Available online at www.sciencedirect.com
ScienceDirect
Veterinary Immunology and Immunopathology 122 (2008) 241–249
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Development and characterization of monoclonal antibodies against FMD virus type Asia-1 and determination of antigenic variations in the field strains

Samir Kumar Rana [a,*], Tamishraha Bagchi [b]

[a] *Research & Development, National Dairy Development Board, Anand 388001, Gujarat, India*
[b] *Department of Microbiology and Biotechnology Centre, MS University of Baroda, Vadodara 390002, Gujarat, India*

Received 1 August 2007; received in revised form 13 November 2007; accepted 8 January 2008

Abstract

Twelve mouse monoclonal antibodies (MAbs) were developed against an Indian vaccine strain of foot and mouth disease virus (FMDV) type Asia-1 WBN 117/85. The MAbs were tested for their ability to bind to whole virus particle, trypsin-treated 146S (TT-146S) virus particle, sub-viral (12S and disrupted virus) antigens by ELISA and to neutralize virus infectivity in cell culture. Extensive characterization of MAbs revealed the existence of three different groups based on the binding of non-overlapping epitopes. Eight type Asia-1 specific MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11 and RC10/M), which formed group 1 (G1), were found to bind a neutralizing, trypsin-sensitive (TS) and conformational epitope. Two MAbs (WB8 and WC3) in group 2 (G2) were found to bind a non-neutralizing, trypsin-resistant, conformational and 12S-specific epitope, which was intertypically conserved in all the four serotypes of FMDV (O, A, C and Asia-1) prevalent in India. Two MAbs (KG10 and KF10), which formed group 3 (G3), were found to be against a non-neutralizing, TS and conformational epitope, common to types Asia-1 and A. Members of G1 were IgG2a isotype, while those of G2 and G3 were IgG1 and IgG2b isotypes, respectively. Antigenic analysis of 31 FMDV type Asia-1 field isolates and two vaccine strains, using a panel of type Asia-1-specific MAbs, revealed antigenic similarity of the virus isolates tested and non-existence of neutralization escape mutants. The developed MAbs have practical utility, especially in the manufacture of FMD vaccine, diagnosis and FMDV characterization.
© 2008 Elsevier B.V. All rights reserved.

Keywords: Foot and mouth disease virus; Monoclonal antibodies; Neutralizing activity; Serotype Asia-1; TS epitope

1. Introduction

Foot and mouth disease (FMD) is a highly contagious and acute viral infection of cloven-footed animals. It is caused by the FMD virus, which belongs to the genus apthovirus in the family Picornaviridae and exists as seven antigenically distinct serotypes, *viz*. O, A, C, Asia-1, SAT-1, SAT-2 and SAT-3. Incidence of types O, A and C has been recorded in different parts of the world; however, incidence of types Asia-1 and SAT-1 to 3 is mainly restricted to Asia and southern Africa, respectively. In India, outbreaks due to three serotypes, *viz*. O, A and Asia-1 are common; but outbreaks due to type C have not been recorded in India since 1995.

Intact FMDV is composed of 60 copies each of four structural polypeptides, VP1–VP4 (Bachrach et al., 1977). VP1 is the most exposed polypeptide on the surface of the virion compared to VP2 and VP3, while

* Corresponding author. Present address: Scientist III (R&D), Indian Immunologicals Ltd., Rakshapuram, PO Gachibowli, Hyderabad 500032, India. Tel.: +91 40 64505486; fax: +91 40 23005958.
E-mail address: ranask_1960@yahoo.co.in (S.K. Rana).

0165-2427/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2008.01.001

polypeptide VP4 is located internally (Morrell et al., 1987). The sedimentation coefficient of the intact virion lies between 140S and 146S (Bradish et al., 1952). VP1 is reported to harbor major immunogenic sites as trypsin treatment of the whole virus particle results in cleavage of VP1 with a drastic reduction in infectivity and immunogenicity (Wild et al., 1969). The amino acid region 141–160 of VP1 forms antigenic sites, the main target of virus-neutralizing antibodies (McCullough et al., 1987; Mateu et al., 1990). In addition to the antigenic sites on VP1, several other antigenic sites have also been identified on VP2 and VP3 of FMDV types O, A and C (Xie et al., 1987; Lea et al., 1994). VP1 is more variable, whereas VP2 is relatively conserved (Jackson et al., 2003; Freiberg et al., 2001). Most FMDV-neutralizing MAbs recognize conformational epitopes within the 140–160 amino acid region of VP1. At least four independent trypsin-sensitive (TS), neutralizing antigenic sites are reportedly present on FMDV type Asia-1; all these sites being dependent on the native conformation of the virus. When compared to other types (O, A and C), type Asia-1 has more TS-neutralizing sites on its surface (Sanyal et al., 1997).

MAbs can contribute towards a better understanding of the antigenic structure of FMDV, particularly the important epitopes involved in conferring protection; therefore, this study was undertaken to develop and characterize MAbs against FMDV type Asia-1 (WBN-117/85). Subsequently, reaction profiles of these MAbs were studied with representative field isolates of serotype Asia-1 to determine the binding specificity of the MAbs with different isolates and establish the minor antigenic variation among the field virus isolates in India.

