MONOCLONAL ANTIBODIES TO AN INDIAN STRAIN OF TYPE A FOOT-AND-MOUTH DISEASE VIRUS

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Received January 21, 1999; accepted March 22, 1999

Summary. – A set of five neutralizing monoclonal antibodies (MAbs) to an Indian strain (IND17/77) of type A (subtype A22) foot-and-mouth disease (FMD) virus (FMDV) was used in the study. Four of the MAbs (27S, 37S, 85S, and 143S) identified a trypsin-sensitive (TS) epitope(s) and were specific for VP1, while the remaining MAb (145S) reacted with a trypsin-resistant (TR) epitope and was specific for VP3 in Western blot analysis. Both the epitopes (TS and TR) were conformation-independent in nature. Results obtained in MAb-competition enzyme-linked immunosorbent assay (ELISA), and profiling of the (MAb) neutralization-escape mutants in ELISA and cross-neutralization test revealed two overlapping TS epitopes (27S/37S and 85S/143S) on the virus. Variation at both these epitopes was observed in some field isolates of serotype A. Comparison of deduced amino acid sequence in the VP1 region (aa 140-213) between the parent virus and the mutants identified Gly_{148} and Arg_{153} as critical for the formation of both the TS epitopes. Substitution of R_{153} by Gly or Ser was observed in mutants with no reactivity for the MAbs 85S/143S. However, these mutants maintained partial reactivity with MAbs 27S/37S, and substitution of Gly_{148} by Glu eliminated both the epitopes. No amino acid substitution was observed in the VP1 region of aa 200-213. Efficient neutralization of the MAb neutralization escape mutants (MAb-resistant (MAR) mutants) by bovine vaccinate serum (BVS) indicated involvement of other epitopes on the virion surface in eliciting neutralizing antibodies following vaccination.

Key words: FMDV; serotype A; neutralization-escape mutant; trypsin-sensitive epitope; trypsin-resistant epitope; 1D gene

Introduction

FMDV occurs as 7 immunologically distinguishable serotypes (O, A, C, Asia1, SAT1, SAT2, and SAT3) and more than 65 subtypes (Pereira, 1977). High mutability and potential for rapid variation of the virus, as indicated by such a large number of serotypes and subtypes, is a major drawback in the control of FMD. In India, the disease is endemic and outbreaks occur due to serotypes O, Asia1, A(A22), and C. Outbreaks due to type C have not been reported during the last 3 years. In a situation like in India, it is important to monitor regularly the antigenic nature of the isolates causing outbreaks in relation to respective vaccine virus strains. The serological tests like complement fixation, neutralization and ELISA, based on polyclonal sera raised against reference vaccine virus, do not recognize minor antigenic changes. MAbs have great potential in determining minor antigenic differences between the isolates as they are epitope-specific.

Analysis of MAR mutants which plays a central role in the identification of antigenic sites/epitopes in picornaviruses (Mateu, 1995) has helped in identifying several

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Abbreviations: AEI = acetylethyleneimine; BVS = bovine vaccinate serum; ELISA = enzyme-linked immunosorbent assay; FMD = foot-and-mouth disease; FMDV = FMD virus; GPS = guinea pig serum; MAb = monoclonal antibody; MAR = monoclonal antibody-resistant; TR = trypsin-resistant; TS = trypsin-sensitive; TT-146 S = trypsin-treated virus

epitopes and their critical amino acid residues in types O (Xie *et al.*, 1987), A (Thomas *et al.*, 1988a; Bolwell *et al.*, 1989; Baxt *et al.*, 1989), and C of FMDV (Mateu *et al.*, 1987, 1989, 1990). Recently, Sanyal *et al.* (1997) have reported 4 TS antigenic sites on type Asia1 FMDV using MAR mutants.

Results with type O FMDV (O1 K) have identified 5 functionally independent neutralizable antigenic sites (Xie *et al.*, 1987; McCullough *et al.*, 1987; Kitson *et al.*, 1990; Crowther *et al.*, 1993), viz. three (one TR and two TS) sites on VP1, and one (TR) site each on VP2 and VP3 of the virus. The critical amino acids involved in the formation of important antigenic sites on VP1 are 144, 148, 149, 154, and 208. In A22 FMDV, Bolwell *et al.* (1989) have identified as critical amino acids Gly_{149} and Arg_{154} (an equivalent position of Gly_{148} and Arg_{153} in this study) on VP1 in the formation of the most important antigenic site by sequencing MAR mutants.

