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HYBRIDOMA CELL LINES SECRETING MONOCLONAL ANTIBODIES TO FOOT-AND-MOUTH DISEASE VIRUS TYPE ASIA-1

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Summary. — Various immunizing regimens, cell culture requirements and cell fusion conditions were examined for efficient production of hybridomas secreting anti-foot-and-mouth disease virus (FMDV) antibodies. A highly sensitive streptavidin-biotin — based enzyme-linked immunosorbent assay (ELISA) was used for screening of hybridomas for specific antibody production as well as for determining the serotype specificity of the antibodies. Six hybridoma cell lines generating antibodies to FMDV type Asia-1 (vaccine strain 63/72) were produced by fusion of SP2/0 mouse myeloma cells and spleen cells from mice immunized with the inactivated viral antigen. The monoclonal antibodies (MoAb) from four producer clones reacted in ELISA specifically with the intact virus antigen of type Asia-1 without any cross-reactivity with strains of other virus types 0, A and C. Two other clones positive for anti-Asia-1 virus antibodies in ELISA cross-reacted with type C virus strain. The MoAb from three of the four Asia-1 virus type specific clones neutralized the infectivity of the immunizing viral strain. One neutralizing MoAb reacted with the separated VP1 from the immunizing viral strain in immunoblotting.

Key words: *foot-and-mouth disease virus*; *type Asia-1*; *monoclonal antibody production and characterization*

Introduction

Successful production of monoclonal antibodies (MoAb) by hybridoma technology initially described by Kohler and Milstein (1975) relies mostly on efficient immunization schedules, cell culturing regimes, cloning conditions and finally on use of rapid and sensitive methods to detect specific antibody production by the hybridomas. The production of hybridomas secreting antibodies against small viruses such as foot-and-mouth disease virus (FMDV) was more difficult than the production of hybridomas generating antibodies against large viruses.

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Efficient protocols for culturing of hybridoma cell lines secreting antibody specific for FMDV and reliable cloning methods for the hybridoma culture (McCullough *et al.*, 1983*a*,*b*) and a rapid enzyme-linked immunosorbent assay (ELISA) for screening of anti-FMDV sera and hybridoma cell products (McCullough and Parkinson, 1984*a*, 1984*b*) were described. Hybridoma cell lines secreting MoAb to FMDV types 0, A and C were successfully generated using different immunization, cell culture and screening protocols (Brocchi *et al.*, 1982; Brocchi and De Simone, 1982; De Simone *et al.*, 1982; Yavin *et al.*, 1982). Although different workers used different protocols, many combination of variables may work and no procedure can be markedly superior to the others.

MoAb to different strains of FMDV subtypes O_1, O_{10}, A_{24}, SAT2 and SAT3 were produced (McCullough and Butcher, 1982, DeSimone *et al.*, 1983; McCullough *et al.*, 1983*a*,*b*) and characterized (McCullough *et al.*, 1985). It has also been shown by Duchesne *et al.*, (1984) that the neutralizing MoAb raised against intact FMDV subtype O_1 Aulendorf reacted with an epitope in intact virus as well as in isolated denatured VP1 from different strains of the same subtype. At least two other different neutralizing epitopes of FMDV had been identified using MoAb (Meloen *et al.*, 1983; Ouldridge *et al.*, 1984).

In this part of the world, foot-and-mouth disease is caused by type Asia-1 virus in addition to virus type 0. A and C is prevalent among various species of animals. Anti-FMDV monoclonal antibodies would be very useful for identification of variant viruses within the same subtype, for routine vaccine control purpose and for molecular characterization of the virus. Although MoAb have been produced and used against all other serotypes of FMDV, there has been no report of attempts made to produce MoAb to Asia-1 type virus. In this paper, we describe the production of hybridoma cell lines secreting MoAb to FMDV type Asia-1 (vaccine strain 63/72) using different immunization regimens, cell fusion and culture conditions. We also report on the evaluation of the specificity of neutralizing MoAb by recognition of epitopes on intact virius as well as on isolated VP1.

Materials and Methods

Cells. SP2/0-Ag 14, a non Ig producing mouse myeloma cell line derived from X63-Ag 8x BALB/c (Shulman *et al.*, 1978) was obtained from the National Institute of Immunology, New Delhi.