2. Materials and methods

2.1. Virus and antigen

Three vaccine strains of FMDV type Asia-1, *viz.* WBN-117/85, IND-63/72 and IND-8/79, were obtained from Indian Immunologicals Ltd., Hyderabad, India. FMDV type Asia-1 WBN-117/85 was used to develop the MAbs. This strain was propagated in a BHK-21 cell line, inactivated using binary ethyleneimine (BEI) and the 146S antigen was purified by CsCl density gradient centrifugation (Bachrach et al., 1964).

The trypsin-treated 146S (TT-146S) particle of type Asia-1 WBN-117/85 was prepared as per Barteling et al. (1979) with a few modifications. Concentration of purified 146S was adjusted to 200 μg/ml in PBS (pH 7.2) and 50 μl of trypsin solution (2 mg/ml) was added and the mixture was incubated at 37 °C for 15 min.

To prepare the 12S subunit, the concentration of 146S was adjusted to 200 μg/ml and treated with 1 M HCl for 15 min at room temperature, followed by addition of 1 M Tris (10%, v/v).

During preparation of the disrupted antigen, the 146S antigen concentration was adjusted to 200 μg/ml in PBS; subsequently, SDS (10% stock) and 2-mercaptoethanol were added at the rate of 10 and 2%, respectively. The mixture was incubated in a boiling water-bath for three min.

2.2. Field isolates

Thirty-one FMDV field isolates of type Asia-1, recovered from clinical cases of FMD-infected cattle and buffaloes in 11 geographically distinct states of India over a period of 14 years (1987–2000), were adapted to the BHK-21 cell line. Virus-infected cell culture supernatants were used (Table 1).

2.3. Development of monoclonal antibodies

2.3.1. Immunization of mice

Six Balb/c mice (6–8 weeks old, female) were immunized with 50 μg of purified 146S antigen of type Asia-1 WBN-117/85. The primary immunization was given intraperitoneally with Freund's complete adjuvant followed by three booster doses at 28-day intervals with Freund's incomplete adjuvant. Immune mice were randomly bled 7 days after the booster immunization and the sera tested by indirect ELISA (McCullough et al., 1985) for the presence of virus-specific antibodies. The mouse showing the greatest antibody response was given the final booster (50 μg/mouse) without adjuvant, intravenously for 3 consecutive days before the day of fusion.

2.3.2. Preparation of hybridoma

Splenocytes from the mouse with the highest antibody titer to 146S antigen of type Asia-1 WBN-117/85 were fused with NS1 mouse myeloma cells, as described by Kohler and Milstein (1975) with a few modifications. Primary screening of hybridomas was done by both indirect and sandwich ELISAs. The secreting polyclones were subcloned twice by a limiting dilution method. Isotyping of the MAbs was carried out using a commercial kit (Sigma, St. Louis, MO, USA). MAbs concentration was estimated by indirect ELISA (McCullough et al., 1987).

Table 1
Details of FMD virus samples of Asia-1 included in the study

Serial number	Laboratory reference number of virus sample	Outbreak date	Origin of the specimen			Species	Specimen collected
			Village	District	State		
1	Asia-1, GUKh-1/87	February 1987	Ravipura	Kheda	Gujarat	C	TE
2	Asia-1, APKh-2/88	August 1988	Nachapalli	Khammam	Andhra Pradesh	B	TE
3	Asia-1, KEC-3/88	August 1989	Taliparamba	Cannanore	Kerala	C	TE
4	Asia-1, KAKo-4/90	January 1990	Hosahudya	Kolar	Karnataka	NA	NA
5	Asia-1, MAP-5/90	December 1990	Wagalwadil	Pune	Maharashtra	C	TE
6	Asia-1, KEC-6/91	September 1991	NA	Cannanore	Kerala	C	TE
7	Asia-1, GUM-7/92	January 1992	Patan	Mehsana	Gujarat	C	FE
8	Asia-1, MPK-8/92	January 1992	Khidikya	Khandwa	Madhya Pradesh	C	TE
9	Asia-1, MPKr-9/92	February 1992	Bhulgonw	Khargove	Madhya Pradesh	C	TE
10	Asia-1, APR-10/92	May 1992	Nandipalli	Rangareddy	Andhra Pradesh	NA	NA
11	Asia-1, MAC-11/92	August 1992	Madheli	Chandrapur	Maharashtra	C	TE
12	Asia-1, KAN-13/93	September 1993	Pandavapur	Mandya	Karnataka	NA	TE
13	Asia-1, TNN-14/93	September 1993	Bokka Farm	Nilgiris	Tamil Nadu	NA	TE
14	Asia-1, GUSk-15/94	February 1994	Lakshipura	Sabarkantha	Gujarat	C	TE
15	Asia-1, WBP-16/94	August 1994	Telidih	Purulia	West Bengal	NA	TE
16	Asia-1, MAB-17/94	September 1994	Goregaon(E)	Mumbai	Maharashtra	B	FE
17	Asia-1, APN-18/94	November 1994	AHC(D.LAB)	Nizamabad	Andhra Pradesh	NA	TE
18	Asia-1, TPA-19/94	November 1994	NA	Agartala	Tripura	C	TE
19	Asia-1, WBN-20/94	November 1994	SLF, Mohanpur	Nadia	West Bengal	C	TE
20	Asia-1, MAA-21/94	December 1994	Koliwadi	Ahmedanagar	Maharashtra	C	TE
21	Asia-1, MAN-22/94	December 1994	Shingave	Nasik	Maharashtra	C	TE
22	Asia-1, KAB-23/94	December 1994	Damlun	Bangalore	Karnataka	C	TE
23	Asia-1, GUKt-24/94	December 1994	Anjar	Kutch	Gujarat	C	TE
24	Asia-1, MAJ-25/95	February 1995	Faizpur	Jalgaon	Maharashtra	C	TE
25	Asia-1, TNDg-26/95	March 1995	Vilanpatti	Dindigul	Tamil Nadu	C	TE
26	Asia-1, GOA-27/95	July 1995	Goa Vehla	North Goa	Goa	C	TE
27	Asia-1, GUR-28/98	May 1998	Peddock	Rajkot	Gujarat	C	TE
28	Asia-1, DED-29/98	March 1998	Gazipur	Delhi	Delhi	NA	TE
29	Asia-1, GUBr-32/99	March 1999	Chathpor	Bharuch	Gujarat	C	TE
30	Asia-1, GUBv-30/99	June 1999	Parvadi	Bhavnagar	Gujarat	C	TE
31	Asia-1, GUSk-31/2000	April 2000	Bayad	Sabarkantha	Gujarat	B	TE