In the present study, we report characterization of MAbs to an Indian strain (IND17/77) of type A (subtype A22) FMDV and identification of two virion conformation-independent TS epitopes involving critical aa 148 and 153 of VP1 and a conformation-independent TR epitope on VP3 of FMDV.

Materials and Methods

Reference virus and antigens. FMDV type A (subtype A22) IND17/77 strain, used in vaccine manufacture by Indian Veterinary Research Institute, Mukteswar-Kumaon, was used as the reference virus in the study. The virus was propagated in BHK-21 cell line. The whole virus particles (146 S particles) were prepared by CsCl density gradient centrifugation (Wagner *et al.*, 1970), and concentration of 146 S particles was estimated using the extinction coefficient of 76 (Doel and Baccarini, 1981). Trypsin-treated virus (TT-146 S) particles were prepared as described by Sanyal *et al.* (1997). Formaldehyde inactivation of 146 S particles was done as per Barteling and Vreeswijk (1991), and acetylethyleneimine (AEI) inactivation of 146 S particles was done as per Cowan (1968).

Field isolates. Nine type A (subtype A22) isolates originating from FMD outbreaks in different parts of India were used in the form of infected BHK-21 cell culture fluid.

Polyclonal antisera. Anti-146 S sera against the reference virus (IND17/77) were raised in rabbits and guinea pigs (Pattnaik *et al.*, 1991). A post-vaccination (21 days) serum against the reference virus was raised in hill bulls using monovalent formaldehyde-inactivated aluminium hydroxide gel vaccine.

MAbs. Five mouse MAbs produced against AEI-inactivated 146 S particles of the reference virus were used in the study (Table 1). The method of production of the MAbs was similar to that described previously for FMDV serotype Asia1 (Sanyal *et al.*,1997). Isotype of the MAbs was determined by antibody capture ELISA using anti-mouse isotype antibodies (Sigma). The mouse immunoglobulin (Ig) concentration in the MAbs was estimated by indirect ELISA (McCullough *et al.*, 1987). The MAbs were used in the form of cell hybrid (hybridoma) supernatant.

MAb purification and biotinylation. A MAb was purified using Avidchrome Protein A Antibody Purification Kit (Sigma) and the obtained IgG (IgG2b) was conjugated with biotinamidocaproate-N-hydroxysulfosuccinimide ester (BAC-sulfoNHS) using Immunoprobe Biotinylation Kit (Sigma).

Virus neutralization test was carried out using BHK-21 cell culture system (Rweyemamu *et al.*, 1978), and the log values of neutralization index (log NI) of the MAb/serum were estimated.

ELISA. The reactivity of the MAbs with native 146 S particles and TT-146 S particles was determined in indirect (untrapped antigen) and sandwich (trapped antigen) ELISAs (McCullough *et al.*, 1985). The affinity of the MAbs to inactivated 146 S particles was determined in indirect ELISA. All the antigen preparations were used at concentrations equivalent to 2 μg of complete virus (146 S) particles per ml.

Antibody competition ELISA. The competing ability of anti-FMDV BVS and anti-146 S guinea pig serum (GPS) with the MAbs for binding to the 146 S antigen was assessed in an antibody competition ELISA (Thomas *et al.*, 1988b). The percentage of competition by the sera for binding to the virus in the presence of a MAb i.e., the binding inhibition of a MAb in the presence of competing sera was estimated as follows:

$$\% \text{ of competition} = 100 - \left[\frac{\text{A of MAb + competitor antibody}}{\text{A of MAb}}\right] \times 100$$

Polyacrylamide gel electrophoresis (PAGE) and Western blot analysis of viral proteins were performed according to Laemmli (1970) and Mateu *et al.* (1987), respectively.

Selection and plaque purification of MAR mutants. MAR mutants were selected by the method described by Crowther *et al.* (1993) with some modifications (Sanyal *et al.*, 1997). The mutant populations thus selected were plaque purified (Das *et al.*, 1997), and several plaques were picked up from each population. The reactivity of each plaque with the selecting MAb was assessed in sandwich ELISA (Samuel *et al.*, 1991).