Medium. RPMI-1640 medium containing 2 mmol/l glutamine (pre-mixed powder from Flow Laboratories, U.K.) was supplemented with additional 2 mmol/l glutamine, 2.2 mmol/l sodium bicarbonate, 20 mmol/l Hepes, 2 mmol/l sodium pyruvate, 100 IU/ml benzylpenicillin, 100 μg/ml streptomycin sulphate, 20 μg/ml kanamycin sulphate and 10–15 % (V/V) foetal calf serum (Flow Laboratories, U.K. or Bethesda Research Laboratories, U.S.A.) to give complete RPMI medium.

HAT medium contained also 1×10^{-4} mol/l hypoxanthine, 4×10^{-7} mol/l aminopterin and 1.6×10^{-5} mol/l thymidine. HT medium was the same as HAT medium without the aminopterin. Both HAT and HT media were obtained from Bethesda Research Laboratories, or Gibco Laboratories, U.S.A.). In some cases, the media were supplemented with 10–20 % (V/V) conditioned medium (medium from 72 hr-old culture of mouse myeloma cells or 48 hr-old culture of mouse spleenocytes or thymocytes) and sterilized by filtration through a 0.22 μm size membrane filter.

Virus. BHK21 or C13 cell monolayers were infected with the vaccine strain (63/72) of FMDV type Asia-1 currently used for vaccine production at this Institute and incubated at 37 °C in Eagle's medium (Glasgow modification) free of serum. The infected cultures were harvested when cytopathogenic effect (CPE) was maximal. The virus after clarification was inactivated by 1 mmol/l binary ethyleneimine (BEI) for 24-hr at 25 °C.

The inactivated virus was concentrated by precipitation with polyethylene glycol (PEG) 6000 and the resuspended precipitate was partially purified by centrifugation at 16000 rev/min for 30 min. The virus was further purified by centrifugation through 10—50 % linear sucrose gradient according to the method of Grubman *et al.*, (1979).

Inactivated and purified virus (intact 146S virus particles) was used both as inoculum for mice and as solid phase antigen in ELISA.

Immunization of mice. Five to seven weeks old BALB/c mice were primed subcutaneously (s.c.) on neck or intradermally (i.d.) on footpads with 20—30 μg per mouse of virus antigen (146S) emulsified in Freund's complete adjuvant followed by two booster injections in Freund's incomplete adjuvant s.c. at 4 weeks and 12 weeks with the same amount of antigen. The final injection was given 4 weeks later intravenously (i.v.) or intrasplenically (i.s.) with 20—40 μg of viral antigen in phosphate buffered saline (PBS). The spleens were collected after a further three days.

Production of hybridomas. Spleenocytes from the immunized mice were hybridized with SP2/0-Ag 14 mouse myeloma cells using a modification of the general procedure of Kohler and Milstein (1975). Cell fusion was carried out using about 1×10^8 spleenocytes and 2×10^7 mouse myeloma cells by means of polyethylene glycol (PEG 1450, 41 % W/V). After fusion, the cells were resuspended in RPMI complete medium and distributed in 1 ml aliquots into 4 × 24-well (w) plates (Nunc, Denmark). After 24-hr incubation (37 °C, 9 % CO_2), HAT medium was added (1 ml/well) and the plates were again incubated. One half of the medium was replaced by HAT medium on days, 3, 6, 9, 12, 15 and 18. Starting on day 21, HAT medium was gradually replaced by HT medium over a period of 8 days and then with normal complete RPMI medium. When hybridomas had grown to about 30—50 % confluence, aliquots of the culture supernatants were collected and tested for antibody activity by ELISA and incidentally in a micro-neutralization test. Positive hybridomas secreting antibodies were cloned at least twice by limiting dilutions (0.5—1.0 cell/well) in 96-W plates (Nunc, Denmark) using mouse thymocytes as feeder cells. Selected positive clones were expanded and frozen in liquid nitrogen. Monoclonal antibodies were produced either in cell culture or in pristane (Sigma Chemical Co., U.S.A.) treated mice by passaging the hybridomas as ascitic tumours.

Enzyme-linked immunosorbent assay (ELISA) methods. Two methods of streptavidin-biotin-based ELISA and one method of ELISA based on immunoglobulin class specific sera and IgG-peroxidase conjugate were used. The details of each method are given below.