NA = not available; C = cattle; B = buffalo; TE = tongue epithelium; FE = foot epithelium.

2.4. Characterization of monoclonal antibodies

2.4.1. Virus neutralization test

The virus neutralization test was carried out *in vitro* using a BHK-21 cell culture system (Rweyemamu et al., 1978) and the virus neutralization titer of all MAbs against the homologous virus Asia-1 WBN-117/85, as well as against heterologous viruses of three other serotypes (O, A and C), was determined.

2.4.2. ELISA

The reactivity of MAbs with native 146S antigen, TT-146S antigen and disrupted virus (DV) antigen was determined in indirect (untrapped antigen) and sandwich (trapped antigen) ELISAs (McCullough et al., 1985) using a concentration of 2 μg/ml antigen.

2.4.3. Western blotting

The specificity of the MAbs against FMDV polypeptides was determined using the protocol described by Towbin et al. (1979) with certain modifications.

2.5. MAb-profiling of the field virus isolates

A MAb-profiling technique, as described by Samuel et al. (1991), was used to assess their reactivity with selected field isolates. The relationship with the reference strains was expressed as a percentage for the particular MAbs against the reference/homologous virus in the reaction ranges >76, 46–75, 20–45 and <20%. A total of 34 FMDV isolates of Asia-1 serotype, *viz.* the homologous virus WBN-117/85, two other vaccine strains (IND-63/72 and IND-8/79) and 31 field isolates were included. In addition to MAb-profiling, all

Table 2
Characteristics of the MAbs developed against FMD virus

Characteristics	Group 1 (G1)	Group 2 (G2)	Group 3 (G3)
Identification number of MAbs	RF7, RF8, RD10, RE11, RC11, RC10/O, RB11, RC10/M	WB8, WC3	KG10, KF10
Reactivity to 146S of Asia-1	Yes	Yes	Yes
Reactivity to tissue culture supernatant of FMD virus types in ELISA	With Asia-1	With O, A, C and Asia-1	With A and Asia-1
Reactivity to viral polypeptides in Western blot	No	No	No
Reactivity to trypsin-treated 146S of Asia-1	No	No	No
Reactivity to 12S subunit of Asia-1	No	Yes	No
Reactivity to DV antigen of Asia-1	No	No	No
Neutralization of virus in cell culture	Only type Asia-1	No	No
Mouse immunoglobulin isotypes	IgG2a	IgG1	IgG2b

31 type Asia-1 isolates were compared using bovine vaccinate sera (BVS) raised against the homologous vaccine strain (WBN 117/85) in liquid-phase blocking (LPB) ELISA (Hamblin et al., 1986) and *r* values were determined.

3. Results

3.1. Development of monoclonal antibodies

Three stable hybridomas, designated R, W and K, of 46 hybridomas exhibited positive reactivity against FMDV type Asia-1 WBN-117/85 in ELISAs on primary screening. Twelve stable monoclones obtained from three hybridomas after single-cell cloning were given suitable identification numbers, as outlined in Table 2.

3.2. Characterization of monoclonal antibodies

All 12 MAbs were further characterized to determine the isotype, antibody concentration, ability to neutralize FMDV, specificity to the homologous virus type Asia-1 and heterologous virus types, such as O, A and C, plus reactivity with several field isolates.