Reverse transcription-polymerase chain reaction (RT-PCR). RNA was extracted by guanidine isothiocyanate method using RNeasy Total RNA Isolation Kit (Qiagen). Two oligonucleotide primers (pNK61 and A-$1C_{562}$) reported earlier (Knowles and Samuel, 1994) were used for RT-PCR amplification of the 1D genomic region of viral RNA following the method described earlier (Tosh *et al.*, 1997). A PCR product of approximately 860 bp was obtained.

Nucleotide sequencing. The PCR products (cDNA copies of 1D gene) were purified using Wizard PCR Prep Kit (Promega) to remove residual primers, free nucleotides and enzymes. The cycle sequencing of 1D gene with Cycle Sequencing Kit (Pharmacia) was performed (Knowles and Samuel, 1994) in a thermocycler (Hybaid, UK). The cycle sequenced samples were electrophoresed in 6% polyacrylamide gel using CastAway Precast Sequencing Gels (Stratagene). After electrophoresis, the gels were stained with silver nitrate (Silver Staining Kit, Promega).

MAb-profiling of field isolates. The MAb-profiling technique of Samuel *et al.* (1991) was used for assessing the reactivity of the MAbs with the field isolates and percent relationship values were calculated.

Table 1. Characteristics of the MAbs against FMDV A22 subtype used in this study

MAbs	Isotype	Conc. (μg/ml)	VNT (log NI)	*Reactivity in ELISAs				Western blot status
				Indirect		Sandwich		
				146 S	TT-146 S	146 S	TT-146 S	
27 S	IgG2b	0.2	3.5	3.0	–	2.7	–	VP1
37 S	IgG2b	0.12	3 5	2.7	–	3.0	–	VP1
85 S	IgG2b	0.16	2.5	0.9	–	0.9	–	VP1
143 S	IgG2b	0.58	4.5	1.2	–	1.2	–	VP1
145 S	IgG2a	1.00	2 0	1.8	1.8	1.8	1.6	VP3

*Reactivity expressed as negative log of dilution of MAb showing 50% of the maximum A_{490}.
VNT = virus neutralization test; TT-146 S = trypsin-treated 146 S particles.

Table 2. Reaction profile of the MAR mutants in sandwich ELISA and cross-neutralization test

Selecting MAb	MAR mutants	MAbs				MAbs			
		27S	37S	85S	143S	27S	37S	85S	143S
		ELISA				Cross-neutralization			
27S	Plq. 3.1	P	P	C	C	+	+	–	–
	Plq. 4 1	C	C	C	C	–	–	–	–
37S	Plq. 3	P	P	C	C	+	+	–	–
	Plq. 11	P	P	C	C	+	+	–	–
85S	Plq. 6.1	P	P	C	C	++	++	–	–
	Plq. 7.1	P	P	C	C	++	++	–	–
143S	Plq. 3 4	P	P	C	C	++	++	–	–
	Plq. 4.1	P	P	C	C	++	++	–	–
	Plq. 5.2	P	P	C	C	++	++	–	–

C = complete mutants (< 20% reactivity) (Samuel *et al.*, 1991)
P = partial mutants (20–75% reactivity) (Samuel *et al.*, 1991).
(++) = log NI ł2.0; (+) = log NI = 1.2; (–) = log NI <0.5.

Results

Specificity of the MAbs

The Ig isotyping results showed that four of the five MAbs belong to IgG2b subclass and the remaining one belongs to IgG2a subclass. The mouse Ig concentration in hybridoma supernatants ranged from 0.12 to 1.00 μg/ml and the homologous virus neutralizing activity of the MAbs, as determined by the *in vitro* neutralization test, varied from log NI of 2.0 to 4.5 (Table 1).

Binding of the MAbs to native 146 S (complete virus) particles and TT-146 S particles was assessed in indirect and sandwich ELISAs (Table 1). All the MAbs showed almost equal reactivity as judged from the titers with the whole virus particles in both the ELISAs. However, the MAbs 27S, 37S, 85S, and 143S did not react with the TT-146 S particles and hence were classified as against TS epitope(s) on the virus particle. MAb 145S reacted equally with both the native and TT-146 S particles, hence it was classified as against a TR epitope on the virus particle.