Double antibody sandwich ELISA. This method was used for initial screening of hybridomas for production of virus specific antibodies. Micro-ELISA plates (Nunc, Denmark or Dynatech Laboratories, U.S.A.) were sensitized with 50 μl/well of viral antigen (146S) in carbonate coating buffer at 40 μg/ml protein concentration overnight at 4 °C. The wells were washed four times with washing buffer (PBS containing 0.05 % bovine serum albumin (BSA). The wells were then saturated in the presence of 3 % BSA in PBS for 2 hr at 25 °C. The test samples (hybridoma culture supernatants) were then added (50 μl/well) and the plates incubated for 1 hr at 25 °C. The wells were washed as per above procedure. Biotinylated goat anti-mouse immunoglobulin (Bethesda Ressearch Laboratories, U.S.A.) diluted in dilution buffer (PBS containing 1 % BSA) was added (50 μl/well) and the plates were incubated for 1 hr at 25 °C. The wells were washed again and diluted streptavidin-peroxidase conjugate (Bethesda Research Laboratories U.S.A.) was added (50 μl/well) and the plates incubated for 30 min at 25 °C. The wells were washed six times and the substrate solution containing O-Phenylenediamine (1 mg/ml), hydrogen peroxide (0.012 %) in citrate buffer (0.1 mol/l, pH 4.5) was added (100 μl/well). The plates were incubated for 30 min at 25 °C in dark and read immediately in an ELISA minireader (Dynatech Laboratories, Singapore) at 410 nm.

Triple antibody sandwich ELISA. This method was used to determine the serotype specificity of the MoAb. The plates were sensitized with FMDV type specific guinea-pig immune serum IgG (50 μl/well) overnight at 4 °C. The wells were washed and saturated with BSA as described before. Then purified homologous type specific virus antigen (146S) at a concentration of 40 μg/ml was added (50 μl/well) and the plates incubated for 1 hr at 25 °C. The rest of the procedure

from addition of test samples (hybridoma culture supernatants) was the same as described under double antibody sandwich ELISA.

ELISA to determine the immunoglobulin (Ig) class of MoAb. The wells of the plates presensitized with purified FMDV type Asia-1 (146S) were saturated with BSA, washed and reacted with the MoAb in culture supernatants (50 µl/well) for 2 hr at 25 °C. With each MoAb in sample, replicate wells for each Ig class reagent were used. To each replicate well, 50 µl of class specific rabbit anti-mouse IgG or IgM sera (Sigma Chemical Co., U.S.A.) was added. The plates were incubated for 2 hr at 25 °C and washed again. Diluted goat anti-rabbit IgG — peroxidase conjugate was then added (50 µl/well) and the plates incubated for 2 hr at 25 °C. After washing the plates, the substrate solution (same as used for other methods of ELISA) was added (100 µl/well). After colour development at 25 °C (within 30 min), the Ig class specificity of the MoAb was determined.

Virus neutralization test. The virus neutralizing activity of the MoAb was determined in a microplate test system using pig-kidney cells (IB-RS-2). The MoAb were tested in two-fold dilution steps against 100 $TCID_{50}$ of virus. The end-points (50 %) were computed according to Reed and Muench method (1938) and the antibody titres were expressed as the negative log of the highest MoAb dilutions at 50 % end-points resulting in virus neutralization. The samples were considered positive if the antibody titres ($log_{10}SN_{50}$ values) exceeded 0.6.

Immunoblotting. The viral structural proteins were separated from the purified virus using SDS-PAGE slab gel electrophoresis using discontinuous buffer system of Laemmli (1970) with a resolving gel of 10 % in the presence of 6 mol/l urea. The separated viral structural proteins were transferred from SDS-PAGE slab gel to nitrocellulose sheets using a method similar to that described by Symington *et al.* (1981) with a transblot apparatus. Nitrocellulose paper (BA85 from Schuer and Schuell, Germany) was used. Transfer buffer contained 20 mmol/l Tris base, 150 mmol/l glycine and 20 % methanol. The transfer was carried out for 16—20 hr at 6 to 7 V/cm and at 20 °C. (Gershoni and Palade, 1982). Protein transfer was verified by staining a strip of the nitrocellulose sheet with amido black and staining of the SDS-PAGE gels by Comassie Brilliant Blue.

Strips cut from nitrocellulose sheets were incubated for 2 hr at 37 °C in 10 mmol/l phosphate buffer, 150 mmol/l NaCl (pH 7.4) containing 5 % defatted milk powder (Johnson *et al.*, 1984) to saturate the remaining protein-binding sites on the nitrocellulose. After blocking, the strips were reacted with MoAb in PBS containing 5 % milk powder for 2 hr at 37 °C. The strips were then washed in PBS to remove unbound products. The rest of the procedure using biotinylated goat anti-mouse immunoglobulin and streptavidin-peroxidase conjugate was same as described under ELISA methods. The colour reaction was developed with 4-chloro-1-naphthol and hydrogen peroxide as described by Hawkes *et al.* (1982). The reaction was stopped after 20 min by washing with distilled water. The strips were then dried between filter papers and stored in the dark.