Isotyping of MAbs showed that eight of the 12 MAbs belonged to IgG2a, two belonged to IgG1 and the remaining two belonged to IgG2b (Table 2). A concentration of 4–6 mg/ml of representative MAbs was obtained when scaled-up and affinity-purified.

To determine specificity, the MAbs were checked for their ability to bind 146S antigen in indirect and sandwich ELISAs. All 12 MAbs reacted with intact 146S antigen of FMDV type Asia-1 WBN-117/85 and OD values ranged from 1.0 to 2.0. The results indicate that all the hybridomas developed were capable of producing antibodies against the epitopes of FMDV type Asia-1.

MAbs were tested in sandwich ELISA to determine their cross-reactivity with three other types, O, A and C, prevalent in India. The whole virus antigen of all four serotypes was trapped using immune rabbit sera against the 146S antigen of the respective serotypes. The results indicated that eight MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11 and RC10/M) tested were type-specific since they reacted only with FMDV type Asia-1 without cross-reacting with any of the other three serotypes of FMDV. In contrast, two MAbs (WB8 and WC3) were found to cross-react with all four serotypes (*viz*. O, A, C and Asia-1) of FMDV in ELISA, indicating that these MAbs recognized a group-specific epitope conserved across all four serotypes. Another two MAbs (KG10 and KF10) were found to react with two serotypes (*viz*., Asia-1 and A), indicating that these MAbs recognized an epitope common to both serotypes.

3.2.1. Identification of neutralizing MAbs

Efficacy of MAbs to neutralize all four serotypes (O, A, C and Asia-1), when tested in a micro-serum neutralization test, indicated that eight MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11 and RC10/M) were capable of neutralizing FMDV type Asia-1, with neutralization indices ranging from 1.5 to 1.8. However, none of these MAbs could neutralize the other three serotypes. The remaining four MAbs (WB8, WC3, KG10 and KF10) failed to neutralize any of the four serotypes tested.

3.2.2. Reactivity of MAbs with viral polypeptides in Western blotting

On Western blot analysis, none of the MAbs were found to react with the polypeptides of FMDV type Asia-1, indicating that they probably recognize the conformational epitope but not the linear epitope.

3.2.3. Reactivity of MAbs to TT-146S antigen

Ten MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11, RC10/M, KG10 and KF10) of total 12 could not bind with TT-146S antigen of the homologous virus when tested in indirect ELISA. The reactivity of two MAbs (WB8 and WC3) was found to marginally reduce when reacted with TT-146S antigen in comparison to intact 146 virus particle of FMDV type Asia-1 WBN-117/85.

Reactivity of all eight MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11 and RC10/M) was further assessed by allowing a serially diluted hybridoma supernatant to react with a constant dose of virus in sandwich ELISA. No variation in the reactivity with TT-146S was observed against serially diluted MAbs. However, reactivity with whole virus particle gradually declined at higher dilutions of MAbs, confirming that all these MAbs were against TS epitope and reactive in both indirect and sandwich ELISA formats.

3.2.4. Reactivity of MAbs with 12S subunit

When reactivity of the MAbs was assessed with 12S subunit of FMDV type Asia-1 by sandwich ELISA, 10 MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11, RC10/M, KG10 and KF10) did not exhibit any reactivity. However, two MAbs (WB8 and WC3) exhibited comparable reactivity with both 12S subunit and intact 146S antigens.

3.2.5. Reactivity of MAbs with DV antigen

None of the 12 MAbs was found to react with DV antigen of FMDV type Asia-1 in both sandwich and indirect ELISAs. In a further investigation, eight neutralizing MAbs were subjected to 11 serial, twofold dilutions in sandwich ELISA to assess their reactivity with the 146S and 12S subunit, as well as with DV antigens. Reactivity of MAbs with 12S and DV antigens could not be detected in any of the dilutions. However, reactivity with whole virus particle gradually declined at higher dilutions of MAbs, further confirming that these eight MAbs (RF7, RF8, RD10, RE11, RC11, RC10/O, RB11 and RC10/M) were against 146S-specific conformational epitope. The characteristics of the MAbs are summarized in Table 2.

On the basis of above findings, the 12 MAbs could be divided broadly into three groups. Eight type Asia-1 specific MAbs, *viz.* RF7, RF8, RD10, RE11, RC11, RC10/O, RB11 and RC10/M against neutralizing, TS and conformational epitope formed group 1 (G1). Two MAbs, *viz.* WB8 and WC3 against non-neutralizing, trypsin-resistant and 12S-specific epitope conserved in all four serotypes formed group 2 (G2). The other two MAbs, *viz.* KG10 and KF10 against non-neutralizing, TS and conformational epitope common to types Asia-1 and A formed group 3 (G3) (Table 2).

3.3. MAb-profiling of Asia-1 field isolates

A total of 34 FMDV type Asia-1 isolates, including the homologous strain, were subjected to MAb-profiling with the MAbs of G1, MAbs of G2 and MAb KG10 of G3 (Table 2). Reactivity values of each virus versus those of the parent/reference virus with the MAb panels, expressed as a percentage, are depicted in Fig. 1. The reaction profile was divided into four ranges on the basis of percentage reactivity of the virus isolates with different MAbs, as described by Samuel et al. (1991). This simplified approach enabled us to divide the isolates into two groups based on the reactivity spectrum with the MAb panel.

