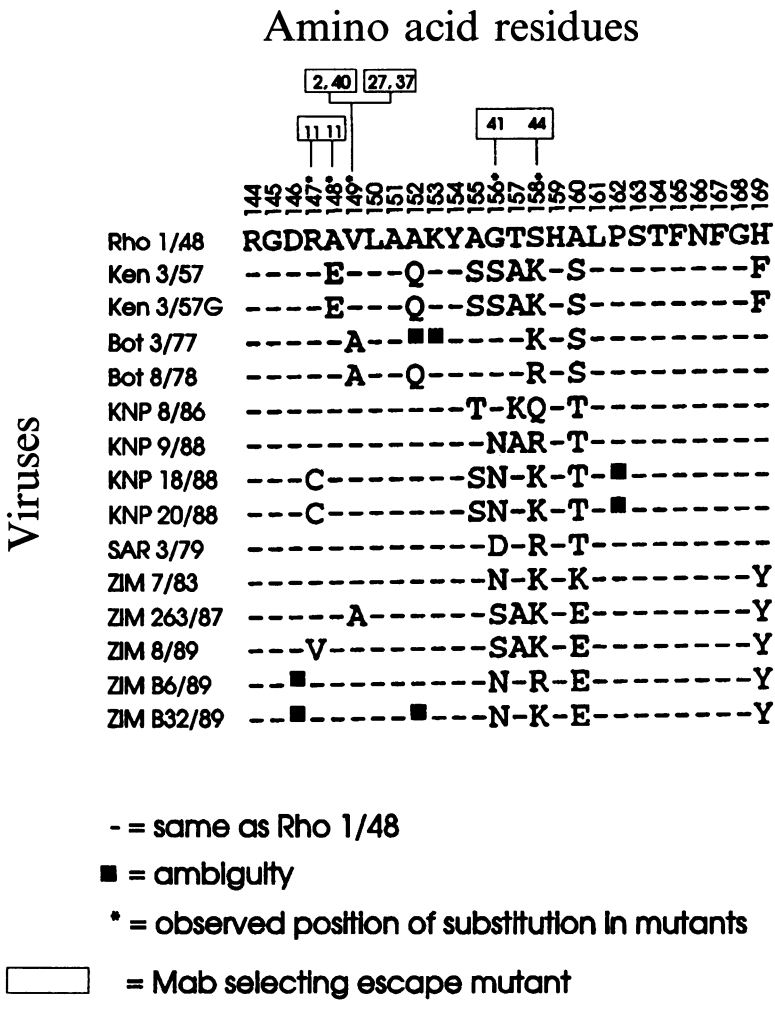


Fig. 4. Comparison of aminoacid sequence data of Rho 1/48 to field isolates of SAT 2 FMD viruses from 144 to 159 of VP1.

in Table 5 and Figure 3. This change was not observed in field strains where sequence data was available (37 isolates). For mutants selected by MAbs 27 and 37, a valine to alanine (V to A) change at 149 of VP1 was observed. These mutants were neutralized by MAbs 2, 25, 40, 48 and 64 as shown in Table 5 indicating that MAbs 7 and 11 were affected by the same mutation. The binding data (Figure 3) indicates a difference between epitopes for MAbs 11, 27, 28 and 37 with that identified by MAb 7. In field strains where MAbs 27, 28 and 37 failed to bind, the same change in amino acid 149 (V to A) was observed and no other. For mutants selected by MAb 11, different amino acid changes at 147 (arginine to tryptophan, R to W) or 148 (alanine to serine, A to S or alanine to valine, A to V) were detected. Mutants selected by MAb 7 showed no changes in VP1. Changes in mutants selected by Mab 41, (glycine to aspartic acid, G to D) at 156 and for MAb 44, (serine to leucine, S to L), at 158 were detected. Figure 4 shows the amino acid sequence of Rho 1/48 from 144 to 169 of the loop region of VP1 compared with

data from other type SAT 2 isolates. The positions of the amino acid changes determined for the mutants is shown.

Virus neutralization tests on escape mutants

Table 5 shows the results for the neutralization of each of the escape mutants by MAbs. MAb 2 and 40 escape mutants were no longer neutralized by any of the MAbs. MAb 7, 11, 27 and 37 escape mutants had the same neutralization profile, where all MAbs except 7, 11, 27, 28 and 37 neutralized the viruses. MAb escape mutants 41 and 44 are neutralized by all MAbs except 41 and 44. Polyclonal guinea-pig anti-type SAT 2 serum neutralized all the mutants.