The reactivity of the MAbs to individual viral structural polypeptides was assessed by Western blot analysis. The four MAbs (MAbs 27S, 37S, 85S, and 143S) identifying TS epitope(s) were specific for VP1 and the MAb145 S identifying TR epitope was specific for VP3 polypeptide of the virus (results not shown).

Isolation and characterization of MAR mutants

MAR mutants were isolated on the basis of their ability to grow in the presence of the MAbs identifying TS epitope(s). Nine mutant populations were isolated and their reactivity with homologous as well as heterologous MAbs was assessed in ELISA and virus neutralization test. Reaction profiles of the mutants with the MAbs were analyzed in sandwich ELISA as per Samuel *et al.* (1991). It was observed that both the partial and complete mutants, resistant to MAbs 27S and 37S, did not recognize MAbs 85S and 143S in ELISA (Table 2). However, the mutants, resistant to MAbs 85S and 143S, maintained partial reactivity (32–57%) with the other two MAbs (27S and 37S). A complete mutant population (<10% reactivity), resistant to MAb 27S (Plq. 4.1), failed to react with the remaining three MAbs (37S, 85S, and 143S). In cross-neutralization test, mutants, resistant to MAbs 85S and 143S, were neutralized (log NI ≥2.0) by MAbs 27S and 37S, whereas mutants, resistant to MAbs 27S and 37S, were not neutralized (log NI <0.5) by MAbs 85S and 143S. As expected, the partial mutants (29–64% reactivity), resistant to MAbs 27S (Plq. 3.1) and 37S (Plq. 3 and Plq. 11), as observed in ELISA, were not completely resistant to neutralization by the homologous as well as heterologous (27S/37S) MAbs (log NI = 1.2).

MAb-competition ELISA

Antibody competition ELISA was performed using the biotin-labeled MAb 37S to see whether the MAbs recognize

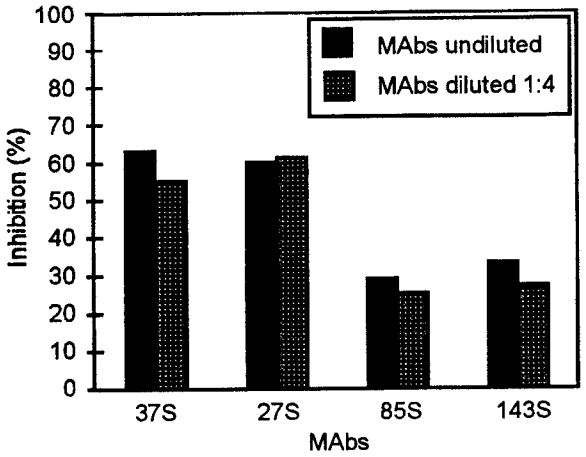

Fig. 1
Binding inhibition of biotinylated MAb 37S by homologous and heterologous MAbs in ELISA

Table 3. Reaction profile of the FMDV A22 subtype field isolates with the MAbs

Field isolates	MAbs				
	27	37S	85S	143S	145S
Reference virus	++	++	++	++	++
IND86/86	++	++	++	++	++
IND99/87	++	++	++	++	++
IND145/87	++	++	++	++	++
IND396/88	++	++	++	++	++
IND125/86	+	+	++	++	++
IND53/86	–	–	–	–	++
IND56/88	–	–	–	–	++
IND61/88	–	–	–	–	++
IND100/88	–	–	–	–	++

(++) = homologous reactivity (⩾76%); (+) = reduced reactivity (20–75%); (–) = no reactivity (<20%).

Table 4. Neutralization of the MAR mutants by BVS

Parent virus/mutants	Log NI with BVS diluted		
	1:32	1:64	1:128
A22 vaccine virus[a]	4.5	3.5	2.5
MAR 27S, Plq. 3.1	3.5	2.5	2.0
MAR 27S, Plq. 4.1	4.0	3.5	2.5
MAR 37S, Plq. 3	3.0	2.5	1.5
MAR 37S, Plq. 11	4.0	3.0	2.5
MAR 85S, Plq. 6.1	4.0	3.5	2.5
MAR 85S, Plq. 7.1	4.5	3.5	2.5
MAR 143S, Plq. 3.4	4.5	3.0	2.5
MAR 143S, Plq. 4.1	4.5	3.5	2.5
MAR 143S, Plq. 5.2	3.0	3.0	2.0

[a]Parent virus.

the same or different epitope(s) in the TS area of VP1. Binding of the biotinylated MAb 37 S to the virus was inhibited by >60% by the homologous MAb as well as MAb 27S, while MAbs 85S and 143S inhibited binding of the biotinylated MAb by 30–33% (Fig. 1).