Twenty-nine of the 34 (85.3%) virus isolates exhibited homologous affinity (>76%) with most of the MAbs from G1. Five field isolates, three from the State of Gujarat (GUSk-31/2000, GUBr-32/99 and GUR-28/98) and one each from the States of Maharashtra and Goa (MAC-11/92 and GOA-27/95) showed reactivity ranging from 46 to 75%. No isolate could be identified which was non-reactive to this MAb panel. More than 76% reactivity was found in 85 and 88% of the Asia-1 field isolates with MAbs WB8 and WC3, respectively. The remaining isolates exhibited reduced reactivity (46–75%) with both WB8 and WC3 of G2. All the 34 Asia-1 isolates, except one, displayed homologous affinity to another non-neutralizing MAb KG10, exhibiting reactivity >76%. Only one isolate collected from Gujarat (GUR-28/98) exhibited marginally reduced reactivity (46–75%) with this MAb. On strain differentiation, significance of r values was interpreted as per the criteria laid down by Samuel et al. (1990) and all the field isolates tested were found to be homologous to the vaccine strain, Asia-1 WBN 117/85 (Fig. 1).

4. Discussion

In this study, a panel of 12 FMDV type Asia-1 specific MAbs were produced and characterized using various ELISA, neutralization and Western blotting techniques. Eight 146S-specific MAbs that formed G1 were found to have reasonable neutralizing ability, as evident from the neutralization indices (1.5–1.8). However, the remaining four MAbs belonging to G2 and G3 were non-neutralizing.

Sr.		Monoclonal antibodies											'r' values
No.	FMD Virus isolates	RF7	RF8	RD10	RE11	RC11	RC10/O	RB11	RC10/M	WB8	WC3	KG10	in LPB
1	Asia-1, WBN-117/85	●	●	●	●	●	●	●	●	●	●	●	1.00
2	Asia-1, GUKh-1/87	●	●	●	●	●	●	●	●	●	●	●	1.00
3	ASIA-1, APKh-2/88	●	●	●	●	●	●	●	●	●	●	●	1.00
4	ASIA-1, KEC-3/88	●	●	●	●	●	●	●	●	●	●	●	1.00
5	ASIA-1, KAKo-4/90	●	▲	●	●	●	●	▲	●	●	●	●	0.79
6	ASIA-1, MAP-5/90	●	●	●	●	●	●	●	●	●	●	●	1.00
7	ASIA-1, KEC-6/91	●	●	●	●	●	●	●	●	▲	●	●	1.00
8	ASIA-1, GUM-7/92	●	●	●	●	●	●	●	●	●	●	●	1.00
9	ASIA-1, MPK-8/92	●	●	●	●	●	●	●	●	●	●	●	0.70
10	ASIA-1, MPKr-9/92	●	●	●	●	●	●	●	●	●	●	●	0.48
11	ASIA-1, APR-10/92	●	●	●	●	●	●	●	●	●	●	●	1.00
12	ASIA-1, MAC-11/92	▲	▲	▲	▲	▲	▲	▲	▲	●	●	●	1.00
13	ASIA-1, KAN-13/93	●	●	●	●	●	●	●	●	●	●	●	1.00
14	ASIA-1, TNN-14/93	●	●	●	●	●	●	●	▲	●	●	●	1.00
15	ASIA-1,GUSk-15/94	●	●	●	●	●	●	●	●	●	●	●	0.68
16	ASIA-1, WBP-16/94	●	●	●	●	●	●	●	●	●	●	●	1.00
17	ASIA-1, MAB-17/94	●	●	●	●	●	●	●	●	●	●	●	1.00
18	ASIA-1, APN-18/94	●	●	●	●	●	●	●	●	●	●	●	1.00
19	ASIA-1, TPA-19/94	●	●	●	●	●	●	●	●	●	●	●	1.00
20	ASIA-1, WBN-20/94	●	●	●	●	●	●	●	●	●	●	●	1.00
21	ASIA-1,MAA-21/94	●	●	●	●	●	●	●	●	▲	▲	●	1.00
22	ASIA-1, MAN-22/94	●	●	●	●	●	●	●	●	▲	▲	●	1.00
23	ASIA-1, KAB-23/94	●	●	●	●	●	●	●	●	●	●	●	1.00
24	ASIA-1,GUKt-24/94	●	●	●	●	●	●	●	●	▲	▲	●	0.89
25	ASIA-1, MAJ-25/95	●	●	●	●	●	●	●	●	●	●	●	0.89
26	ASIA-1, TNDg-26/95	●	●	●	●	●	●	●	●	●	●	●	1.00
27	ASIA-1, GOA-27/95	●	●	▲	▲	▲	▲	▲	●	●	●	●	0.83
28	ASIA-1,GUR-28/98	▲	▲	▲	●	▲	▲	▲	●	●	●	▲	0.55
29	ASIA-1,DED-29/98	●	●	●	●	●	●	●	●	●	●	●	1.00
30	ASIA-1,GUBr-32/99	▲	▲	▲	▲	▲	▲	▲	●	●	●	●	1.00
31	ASIA-1,GUBv-30/99	●	●	●	●	●	●	▲	●	●	●	●	1.00
32	ASIA-1, GUSk-31/2000	▲	▲	▲	▲	▲	▲	▲	▲	●	●	●	1.00
33	ASIA-1, IND-63/72	●	●	●	●	●	●	●	●	▲	▲	●	Not Done
34	ASIA-1, IND-8/79	●	▲	●	●	●	●	●	●	●	●	●	Not Done
	● : 76 % and above reactivity			▲ : 46-75 % reactivity				LPB = Liquid phase blocking ELISA					