DISCUSSION

This paper is the first to describe the production and characterization of MAbs against a type SAT 2 FMD virus. The data obtained by serological and biochemical tests gives the first insight into the nature of antigenic sites on SAT 2 viruses. The data show that all the epitopes identified were associated with the VP1 loop region on the virus. This region has been shown to be important in other serotypes of FMD virus in that it shows high amino acid sequence variations [18], contains linear epitopes which are trypsin sensitive [19] and has neutralizable epitopes [20]. The SAT 2 MAbs identified both conformational and non-conformational epitopes, as judged by reaction with denatured virus and different antigens in the sandwich and indirect ELISA. The escape mutant data also indicates that the epitopes identified are all involved with the VP1 loop region of Rho 1/48. All the MAbs inhibited haemagglutination by the parental virus. This is in keeping with the evidence [21] that sites involved with HA are trypsin sensitive. Although HAI activity was associated with binding to single epitopes, as shown by the MAbs which did not bind to viruses in ELISA failing to give HAI, we did not identify a specific epitope involved in HAI. It is likely that the HAI observed was mediated through any of the MAbs reacting in the loop region preventing the guinea-pig red blood cell receptor/virus interaction by steric interference. This was also indicated because none of the mutant viruses lost their ability to haemagglutinate (data not shown) which may show that the virus-specific epitope(s) which interact with the guinea-pig red blood cell receptor are near to, but outside the loop. The data for the epitopes may be examined in more detail. A non-conformation-dependent site (linear epitope) was identified by MAbs 2, 25, 40, 48 and 64 which was conserved in all 73 SAT 2 field strains studied, thus the integrity of the site must be important. Evidence that the epitope was not conformation-dependent comes from the binding data where the site is unaffected by denaturation and that reaction 12S subunits is maintained. The site was also unaffected when virus was directly attached to microtitre plates, a process which has been shown to alter MAb reactivity with conformation-dependent sites of FMD type O [22]. Escape mutants selected by two of the MAbs from this group had an amino acid change at 149 on VP1 from a valine to glutamic acid eliminated neutralization and binding with all the other MAbs. This indicates that the V–E change causes a drastic alteration in the loop region which affects both the linear epitope detected by MAb 11 and conformation-dependent epitopes associated with the loop, as discussed below. This effect was not observed for the other

mutant viruses, where only the reactions of the selecting MAb or those characterized as being in the same group, was eliminated.

The binding data indicate that MAbs 27, 28 and 37 react with a conformation-dependent epitope. The MAbs did not bind to denatured virus and the reaction with whole virions in the non-capture system was affected. This site is not conserved in field strains and viruses may be distinguished using these MAbs. The amino acid change in mutants at position 149 of VP1 is interesting because this is the same position as the first group of MAbs recognizing the linear site, however the change is from a valine to alanine. The same change is the only one observed in field isolates where no reaction is observed with MAbs, 27, 28 and 37. This change has no effect on the binding or neutralization of MAbs from the first group examined (Figure 3 and Table 5). The interaction of the valine residue may be important in maintaining the three-dimensional structure either in the loop region or more probably between the loop region and an interaction between amino acids in VP2/3 on the capsid surface, as has been observed in type O virus [23]. The epitope identified by MAbs 41 and 44 is conformation-dependent and specific for homologous virus. This mono-specificity for the virus used to prepare MAbs has not been observed for MAbs against other serotypes. The escape mutants implicate amino acids at 156 or 158 being important to this epitope. This is interesting since analysis of sequence data shows that type SAT 2 viruses contain three extra amino acids in VP1 (compared to types O, A C) at positions 157 to 159 on VP1. This region is also highly variable in SAT 2 viruses as compared to the rest of VP1 as indicated in Figure 4. This site is dependent on conformation so that interactions of amino acids in this region with others needs to be examined. One problem associated with producing efficient vaccines against SAT 2 is that antigenic variation in field strains appears more important in this serotype compared to types O, A and C. One explanation for this could be that this site is immuno-dominant. Since the epitope defined by MAbs 41 and 44 is unique to that SAT 2 serotype through the incusion of additional sequence; is specific for parental virus and is in the region of the VP1 loop where high variation is found in field isolates; then antibodies produced following vaccination may be strain-specific and result in poor cross-protection. Although this is speculative, the preparation of multiple monoclonal antibody escape mutants and their exploitation in virus neutralization tests on cattle antisera will test this hypothesis. MAbs recently received from S. Africa, produced against three different isolates of SAT 2, show examples of antibodies which react only with the virus that was used to prime the mice, as for 41 and 44. MAbs 7 and 11 are of interest because they react with conformation- and non-conformational dependent epitopes, respectively. Serological evidence suggests that there are only small differences in their reaction in the HAI and sandwich ELISA. The ELISA study on 73 field isolates related MAbs 7 and 11 most strongly to 37, 27 and 28, a conformation-dependent group. Critical amino acid substitutions at positions 147 and 148 were found for MAb 11 mutants, whereas no amino acid changes were found in VP1 of the MAb 7 mutants indicating that amino acids on VP2 or VP3 are involved with those on VP1. Changes on the linear epitope selected by MAb 11 affected the binding of MAb 7, as seen by the reaction of the MAbs of the respective mutants and thus may interfere with the interaction of the loop with other amino acids, modifying

the conformation requirement of MAb 7 for that site. Future work will involve preparation of MAbs against other isolates of type SAT 2 FMD viruses and their use in serological and biochemical tests to further characterize virus epitopes. The present MAbs have proved valuable reagents in the rapid analysis of type SAT 2 field isolates in epidemiological studies. The existing MAb escape mutants have also proved invaluable in the rapid characterization of MAbs obtained from another laboratory.

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