Results

Virus specific immunoglobulin activity could be detected in the sera of all immunized mice by double antibody sandwich ELISA. Spleens collected from two or three immunized mice were used for preparing a pool of spleenocytes in each cell fusion experiment. After hybridization and plating with HAT medium in 24-well plates, growth of the hybridomas was observed in 53 % of the wells. Eight of a total number of 202 hybridomas (4 % specific efficiency) produced viral antigen specific antibodies as detected by ELISA. The antibodies produced by three of the eight ELISA positive hybridomas also neutralized the virus infectivity (Table 1).

Two of the 8 positive polyclonal hybridomas were weak antibody producers and hence not followed further. After cloning of the six strongly positive polyclonal hybridomas in 96-well plates, 164 wells contained cell clones. These clones were screened by ELISA yielding 86 positive clones (Table 2). Thus, from the number of positive wells in the two-step cultures,

Table 1. Production of polyclonal hybridomas secreting antibodies to foot-and-mouth disease virus type Asia-1

Cell fusion number*	Number of wells seeded	Number of Hybridomas Produced		
		Total	ELISA positive	Virus neutralization positive
1	96	51	1	0
2	96	49	1	0
3	96	47	2	1
4	96	55	4	2

* In each fusion, the mice had been immunized according to the same schedule. However, in fusion number 3, the final injection was given intrasplenically.

it was estimated that 1 in 64 hybrid cells was a specific antibody producer. After recloning the positive clones, six subclones named 1-A5, 1-B1, 11-A6, 11-D3, 11-C2 and 11-C5 which gave at least 5 times the control absorbance values were selected for further studies.

The 6 producer clones selected were assayed by triple antibody sandwich ELISA for their reactivity against virus particles of different serotypes. Positive reaction was obtained only with virus type Asia-1 antigen in case of 4 clones namely 1-A5, 11-D3, 11-C2 and 11-C5. Whereas, two other clones named 1-B1 and 11-A6 apart from reacting with virus type Asia-1 antigen, cross-reacted with virus type C antigen (Table 3). ELISA carried out using class specific rabbit anti-mouse IgG or IgM sera showed that the MoAb produced by all the 6 clones belonged to IgG class. Culture supernatants with MoAb from the 6 producer clones were tested for their capacity to neutralize different virus types, namely O, A, C and Asia-1. Three clones named 1-A5, 11-C2 and 11-C5 neutralized specifically the infectivity of the immunizing strain of virus type Asia-1 without any reactivity with other virus types. The other 3 clones (1-B1, 11-A6 and 11-D3) although positive in ELISA were unable to neutralize the virus (Table 3).

Table 2. Limit dilution cloning of FMDV antibody-secreting polyclonal hybridomas

Polyclonal hybridoma	Number of wells seeded	Number of cells seeded per well	Number of Clones Produced		
			Total	ELISA positive	Virus neutralization positive
A-5	96	1.0	30	13	9
B-1	96	0.8	25	9	0
A-6	192	0.5	23	11	0
D-3	96	1.0	26	14	0
C-2	192	0.9	31	17	12
C-5	96	0.9	29	22	15

Table 3. Characterization by ELISA and virus neutralization test of the specificities of monoclonal antibodies produced against foot-and-mouth disease virus type Asia-1

Test system	Virus serotype	Clone					
		1-A5	1-B1	11-A6	11-D3	11-C2	11-C5
ELISA	Asia-1	0.44*	0.38	0.23	0.21	0.48	0.50
	C	0.04	0.27	0.16	0.04	0.06	0.06
	A	0.03	0.08	0.06	0.02	0.02	0.03
	0	0.03	0.06	0.04	0.03	0.03	0.04
Virus neutralization	Asia-1	1.65+	—	—	—	2.55	2.55
	C	—	—	—	—	—	—
	A	—	—	—	—	—	—
	0	—	—	—	—	—	—

* Absorbance value at 410 nm
+ Log_{10} antibody titres
— Log_{10} antibody titre < 0.6