Fig. 1. Reaction profile of Asia-1 isolates with the MAb panels and their *r* values in LPB ELISA.

Relative reactivity of the MAbs towards intact viral and sub-viral antigens in ELISA generally builds the basis for classification and nature of the antigenic sites recognized by the MAbs (Xie et al., 1987). All eight virus-neutralizing MAbs that belonged to G1 were reactive only with intact 146S antigen but not with TT-146S antigen and sub-viral particle. This implies that MAbs of G1 recognize the TS-neutralizing site only, the binding of which depends upon the native conformation of the virus. Failure of this group of MAbs to react with the DV antigen on Western blot further proved the conformational nature of the epitope recognized. It is presumable that the antigenic site identified by the MAbs that belonged to G1 may be one of the four TS neutralizable sites reported by Sanyal et al. (1997). The presence of similar conformational and TS sites on FMDV types O and C were reported by Crowther et al. (1993) and Mateu et al. (1990), respectively. Neutralizing and conformational independent epitopes have also been reported (Sanyal et al., 1997), although conformational-dependent and -neutralizing epitopes are predominantly present on type Asia-1 virus. Grazioli et al. (2003) reported FMDV type Asia-1 MAbs specifically recognizing neutralizing, TS and linear epitope in the G–H loop of VP1. However, MAbs recognizing similar antigenic sites on type Asia-1 could not be identified in this study.

Under these circumstances, it would appear that the MAbs of G1 are probably against the neutralizing epitope in VP1 rather than the linear epitope. However, mapping of this epitope may be possible by isolating

and sequencing the neutralization escape mutants against these MAbs.

Surprisingly, MAbs belonging to the G2 recognizing, non-neutralizing epitopes were found to have a very marginally reduced binding affinity with TT-146S antigen of the homologous type. In addition, they also reacted with the 12S subunit of type Asia-1 virus but failed to react with DV antigen. The MAbs also failed to react with denatured antigen on Western blot, suggesting the conformational nature of the epitope that requires the integrity of the virion be maintained. MAbs WC3 and WB8 were found to react with all four serotypes (O, A, C and Asia-1) prevalent in India. Although the reactivity of the MAbs belonging to G2 with FMDV types O, A, C and Asia-1 agrees with the findings of Freiberg et al. (2001) and Yang et al. (2007), the epitope recognized by the MAbs in G2 may not be the same on VP2 as described by them. It is likely that the MAbs of G2 may recognize epitopes common to 146S and 12S, as described by Butchaiah and Morgan (1997).

The cross-reactive ability of MAb WC3 (G2) has been further demonstrated with 23 strains of serotype O, 23 strains of serotype A, 13 strains of serotype C and 52 strains of serotype Asia-1 originating from different parts of the country (data not shown). Yang et al. (2007) also reported the MAbs as showing cross-reactivity with FMDV types O, A, C and Asia-1. The reactivity profiles of MAbs WC3 and WB8 appear to be unique and, to the best of our knowledge, reported for the first time on type Asia-1. Further studies are required to precisely determine the nature and location of the antigenic site on the FMDV structural protein(s) recognized by the MAbs WC3 and WB8.

Interestingly, a couple of MAbs of G3 (KG10 and KF10) recognized a TS, non-neutralizing and conformational epitope on FMDV types Asia-1 and A (Table 3). Similar observation was also reported by Marquardt and Freiberg (2000) for serotype Asia-1 and other FMDV serotypes. Taken together, these findings support the fact that TS, non-neutralizing and conformational epitope may reside at the N-terminus of VP2, as reported by Freiberg et al. (1999) and Marquardt and Freiberg (2000). The N-terminus of VP2 was found to be disordered in the virus crystals (Acharya et al., 1989), indicating its location on the virus surface, which is a prerequisite for susceptibility to trypsin.

4.1. MAb-profiling of FMDV type Asia-1 field isolates

Antigenic variation in the field isolates can be better assessed by analysis of antigenic sites, employing a panel of type-specific MAbs recognizing different epitopes on the virus particle. A total of 34 FMDV type Asia-1 isolates, including the homologous vaccine strain, were subjected to MAb-profiling with a panel of 11 type Asia-1 specific MAbs in sandwich ELISA and all isolates were found to react well with the group of neutralizing MAbs. This indicates that the epitope/site recognized by the panel of MAbs that formed G1 may be common to all isolates, including the three type Asia-1 vaccine strains, *viz*. WBN 117/85, IND-8/79 and IND-63/72. The similar reactivity pattern of all eight MAbs of G1 with most of the type Asia-1 isolates, except a few, suggests the existence of a type-specific epitope on all the type Asia-1 isolates tested, which was uniformly recognized by all these MAbs.

