et.Med.J.,Giza. Vol.45, No.4. (1997): 449-455.

RODUCTION OF MONOCLONAL ANTIBODIES AGAINST BOVINE ARVOVIRUS[1]

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eceived: 1/6/1997.
ccepted : 4/10/1997.

SUMMARY

Monoclonal antibodies (MAbs) against bovine parvovirus (BPV) were successfully produced after fusing the spleen cells of an immunized mouse with myeloma cells. A total of six hybridomas were found to produce MAbs against BPV with different reactivities using haemagglutination inhibition (HI) test. These clones are: $3B_{10}$, $3C_5$, $3D_6$, $2H_6$ and $1E_6$. The first three clones produced MAbs of IgG class, but the last three clones produced those of the IgM class and all of them contained K light chain.

INTRODUCTION

Bovine parvovirus (BPV) represents one of the autonomous parvoviruses group which are known to cause enteritis, respiratory and reproductive diseases (Siegl, 1976 and Storz et al., 1978). The infection of calves and adult cattle with bovine parvovirus (BPV) has been reported to be widely distributed in many countries of the world likeMaryland (Abinanti and Warfield, 1961), Algeria (Vincent, 1971), Colorado and South Dakota (Bates et al., (1072) Japan (Inaba et al., 1973), Europe (Huck et al., 1975), England, Brazil and Austria (Leary and Storz, 1980), Switzerland (Hassig et al., 1988) and Nigeria (Akpavie, 1990).

In Egypt, nobody has paid attention to the role of parvovirus as a causative agent of calf enteritis. The aim of this study was the production of monoclonal antibodies (MAbs) against the reference HADEN strain of bovine parvovirus for the purpose of using these MAbs in diagnosis of BPV infection among Egyptian newborn cattle and buffalo calves.

[1] This work has been financed by the Finish-Egyptian Agriculture Research Project, Ministry of Agriculture, Egypt.

MATERIAL AND METHODS

1- Virus:

Bovine parvovirus (reference HADEN strain), was kindly supplied through the National Veterinary and Food Research Institute, Helsinki, Finland.

2- Cell line:

a- Meloma cells: NS-1 cell strain of mouse myeloma was used for hybridoma production.

b- Bovine turbinate cells (BT).

3- Laboratory animals:

a- Bulb-C mice for collection of spleenocyte cells from immunized mice and collection of thymocyte from untreated one.

b- Guinea pig: for collection of G. pig RBCs.

Methods:

Procedures for production of monoclonal antibodies:

1- Preparation of purified bovine parvovirus antigen:

Reference HADEN strain of BPV (Finland) was propagated in BT cells using Eagle's minimal essential medium containing 2-3% sheep serum. Five day after infection of BT cells, the whole cultures were frozen and thawed 3 times, centrifuged at 3000 rpm for 30 minutes and the supernatant fluids were collected, measured and kept in one container. This fluid was mixed with 0.5 M sodium chloride (NaCl) and polyethylene glycol 6000 (PEG) at 10% final concentration and kept overnight at + 4°C. The whole mixture was centrifuged at 3000 rpm for one hour and the sedimented pellet was obtained and resuspended in10ml phosphate buffer saline (2% of the original volume). Then sodium chloride was added to a final density of 1.3 g/ml, and the whole sample was centrifuged at 20,000 rpm for 5 hours in a Beckman centrifuge. The band was collected and resuspended in PBS and kept at -20°C. This antigen was titrated in different dilutions in parallel to a negative control antigen and used for immunization of mice.

2- Immunization of mice:

Bulb-C mice were immunized twice with an inoculum containing 20 mg. of virus protein. The virus was mixed with equal volume of Freund's incomplete adjuvent and Quil A saponin. The mice were inoculated subcutaneously, then a second booster dose was given after two months subcutaneously. Three days after the second immunization, the spleen was collected for hybridoma production. The immunization schedule was obtained from Dr. Nils Juntti. University of Uppsala, Sweden.

3- Fusion of spleen lymphocytes and myeloma cells:

Spleen cells and myeloma cells were fused together according to a modified method used at the Wister Institute and the University of Uppsala, Sweden (Galfre and Milstein, 1981).