The binding of virus neutralizing MoAb from clones 1-A5, 11-C2 and 11-C5 to separated virus proteins (VP1, VP2, VP3 and VP4) was analysed by immunoblotting. It was observed that the MoAb 1—A5 reacted with VP1 region (Fig. 1). This indicated that the virus neutralizing MoAb produced by the clone1—A5 recognized specifically the intact VP1 region of the virus. This neutralizing MoAb producing clone 1-A5 was amplified up to a culture volume of one litre. The MoAb was separated from cell culture fluid by precipitation with saturated ammonium sulphate solution and purified by affinity chromatography on protein-A-sepharose CL-4B using 0.1 mol/l acetic acid in 0.15 mol/l NaCl. The specific immunoglobulin activity of the purified MoAb could be detected by ELISA and virus neutralization tests.

About one litre of culture supernatant containing MoAb from 1/A-5 clone was concentrated 100 times in stirred ultrafiltration cell model 8400 (Amicon B. V., Netherlands) using the membrane XM50 with a molecular weight cut off value of 50,000. The estimation of antibody protein in the culture fluid (before filtration) and in the concentrate (after filtration) showed that the MoAb in the cell culture fluid could be effectively concentrated with a maximum recovery of over 90 %. The specific immunoglobulin activity of the concentrated MoAb could be detected by ELISA and virus neutralization tests.

The neutralizing MoAb producer clones when passaged induced ascitic tumours in pristane-treated BALB/c mice containing 1—2 mg/ml of antibody protein (E 280 mg/ml = 1.45). Although specific antibody activity could be detected in ascitic fluid and serum of Freund's adjuvant-treated mice injected intraperitoneally with the producer clones, the growth of the hybridomas was poor and could not be maintained on further passage in mice. Whereas, pristane treatment of mice promoted the growth and successful passage of

the clones in mice. During the course of initial experiments for development of hybrid clones, many combinations of variables with regard to mouse immunization schedules, cell culture requirements, cell fusion and cloning conditions were tried. After trying various mouse immunization schedules, it was observed that an extended mouse immunization protocol over a period of 3—4 months and with a rest period of at least 3 weeks before the final injection of antigen intrasplenically helped in increasing the efficiency of specific hybridoma production with increased affinity of the MoAb.

The examination of various culture conditions revealed that RPMI-1640 medium supplemented with 10 % foetal calf serum (FCS) enabled rapid and efficient growth and viability of myeloma or hybridoma cultures in general. However, increasing the FCS from 10 to 15 % and CO_2 content from 5 to 9 % enabled some slow growing hybridomas to multiply faster. Although Eagle's (Glasgow modification) medium with 10 % FCS supported the growth of the cells, the rate of growth was very low (data not presented). When excess splenocytes were used for cell fusion, no separate preparation of feeder layers in plates prior to fusion was found necessary for the growth of polyclonal hybridomas. The rate of production of growing hybridomas was high when 24-well plates were used instead of 96-well plates for initial plating of cell fusion mixture. Successful maintenance of hybridoma cultures initially required high cell viability (70—100 %) and high cell densities (2×10^4 to 2×10^5 cells/ml).

We failed to get fused cells when we used some commercially available PEG solutions. Similarly, the efficiency of fusion with PEG 4000 was low when compared with PEG 1450 (data not presented). Cloning over preformed mouse thymocyte feeder layers by limiting dilution technique enabled growth of viable cells without the addition of any separate conditioned medium to the complete RPMI-medium. We therefore, do not routinely add any conditioned medium to the RPMI medium for the growth of the clones even during the first few passages. However, some rare clones needed feeder cells or addition of conditioned medium for the first two or three passages. The incorporation of B-lymphocyte growth stimulant 2-mercaptoethanol (2-ME) at a concentration of 5×10^{-5} mol/l did neither enhance the growth of hybrid cultures after fusion, nor was there any beneficial effect on the growth of established hybridoma cultures. We therefore, do not add routinely 2-ME to the medium.

Discussion

The present report documents the development of stable hybrid cell lines secreting MoAb to FMDV type Asia-1. The value of continuous supply of MoAb for diagnosis and antigenic analysis is obvious. These antibodies ensured high sensitivity with minimal non-specific effects over conventionally raised antisera. The neutralizing MoAb produced the by clone 1-A5, reacted with an epitope in separated VP1 of FMDV type Asia-1 by immunoblotting. This site was also recognized by the MoAb in the intact virus by neutralization test and ELISA. These neutralizing antibodies showed no cross-reactivity with other types of FMDV. Whereas Duchesne *et al.* (1984) reported that

anti-FMDV type 0 neutralizing MoAb cross-reacted with type C virus but not with type A virus. At least three neutralization epitopes on VP1 of FMDV have so far been identified using MoAb (Meloen *et al.*, 1983; Duchesne *et al.*, 1984; Ouldridge *et al.*, 1984).