In the reaction with non-neutralizing MAbs of G2 (WC3 and WB8), the majority of isolates exhibited homologous reactivity and none showed a degree of reduced affinity (<45%). This reaction pattern indicates that the intertypically conserved, non-neutralizing site/epitope recognized by these MAbs is conserved in all the isolates of FMDV type Asia-1 in the country.

The reaction pattern of another non-neutralizing MAb of G3 (KG10) with all the 34 Asia-1 isolates confirms the high level of conservation of the epitope located on VP2 of all the isolates collected from various regions of the Indian subcontinent. Although MAbs WC3, WB8 and KG10 are against the intertypically conserved, non-neutralizing epitope, it appears that the epitope recognized by MAb KG10 is different from that recognized by MAbs WB8 and WC3.

To understand whether the relationship of virus isolates, demonstrated by the MAbs, is reflective of the reactivity against BVS, a similar MAb-profiling study was conducted using field isolates and reference vaccine strain in LPB ELISA and *r* values were calculated. The results of LPB ELISA showed that all strains had a good serological relationship with the vaccine strain Asia-1 WBN-117/85. The *r* values were in the range 0.48–1.00 with a mean *r* value of 0.92, indicating the antigenic similarity among the Indian type Asia-1 field isolates. This is in agreement with the MAb-profiling study of Sanyal et al. (2003).

In the present study, two unique sets of MAbs could be developed, both of which may have practical utility in the field of FMD vaccine manufacturing and diagnosis. It is widely believed that the intactness of the 146S antigen is essential for immunogenicity of the FMD vaccine since the 146S antigen can undergo proteolytic cleavage, either during manufacture or storage, thereby making the vaccine poorly immunogenic. The MAbs developed against the TS conforma-

tional epitope could be used to quantify the type Asia-1-specific, intact 146S particles and also for in-process quality control (Van Maanen and Terpstra, 1990). These MAbs could also be used for specific diagnosis and serotyping of FMDV type Asia-1, either in the laboratory or the field, using advanced diagnostic tools. It is conceivable that the pan-serotype (O, A, C and Asia-1) specific MAbs (WC3 and WB8) could be used to develop a diagnostic system, similar to that reported by Van Maanen and Terpstra (1990) and Smitsaart et al. (1990), to give a group-specific diagnosis of FMD which would otherwise require a battery of reagents.

The MAbs developed in the present study will be important in the diagnosis and characterization of FMD virus isolates, as well as for the development and quality control of FMD vaccine.

Acknowledgements

We thank the Management of the National Dairy Development Board for providing the necessary facilities to carry out this research work and Indian Immunologicals Ltd. for supplying the vaccine strains of FMD virus type Asia-1. The authors are grateful to Dr. V.A. Srinivasan, Research Director, Indian Immunologicals Ltd. for his valuable suggestions.

References

Acharya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D., Brown, F., 1989. The three-dimensional structure of foot and mouth disease virus at 2.9 Å resolution. Nature 337, 709–716.

Bachrach, H.L., Trautman, R., Breese, S.S., 1964. Chemical and physiological properties of virtually pure foot and mouth disease virus. Am. J. Vet. Res. 25, 333–342.

Bachrach, H.L., More, D.M., McKercher, P.D., Polatnick, J., 1977. An experimental subunit vaccine for foot and mouth disease. Dev. Biol. Stand. 35, 155–160.

Barteling, S.J., Meloen, R.H., Wagenaar, F., Gielkens, A.L.J., 1979. Isolation and characterization of trypsin resistant O1 variants of foot and mouth disease virus. J. Gen. Virol. 43, 383–393.

Bradish, C.J., Dillon, J.F., Normbeuna, M., 1952. Ultra centrifugational studies on the infective and complement fixing components in the virus system of foot and mouth disease. Proc. R. Soc. (B) 140, 107.

Butchaiah, G., Morgan, D.O., 1997. Neutralization antigenic sites on type Asia-1 foot and mouth disease virus defined by monoclonal antibody-resistant variants. Virus Res. 52 (2), 83–94.

Crowther, J.R., Farias, S., Carpenter, W.C., Samuel, A.R., 1993. Identification of a fifth neutralizable site on type O foot and mouth disease virus following characterization of single and quintuple monoclonal antibody escape mutants. J. Gen. Virol. 74, 1547–1553.

Freiberg, B., Hohlich, B., Haas, B., Saalmuller, A., Pfaff, E., Marquardt, O., 2001. Type-independent detection of foot and mouth disease virus by monoclonal antibodies that bind to amino-terminal residues of capsid protein VP2. J. Virol. Methods 92 (2), 199–205.