On the day before fusion, actively growing two myeloma culture bottles (50 ml) were spilt 1:2 in fresh MEM with 20% foetal calf seurm.

the day of fusion, the immunized mouse and untreated ones were killed by cervical location; the spleen was collected aseptically om the former and thymuses from the latter ce. The spleen and the thymuses were ground parately through metal sieves in separate tri-dishes using the blungers of 1ml plastic ring. Before grinding 5ml of MEM (without CS) were pipetted into the dishes. Disintegrated ells in MEM were then transferred to a 15 ml entrifuge tube and left to stand for 2 minutes to llow large pieces of the organs to settle down. The supernatant was transferred to 50ml plastic entrifuge tubes and the spleen, thymus and NS-1 ells were washed 3 times with serum free EDTA-MEM (0.01% disodium ethylene diamine tra-acetate) by centrifugation at 1500 rpm for 10 min. at 21°C. Approximately equal volumes of packed myeloma and spleen cells (1 myeloma cell to 4 spleen cells) were suspended and combined in the same tube. After centrifugation, the cell pellet was drained carefully and loosened by gentle tapping, and 1 ml. of warm polyethylene glycol 1500 (PEG, Dupont, 50% PEG + 50% serum free MEM + 1% dimethylsulphoxide, DMSO) was slowly pipetted into tube over a period of 1 min. The cells were left to fuse for 1 min. and 10 ml. of MEM plus 20% FCS were then added, while rotating the tube at the same time. The cells were collected by centrifuging carefully at 800 rpm. for 5 min. The supernatant was pipetted off and the thymocytes from the two mice were added as feeder cells. The combined cell mixture was brought to a final volume of 40 ml in MEM with 15% FCS. This mixture was distributed in 0.1 ml aliqoates into the wells of 96-well sterile microtiter plates. The plates were incubated at 37°C in a humid CO_2 cabinet. The following day 0.1 ml aliqoat of HAT (hypoxanthine-aminopterin-thymidine, flow 50x) medium at twice the prescribed concentration were added to each well for selecting hybridomas. Fresh HAT medium (0.05 ml) was added to the cultures at 4 days intervals. after 2 weeks incubation the parvovirus antibody content of the wells was determined using the Haemagglutination Inhibition (HI) test. Clones from positive wells were subcultured and the supernatant media were collected and titrated for HI antibodies against bovine parvovirus (HADEN strain).

4- Isotype analysis:

The isotype analysis for the MAbs was carried out by enzyme-linked immunosorbent assay (ELISA) using mouse hybridomas subtyping Kit (Cell Biology, Boehringer Mannheim, Germany).

Microtiter ELISA plates from Nunc GmbH, Wiesbaden, W. Germany were coated with coating antibody diluted 1:500, 50µl were dispensed to each well of the plates and incubated at room temperature for one hour. Then, the plates were decanted and washed. 200µl of post coating buffer were pipetted into each well and incubated for 15 min. at room temperature, then washed. 50µl of the hybridoma undiluted supernatant were pipetted into each well (one row for each hybridoma)

and the plates were incubated at room temperature for 1 hour and washed. Then 50µl of different conjugate dilutions were added, each type in one column of the plate and incubated for one hour at room temperature and then washed, 50µl of substrate solution were pipetted to each well of the plates and incubated until positive samples showed dark green colour for approximately one hour. Then, the plates were read on MCC titertek Mutiscan at 405 nm and results were evaluated.

RESULTS

A total of 6 hybridomas were found to produce antibodies against bovine parvovirus after fusing the spleen cells of an immunized mouse with myeloma cells by screening the hybridoma against BPV using HI test. The result of HI reactivities of MAbs produced agans BPV are presented in Table (1) and Fig. (1).

The different MAbs showed varying reactivities against BPV as measured by HI test.