The data presented here further showed that anti-FMDV type Asia-1 MoAb produced by clones 11-A6 and 1-B1 were not virus neutralizing in character and cross-reacted with type C viruses but not with type 0 and A virus in ELISA. Such crossreactivity of MoAb is difficult to explain since the crossreactivity between serotypes is not observed with classical type specific antisera except in very low dilutions. The crossreaction of MoAb may be due to a common site on different serotypes of FMDV against which they are directed or a low-affinity interaction which is not seen against the background of multiple high-affinity interactions with a potent polyclonal antibody (Duchesne *et al.*, 1984). Several technical points arising from the development of the hybrid clones deserve mention here. The extended mouse immunization regimen and final injection of antigen intrasplenically might be very useful especially with the FMDV which must be inactivated before use.

The better results obtained with respect to the production of growing hybrids when 24-well plates were used instead of 96-well plates for initial plating of cell fusion mixture corroborated and extended the findings of McCullough *et al.* (1983*a*). This is in contrast with Fazekas de St. Growth and Scheidegger (1980) who reported little difference between 24-well and 96-well plates. In agreement with the findings of McCullough *et al.* (1983*a*), we were unable to determine any beneficial effect of 2-ME on the growth of hybrid cells. The percentage of wells which were positive for both growth and ELISA (4 %) obtained in this study using 24-well plates for initial seeding was higher than that reported (0.8 %) by McCullough *et al.* (1983*a*). In our opinion, the efficiency of producing positive hybrid cells depends on the mouse immunization regimen and sensitive screening methods rather than on the culture conditions especially with the FMDV. Although these factors may not be solely responsible, they were very important (Horenstein *et al.*, 1985). In our experience, cloning by limiting dilution method over mouse thymocyte feeder cell layers in 96-well plates although time consuming worked satisfactorily.

Since the quantity of MoAb secreted by the clones in cell culture fluid is less, their concentration is reqiured is before they are used in serological tests. Ultrafiltration with a membrane of suitable molecular weight cut off value was found an effective method of concentrating MoAb in this study. The initial screening of hybridoma culture supernatants by ELISA is preferred over other tests. There is no consistent methodology for the use of ELISA for such a purpose and different laboratories use different methods. But the low levels of antibodies in some hybridoma culture supernatants may present problems. Screening of such culture supernatants require the use of a very highly sensitive ELISA. So in the present study, the application of straptavidin-biotin-based ELISA system was tried for screening of hybridoma culture supernatants.

The double antibody sandwich ELISA in which the plates were directly coated with the FMDV antigen was useful for screening of the hybridoma cultures. But this method could not be used for determining the serotype specificity of the MoAb as FMDV was conformationally altered to expose internal virion proteins after non-covalent binding to the plate (Meloen *et al.* 1979). Therefore, a triple antibody ELISA system in which the plates were coated with serotype specific anti-FMDV type O, A, C and Asia-1 guinea-pig IgG (capture antibody) followed by binding with homologous type FMDV antigen was used to determine the serotype specificity of the MoAb. The streptavidin-biotin-based ELISA was highly sensitive with minimum non-specific reactions and was very useful for detecting anti-FMDV antibody producing clones. Unlike Voller *et al.* (1979) and McCullough and Parkinson (1984a) who reported that there was an obligatory requirement for Tween-20 in the buffer used for ELISA it was found not necessary to supplement the buffer with Tween-20 to minimise the non-specific reaction in the ELISA system used in the present study. Further there was no need to stop the enzyme reaction in this system before reading.

In conclusion, the availability of hybridoma cell lines secreting MoAb to FMDV type Asia-1 would ensure better specificity and reagent continuity for diagnosis, differentiation of virus isolates and molecular characterization of the virus.

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Explanation to Figure (Plate XVII):

Fig. 1. Monoclonal antibodies produced by clone 1-A5 reacting with separated VP1 of foot-and-mouth disease virus type Asia-1.

a. Immunobloting-Positive reaction is observed with the antibody only with separated VP1 fragment.

b. Separated polypeptides of FMDV type Asia-1.