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Characterization of Monoclonal Antibodies Against Foot and Mouth Disease Virus and Determination of Antigenic Variations in Field Strains

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Abstract: The aim of this project was characterization of monoclonal antibodies (MAbs) against O_1 Man. 69, a vaccine strain of the Ankara Foot and Mouth Disease (FMD) Institute and detection of the antigenic variation in field virus strains. For that purpose,17 MAbs were used. Activities of MAbs against 146S, enzyme treated 146S (146S-T) and 12S antigenic particles of O_1 Man. 69 virus strain were estimated by indirect ELISA. Reaction to viral capsid proteins was detected by Western immunoblotting and detection of the neutralization specificity was done by both in vitro and in vivo neutralization tests and mouse protection assay.

Twenty O type field virus strains collected from several provinces in Turkey were compared with the FMD virus vaccine strain by trapping ELISA.

As a result of characterization studies, neutralizing Mabs against VP1 structural protein were identified.Thirteen field virus isolates were close to each other whereas seven did not share some of the epitopes with the vaccine strain.

Key Words: Monoclonal antibodies, foot and mouth disease virus, characterization.

Şap Virusuna Karşı Monoklonal Antikorların Karakterizasyonu ve Saha Suşlarının Antijenik Varyasyonlarının Belirlenmesi

Özet: Bu projenin amacı Ankara Şap Enstitüsü'nün aşı suşu olan O1 Man 69'a karşı monoklonal antikorlar (MAbs) ın karakterizasyonu ve saha suşlarının antijenik farklılıklarının belirlenmesidir. Bu amaçla 17 MAbs kullanılmıştır. MAbs'ın O1 Man 69 virus suşunun 146S, enzim ile parçalanmış 146S (146S-T) ve 12S partiküllerine karşı aktivitelerini belirlemek amacı ile indirekt ELISA, virusun kapsid proteinlerine karşı reaksiyonu belirlemek amacı ile Western immunoblotting, nötralizasyon özelliğini belirlemek amacı ile in vivo ve in vitro nötralizasyon testleri ve fare pasif koruma testleri uygulanmıştır.

Türkiye'nin çeşitli illerinden temin edilen 20 O tipi şap virusu suşunun aşı suşu ile karşılaştırılması trapping ELISA ile gerçekleştirilmiştir.

Sonuçta VP1 yapısal proteinine özel nötralizan MAbs belirlenmiştir. Bunun yanında 13 saha virus suşu birbirine yakın bulunurken 7 suşun ise aşı suşu ile bazı epitopları paylaşmadıkları tesbit edilmiştir.

Anahtar Sözcükler: Monoklonal antikorlar, şap virusu, karakterizasyon.

Introduction

Monoclonal antibodies (MAbs) are useful reagents because of their single molecular structure. They can be utilized in understanding the neutralization characteristics of the viruses at molecular level. Some MAbs can only neutralize the viruses in the presence of the complement. Not all of the in vitro neutralizing MAbs against the foot and mouth disease virus (FMDV) are able to protect laboratory animals in vivo (1). Furthermore, since each MAbs recognizes only one antigenic determinant of the virus, each of them represents different properties (2). Thus, it is necessary to characterize MAbs before utilisation (3).

Many laboratories have prepared MAbs against FMDV since 1983. Recently in the Ankara FMD Institute, MAb secreting hybridomas against the same virus have been produced (4).

In the control of the disease, antigenic variation in FMDVs is an important factor to be considered. There are several techniques for determining the gene products present on the viral capsid. Trapping ELISA (T-ELISA) is one of them which requires a panel of MAbs (5).

Table 1. FMDV type O field isolates examined.

No	City of origin and year collected
-	Manisa / 1969 (vaccine strain)
01	Konya-1 / 1995
02	Ankara / 1995
03	İçel / 1996
04	Kırklareli-1 / 1995
05	Burdur / 1995
06	Mardin / 1995
07	Kırklareli-2 / 1995
08	Elazığ / 1995
09	Konya-2 / 1995
10	Kırklareli-3 / 1995
11	Konya-1 / 1996
12	Balıkesir / 1994
13	Erzincan / 1995
14	Konya-3 / 1995
15	Samsun / 1996
16	Çanakkale / 1996
17	Bursa / 1996
18	Konya-2 /1996
19	Edirne / 1996
20	İçel / 1996

The aim of this study was to characterize the MAbs against the O_1 Man 69 vaccine strain and to use them for detection of the antigenic variation in field strains in Turkey.

Materials and Methods

Viruses: O_1 Man 69 is the vaccine strain of the Ankara FMD Institute. Ten FMDV field strains were collected from several provinces between 1994 and 1996 (Table 1).The viruses were passaged in baby hamster kidney (BHK) cell-line monolayer cultures three times and samples were stored at -70°C.

Preparation of trypsin-treated virus: Purified 146S antigen of O_1 Man 69 was treated with 50 µl of trypsin sol. Following one hour incubation at 37°C the reaction was stopped by addition of soy bean trypsin inhibitor factor (6).