Freiberg, B., Rahman, M.M., Marquardt, O., 1999. Genetical and immunological analysis of recent Asian type A and O foot and mouth disease virus isolates. Virus Genes 19 (3), 167–182.

Grazioli, S., Fallacara, F., Brocchi, E., 2003. Mapping of neutralizing sites on FMD virus type Asia 1 and relationships with sites described in other serotypes. Report of the Session of the Research Group of the Standing Committee of the European Commission of Foot and Mouth Disease, Appendix 44, pp. 277–287.

Hamblin, C., Barnett, I.T.R., Crowther, J.R., 1986. A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot and mouth disease virus. II. Application. J. Immunol. Methods 93 (1), 123–129.

Jackson, T., King, A.M., Stuart, D., Fry, E., 2003. Structure and receptor binding. Virus Res. 91, 33–46.

Kohler, G., Milstein, C., 1975. Continuous culture of fused cells secreting antibody of predefined sensitivity. Nature (London) 256, 495–497.

Lea, S., Hernandez, J., Blakemore, W., Brocchi, E., Curry, S., Domingo, E., Fry, E., Abu-Ghazaleh, R., King, A.M.Q., Newman, J., Stuart, D., Mateu, M.G., 1994. The structure and antigenicity of a serotype C foot and mouth disease virus. Structure 2 (2), 123–139.

Marquardt, O., Freiberg, B., 2000. Antigenic variation among foot and mouth disease virus type A field isolates of 1997–1999 from Iran. Vet. Microbiol. 74, 377–386.

Mateu, M.G., Martinez, M.A., Capucci, L., Andreu, D., Giralt, E., Sobrino, F., Brocchi, E., Domingo, E., 1990. A single amino acid substitution affects multiple overlapping epitopes in the major antigenic site of foot and mouth disease virus of serotype C. J. Gen. Virol. 71, 629–637.

McCullough, K.C., Crowther, J.R., Butcher, R.N., 1985. Alteration in antibody reactivity with foot and mouth disease virus (FMDV) 146S antigen before and after binding to a solid phase or complexing with specific antibody. J. Immunol. Methods 82 (1), 91–100.

McCullough, K.C., Crowther, J.R., Carpenter, W.C., Brocchi, E., Capucci, L., DeSimone, F., Xier, Q.I., McCahon, D., 1987. Epitope on foot and mouth disease virus particles. I. Topology. Virology 157, 516–525.

Morrell, D.J., Mellur, E.J.C., Rowlands, D.J., Brown, F., 1987. Surface structure and RNA-protein interaction of foot and mouth disease virus. J. Gen. Virol. 68, 1649–1658.

Rweyemamu, M.M., Booth, J.C., Head, M., Pay, T.W., 1978. Micro neutralization tests for serological typing and subtyping of foot and mouth disease virus strains. J. Hygiene (Lond.) 81 (1), 107–123.

Samuel, A.R., Knowles, N.J., Samuel, G.D., Chowther, J.R., 1991. Evaluation of a trapping ELISA for differentiation of foot and mouth virus strains using monoclonal antibodies. Biologicals 19, 299–310.

Samuel, A.R., Ouldridge, E.J., Arrowsmith, A.E.M., Kitching, R.P., Knowles, N.J., 1990. Antigenic analysis of serotype O foot and mouth disease virus isolates from the Middle East, 1981 to 1988. Vaccine 8 (4), 390–396.

Sanyal, A., Gurumurthy, C.B., Venkataramanan, R., Hemadri, D., Tosh, C., 2003. Antigenic characterization of foot and mouth disease virus serotype Asia1 field isolates using polyclonal and monoclonal antibodies. Vet. Microbiol. 93 (1), 1–11.

Sanyal, A., Venkataramanan, R., Pattnaik, B., 1997. Antigenic features of foot and mouth disease virus serotype Asia-1 are related by

monoclonal antibodies and neutralization-escape mutants. Virus Res. 50, 107–117.

Smitsaart, E.N., Saiz, J.C., Yedloutschnig, R.J., Morgan, D.O., 1990. Detection of foot and mouth disease virus by competitive ELISA using a monoclonal antibody specific for the 12S protein subunit from six of the seven serotypes. Vet. Immunol. Immunopathol. 26 (3), 251–265.

Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76, 4350–4354.

Van Maanen, C., Terpstra, C., 1990. Quantification of intact 146S foot and mouth disease antigen for vaccine production by a double antibody sandwich ELISA using monoclonal antibodies. Biologicals 18 (4), 315–319.

Wild, T.F., Burroughs, J.N., Brown, F., 1969. Surface structure of foot and mouth disease virus. J. Gen. Virol. 4, 313–320.

Xie, Q.C., McCahon, D., Crowther, J.R., Belsham, G.J., McCullough, K.C., 1987. Neutralization of foot-and-mouth disease virus can be mediated through any of at least three separate antigenic sites. J. Gen. Virol. 68, 637–647.

Yang, M., Clavijo, A., Surazez-Banmann, R., Avalo, R., 2007. Production and characterization of 2 serotype independent monoclonal antibodies against foot and mouth disease virus. Vet. Immunol. Immunopathol. 115, 126–134.