Sequencing of MAR mutants

The nucleotide sequence at the 3'-end of the 1D genomic region of the parent (reference) virus and the plaque-purified mutants was determined and the deduced amino acid sequence in the VP1 region of aa 140-213 was compared.

All the mutants revealed amino acid replacements in the aa 140-160 of TS region of VP1. The replacements were either at aa 148 or 153. Three mutant populations, resistant to MAb 143S, showed replacement of Arg_{153} by Gly. Two mutant populations, resistant to MAb 85S (Plq. 6.1 and Plq. 7.1), showed replacement of Arg_{153} by Gly/Ser. One partial mutant, resistant to MAb 27S, showed replacement of Arg_{153} by Gly, while its complete mutant showed replacement of Gly_{148} by Glu. The (partial) mutant, resistant to MAb 37S, showed replacement of Arg_{153} by Gly. From these results it is evident that Arg_{153} is not the only critical amino acid involved in binding of MAbs 27S and 37S, as in the case of MAbs 85S and 143S where replacement of Arg_{153} by Gly resulted in complete loss of MAb recognition. However, the mutants did not show any amino acid replacement at another TS region (aa 200-213) of VP1.

MAb-profiling of field isolates

The antigenic variation in field isolates can be better assessed employing a set of MAbs recognizing different epitopes on the virus particle. Conversely, the reaction profiles of field isolates with a set of MAbs in sandwich ELISA (MAb-profiling) can be used to differentiate whether a MAb recognizes the same or different epitope.

A total of 9 field isolates of FMDV serotype A (subtype A22) were subjected to MAb-profiling using all the 5 neutralizing MAbs (27S, 37S, 85S, 143S, and 145S). The results (Table 3) show the percent reactivity (Samuel *et al.*, 1991) of each isolate with the MAb set. All the isolates showed reaction of homology with MAb 145S which identifies a TR epitope on the virus particle. However, the field isolates varied in their reactivity with the four MAbs identifying TS epitope(s). Four isolates, viz. IND 86/86, IND99/87, IND145/87, and IND396/88 showed reaction of homology with all the 5 MAbs, while the isolates IND 53/86, IND56/88, IND61/88, and IND100/88 did not react (<20%) with the MAbs recognizing TS epitope(s) on the virus particle. One of the isolates, IND125/86, showed reaction of homology with MAbs 85S and 143S and a reduced reactivity with MAbs 27S and 37S. This observation revealed that the MAbs (27S, 37S, 85S, and 143S) recognized at least 2 epitopes in the TS region on the

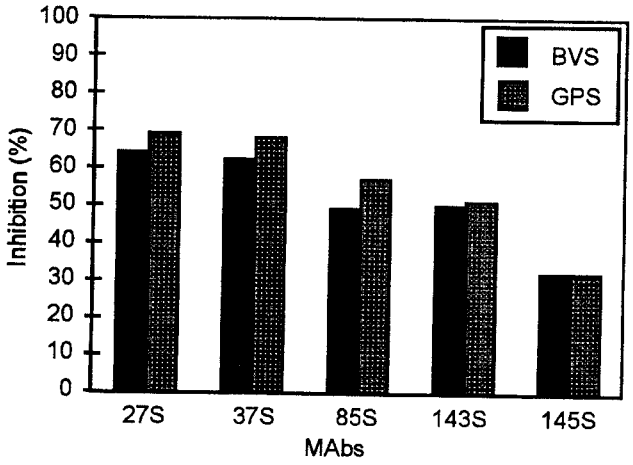

Fig. 2
Binding inhibition of the MAbs in the presence of anti-FMDV polyclonal sera in ELISA