Preparation of 12S subunit particles: Following acidification of one part of O_1 Man 69 146S antigen by treatment with two parts of 0.05M NaH_2PO_4 the mixture was incubated at room temperature for ten minutes. The reaction was stopped by adjustment of the pH to 7.4 by addition of 1N NaOH.

Monoclonal antibodies: They were prepared as described previously (4).

Characterization of MAbs

Indirect ELISA: Indirect ELISA was applied to determine the specificity of the MAbs against the homologous virus by the technique described previously (7).

Western immunoblotting: This assay was performed to determine the reactions of the MAbs against capsid proteins denaturated with sodium dodecyl sulphate (SDS) by the technique described previously (8).

In vitro neutralization: Two methods (with and without complement) were applied to determine the neutralization characteristics of the MAbs (8).

In vivo tests: These assays were applied to determine the activities of the MAbs against the homologous virus in laboratory animals. Two different techniques were used for this purpose:

1- In vivo neutralization measured the efficiency of the helper factors on the protective immune response against FMDV by the technique described previously (9).

2- In vivo passive protection determined the protective functions of the MAbs arrived at the reticulo-endothelial system (RES) against $50LD_{50}$ of homologous virus by the technique described previously (7).

Characterization of FMD field viruses: For this purpose T-ELISA was applied (10). Polyclonal rabbit anti-O_1 Man 69 virus serum was used. FMDV strains passaged in BHK cell cultures were incubated with this serum as shown in Table 1. MAbs and rabbit anti-mouse HRP conjugate were added in consecutive order. Guinea hyperimmune serum were added to the duplicate wells as positive controls. Validation was carried out as described elsewhere (5,11-13).

Results

Characterization of MAbs:

Only one MAb (No 113) reacted with VP1 capsid protein of the homologous virus in the Western immunoblotting test (Figure1).

Table 2. Characterization of MAbs and their reactivity against the homologous O_1 Man 69 antigens in the indirect ELISA.

MAb No.	Ig	146S	146S-T	12S
11	Ig G	+	+	+
20	Ig G	+	+	-
24	Ig M	+	-	+
49	Ig M	+	+	+
51	Ig M	+	-	+
76	Ig G	+	+	+
102	Ig G	+	-	+
103	Ig G	+	-	-
105	Ig G	+	-	-
106	Ig G	+	-	-
113	Ig G	+	-	-
143	Ig M	-	-	-
149	Ig G	+	-	-
152	Ig M	+	-	
154	Ig G	+	-	-
160	Ig G	+	-	-
176	Ig G	+	-	-

(-) : The optical density (OD) at 492 nm below 0.1

(+) : The optical density (OD) at 492 nm over 0.1

Indirect ELISA results indicated that the hybridoma supernatants included mouse immunoglobulines (Ig) against the homologous virus with different activity levels. Whereas, some of the MAbs did not react with some of the antigenic particules of the homologous virus (146S, 146S-T and 12S). Nine MAbs were specific to untreated 146S antigen while one MAb did not react with any of them (Table 2).

Ten MAbs neutralized the homologous virus in the presence of complement (C), while the neutralization reaction was positive with 16 MAbs when C was not added to the system (Table 3).

All of the ten MAbs which reacted specifically with 146S antigen neutralized the virus in vitro, but only three of them protected the mice against the virus (Table 3).

Characterization of O type field virus isolates (Figure 2):

Using the panel of MAbs described above and the standardized methods, 20 O type FMDV field isolates were compared with the vaccine strain (O_1 Man 69). Binding capacities of the MAbs number 103, 106, 113, 143, 149, 152 and 154 to Edirne/96 and İçel/96 isolates were quite low. Kırklareli/95 isolate was not recognized by three MAbs (106,113 and 160). Also MAbs 160 and 176 didn't bind to Bursa/96 and Çanakkale/96 isolates, or MAb 76 to Konya/96.These results indicate that all of the six O type FMDV samples isolated in 1996 have different epitopes from either the vaccine strain and the causative agents of the l995 outbreaks.

Discussion

The purpose of this study was to evaluate some of the specifications of recently produced MAbs and to examine antigenic differences between O type FMDV field isolates by the use of MAbs. Neutralizing MAbs against FMDVs are able to identify the viruses in the same serotype (subtype). Non-neutralizing MAbs usually cross-react (14). Thus, MAbs need to be characterized after production.

VP1, capsid protein of FMDVs, which has a very important role in immunity is trypsine sensitive. In this study ten MAbs did not react with trypsin-treated antigen. This indicates that the MAbs are specific for the epitopes on VP1.

Although MAbs were produced against 146S purified antigen, six of the MAbs also reacted with 12S particles. This can be attributed to the in vivo degradation of 146S particles (15), which should be considered in vaccine formulation.

There are at least five neutralizing epitopes on the O type FMDVs (16). The epitope numbers 1., 3. and 5. are present on trypsine sensitive VP1; the 2nd epitope is present on VP2; epitope 4 is specific to VP3. The antigenic variation in virus strains is related to differences in the amino acid sequences of the neutralizing epitopes on VP1 (16). One (MAb 51) of the MAbs in this study did not neutralize the virus in vivo and in vitro, whereas the rest neutralized the virus (15).