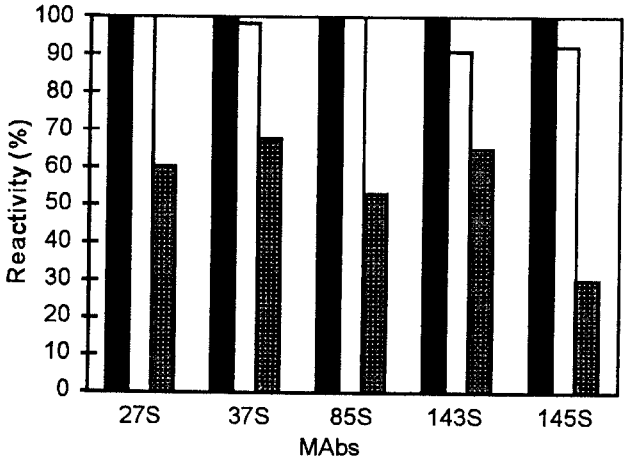

Fig. 3
Effect of inactivation on binding of the MAbs to homologous virus in ELISA
Live 146 S particles (black columns); AEI-inactivated 146 S particles (empty columns); formaldehyde-inactivated 146 S particles (thatched columns).

virus particle in agreement with the results of MAR mutant analysis.

Neutralization of MAR mutants by BVS

Some of the MAR mutants were analyzed in neutralization test using BVS. They were effectively neutralized by BVS (Table 4). This indicates the presence of other epitopes which also elicit virus neutralizing antibodies following vaccination than those identified by the MAbs used in the study.

Polyclonal antibody competition ELISA

The test was performed to assess the ability of convalescent GPS and BVS to inhibit the binding of the MAbs to the virus. It was observed (Fig. 2) that both the GPS and BVS strongly (50–70%) inhibited the binding of the MAbs recognizing TS neutralizable epitope(s), while MAb 145S which identifies a TR epitope was inhibited up to 32% by both GPS and BVS.

Reactivity of MAbs with inactivated whole virus particles

The effect of FMDV inactivants (formaldehyde and AEI) on the structural integrity of the 146 S particles was assessed in indirect ELISA. It was observed that all the MAbs showed a 32–70% reduction in reactivity with formaldehyde-inactivated 146 S particles (Fig. 3) indicating alteration in the TS as well as TR areas exposed on the virion surface following formaldehyde inactivation. Formaldehyde, like trypsin, alters the TS epitopes of FMDV (Capucci *et al.*, 1987). There was no effect of AEI on the binding of the MAbs to the virus.

Discussion

In characterizing the five MAbs produced against FMDV A22 strain we have identified 3 different neutralizing epitopes on the virion surface. Two of these epitopes are TS, conformation-independent and present within the previously identified (Bolwell *et al.*, 1989) major antigenic site (aa 145-154 of VP1) in A22 strain. The other epitope is TR, conformation-independent and present on VP3 of the virus. MAbs 27S, 37S, 85S, and 143S reacted only with 146 S particles but not with TT-146 S particles in both the ELISAs and were specific to VP1 in Western blot analysis, suggesting that they are against TS and conformation-independent epitope(s) on the virus particle. In contrast, MAb 145S reacted equally with both the native 146 S and TT-146 S particles in both the ELISAs and was specific to VP3 in Western blot analysis, suggesting that it recognizes a TR, conformation-independent epitope on the virus particle.

MAbs and MAR virus mutants provide an excellent means for localization of antigenic sites/epitopes on the virion surface (Meloen *et al.*, 1983; Baxt *et al.*, 1984; Stave *et al.*, 1986; Xie *et al.*, 1987; Bolwell *et al.*, 1989; Mateu *et al.*, 1990; Crowther *et al.*, 1993). The analysis of the MAR mutants in ELISA and cross-neutralization test showed that MAbs 27S/37S and 85S/143S identified two overlapping epitopes on the virion surface. The non-reactivity of the mutants, resistant to MAbs 27S/37S, with MAbs 85S/143S and the partial reactivity of the mutants, resistant to MAbs 85S/143S, with MAbs 27S/37S in ELISA revealed that the epitope 85S/143S lies within the (larger) epitope 27S/37S.

This is further evident from the results of MAb-competition ELISA in which binding of the biotinylated MAb 37S to the virus particles was inhibited only up to 30% by MAbs 85S/143S.

Partial sequencing of the 1D gene corresponding to aa 140-213 of VP1 identified replacements/substitutions of amino acids in the VP1 aa 140-160 region of the MAR mutants. The complete mutants resistant to MAbs 85S and 143S revealed a replacement at aa 153 (Arg_{153} by Gly/Ser). The partial mutants, resistant to MAbs 27S (Plq. 3.1) and 37S (Plq. 11), which had no affinity for MAbs 85S and 143S also revealed a replacement at aa 153 (Arg_{153} by Gly). These observations suggest that (1) Arg_{153} is solely critical for the epitope 85S/143S, (2) in addition to Arg_{153}, the epitope 27S/37S involves participation of other amino acids and therefore the replacement at aa_{153} maintained partial reactivity with the homologous MAb (27 S/37 S), and (3) the epitope 85S/143S is located within a larger epitope 27S/37S and therefore partial mutants selected with MAbs 27S and 37S showed no affinity for MAbs 85S/143S. One complete mutant population, resistant to MAb 27S (Plq. 4.1), which also did not react with MAbs 37S, 85S, and 143S, showed a replacement at aa_{148} (Gly_{148} by Glu). This suggests that, in addition to Arg_{153}, Gly_{148} is required for the formation of the epitope 27S/37S, and the replacement at aa_{148} results in elimination of both the epitopes. However, Bolwell *et al.* (1989) have reported the amino acid changes Gly→Arg and Arg→Lys in VP1 in the corresponding positions in type A22 Iraq 24/64 strain using MAR mutants. It has been reported that, in FMDV type C, a single critical amino acid replacement in the FMDV loop at position 146 of VP1 resulted in the loss of many conformation-independent epitopes within the region (Mateu *et al.*, 1990).

Bolwell *et al.* (1989) reported that the most important antigenic site on type A22 FMDV lies within the VP1 aa 145-154. The involvement of C-terminus (aa 200-213) of VP1, either independently or in association with its aa 140-160 region, in the formation of an antigenic site has been reported for FMDV type O (Xie *et al.*, 1987) and subtypes A12 (Baxt *et al.*, 1989) and A10 (Thomas *et al.*, 1988a). However, in the present study, none of the variants/mutants showed an amino acid substitution at the C-terminus of VP1 in agreement with the observation made by Bolwell *et al.* (1989).

MAb-profiling of the field isolates showed that the TR epitope identified by MAb 145S is conserved across the isolates. The reactivity pattern of the field isolates also revealed that the epitope, identified by MAbs 27S and 37S, is different from that identified by MAbs 85S and 143S, similarly to the case of the IND 125/86 isolate. This observation is in agreement with that obtained in the MAR mutant study.

In order to understand the importance of MAb-binding epitopes in eliciting virus-neutralizing antibody response following vaccination/infection, the ability of a BVS and convalescent GPS to inhibit binding of the MAbs to the virus was assessed (Thomas *et al.*, 1988b). It was observed that the antibodies against both the TS epitopes (27S/37S and 85S/143S) were better represented, as compared to the TR epitope identified by MAb 145S, in both GPS and BVS. The inhibition of binding of a MAb to the virus depends on the concentration of antibodies against the particular MAb-binding site in the polyclonal serum (Thomas *et al.*, 1988b; Pattnaik *et al.*, 1996). Therefore, a higher level of inhibition effected by the competing sera (BVS and GPS) on binding of the MAbs identifying TS epitopes to the virus as compared to the MAb identifying TR epitope, suggests dominance of the former epitope (TS epitope) in eliciting neutralizing antibody response following vaccination/infection. Accordingly, the present observation of alteration in the TS epitopes following formaldehyde inactivation of the virus might be the factor responsible for the reduced immunogenicity of formaldehyde-inactivated FMD vaccines. However, the efficient neutralization of the MAR mutants by BVS indicates that there are other epitopes on the virus which also elicit a neutralizing antibody response in animals following vaccination.

The characterization of the antigenic site(s) involved in neutralization of A22 subtype FMDV is far from complete due to availability of limited number of MAbs. Further work using a larger number of MAbs may identify additional antigenic sites on the virion surface.

Acknowledgements. We are thankful to the Director, Indian Veterinary Research Institute, and Indian Council of Agricultural Research for providing necessary facilities to carry out this work. We are also thankful to Drs. N.J. Knowles and A.R. Samuel of World Reference Laboratory for FMD, BBSRC Institute for Animal Health, Pirbright, Surrey, U.K. for providing oligonucleotide primers used in this study.

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