C utilization in in vitro neutralization tests might give some clues about the in vivo influence of these MAbs (3). The results of the in vitro neutralization tests carried out in the presence of C are also indicative of in vivo passive protection by the MAbs. The in vitro neutralization titers

Table 3. Protection capacity of MAbs elicited by FMDV O_1 Man 69 146S antigen after dissemination in vivo against homologous virus and comparison with in vitro neutralization tests.

In vitro neutralization		IN VIVO TESTS	
+C[(a)]	-C[(b)]	Passive PD_{50}/30µl[(c)]	M PD_{50}/30µl[(d)]
2.4	1.7	0	1.0
2.6	2.6	1.5	0
2.6	2.2	1.5	0
2.2	2.0	1.0	1.7
0.9	0	0	0
3.0	3.2	1.2	0
0.6	3.3	0	4.5
3.0	2.0	0	3.6
2.8	3.9	0	1.4
0.6	3.2	0	1.4
3.3	3.2	1.7	6.4
2.4	3.4	2.5	5.4
0	2.2	2.5	3.8
0.6	1.9	0	7.7
0.9	1.9	1.9	3.6
1.5	2.1	0	6.9
1.0	3.0	0	7.7

(a) The MAb dilution which 50% inhibits the unpurified virus infectivity in the presence of normal guinea pig serum (complement).

(b) The MAb dilution which 90% inhibits the purified infective FMDV 146S antigen.

(c) Passive PD_{50} titers of MAbs against a challenge of $100LD_{50}$ virus after inoculation the 30 µl MAbs.

(d) PD_{50} of the MAbs inoculated after neutralization with $100LD_{50}$ virus.

(*) log_{10} antibody titers.

of most of the MAbs which neutralized the virus in vivo were reasonably high.

T-ELISA with the MAbs is widely used as a valuable tool in rapid discrimination of FMDVs (13). Neutralizing MAbs identify the viruses of the same subtype, however, the others can cross-react with many subtypes of the same serotype. That is why all the existing MAbs should be used in the same test to detect the antigenic variation in field viruses (14). In this study 17 MAbs were used for detection of the antigenic differences in 20 field virus isolates. The presence of antigenic differences among field strains has been documented with these assays (17). This work is the first trial carried out with T-ELISA in the FMD Institute, Ankara. The authors suggest this survey be repeated with increased numbers of MAbs and field virus isolates.

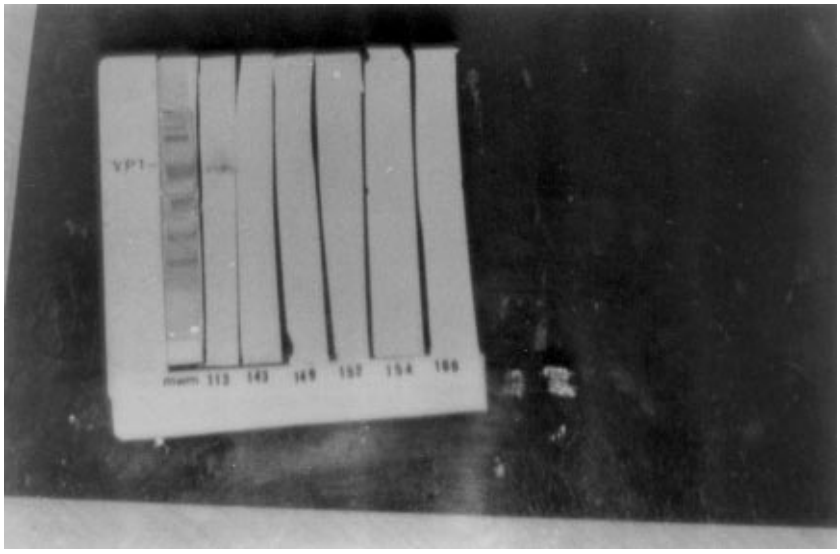

Figure 1. Western blot after separation of the structural proteins of denaturated purified FMDV O_1 Man 69 146S antigen using SDS-PAGE with Laeammli's discontinuous buffer system and 10% resolving gel.

MAbs	VIRUSES																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
11				■	■				■							■				■
20		■	■	■						■				■	■	■	■	■	■	■
24																				
49					■		■		■			■	■							
51		■									■	■								
76		∇	■			■											*******			
102	■	■		∇		■		■		∇	■									■
103	■				■														∇	******
105		■	■									■	■		■			■		
106		■		■					■	******									******	■
113	■	■	■	■				■		******					■			■	■	
143	■	■	■															■	∇	■
149																			■	******
152									■										******	∇
154	■	■	■			■	******	■	■		■				■	******		■	∇	■
160		■	■	∇	■				■	******					■	******	******	■		
176	∇	■	■						■		■				■					

Binding of MAbs compared with homologous reaction:

	: 75-100% homologous (same reaction as with virus used to prepare MAbs)
■	: 74-45% homologous but not identical
******	: 44-20% identical but thereare some differences
∇	: 20% not reactive (heterologous)

Figure 2. Antigenic profiling of type O FMDVs by trapping ELISA using MAbs against O_1 Man 69

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