Table (1): HI reactivities of MAbs produced against bovine parvovirus (HADEN strain)

MAbs	HI titers
3B10	32
3C5	16
3D6	16
2H5	8
2H6	8
IE6	8

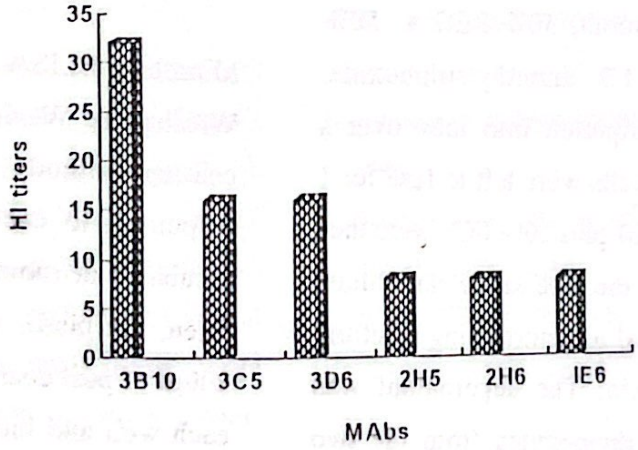

Fig. (1): HI titers of MAbs produced against bovine parvovirus (HADEN strain)

Table (2): Results of enzyme immunoassay for the determination of immunoglobulin class, subclass and light chain of mouse monoclonal antibodies produced against bovine parvovirus.

Clones	Ig		
	Class	Subclass	Light chain
3B10	IgG	IgG_1	K
3C5	IgG	IgG_{2a}	K
3D6	IgG	IgG_{2a}	K
2H5	IgM		K
2H6	IgM		K
1E6	IgM		K

identifying of class, subclass and light chain e of mouse monoclonal antibodies munoglobins) a mouse hybridoma subtyping was used. The resutls of the test were shown in ble (2). The data in the table showed that 3 nes of the produced MAbs were of the IgG s, subclass IgG_1, IgG_2a containing K light in and 3 clones of the produced MAbs were of M class containing also K light chain.

SCUSSION

ost enteric problems in newborn calves are inly attributed to microbial, nutritional, rasitic and hygienic factors. The rate of losses the early stage of life ranges from 80-100% and is occurs as the well-known pneumoenteritis ndrome. Bovine parvovirus is one of the viral tiologic agents causing pneumoenteritis among alves and adult cattle allover the world (Siegl, 976).

In Egypt, although there are many studies about viruses causing enteritis in newborn calves, nobody has paid attention to the role of parvovirus as a cause of enteritis among Egyptian newborn calves.

Bovine parvovirus was referred to as HADEN or haemadsorbing enteric virus (Abinanti and Warfield, 1961). Diagnosis of BPV often depends on viral HA activity (Freeman et al., 1986).

Parvoviruses can be divided into different variant types using HI assays with monoclonal antibodies. The majority of the MAbs prepared against Raccon dog parvoirus were directed to antigenic sites common to all the parvoviruses studied (Parrish and Carmichael, 1986). MAbs were previously used successfully in diagnostic purposes of Raccon dog and blue Fox parvoviruses (Veijlanien. (1987).

From the aforementioned information, the main objective of the present work was production of monoclonal antibodies (MAbs). BPV (HADEN strain) has an outer haemagglutinin epitope which interacts with monoclonal antibodies which inhibited the agglutination of G. pig RBCs to the virus.

BPV monoclonal antibodies (MAbs) against the reference HADEN strain of BPV were produced. Six different clones were obtained producing MAbs against BPV. Three of them are producing MAbs of IgG class (one subclass IgG1 and two IgG2a). These clones are 3B10, 3C5 and 3D6. The other three clones produced MAbs of IgM isotype (Table 2). Because of the more specificity and high affinity of IgG1 immunoglobulin, the clone 3B10 was selected for diagnostic purposes of BPV infection among Egyptian newborn calves.

The MAbs produced in this work exhibited variable degrees of inhibition to the HA activity of the reference HADEN strain of BPV (Fig. 1). It is very interesting to find out that some of the clones were producing MAbs of IgM, and this may be attributed to the type of the technique of immunization of the Bulb-C mice with parvovirus antigen. The specificity of the reaction showed by these clones confirmed the lower titers given in HI test as presented in Table (1).

As shown in this study, monoclonal antibodies are of valuable help in analyzing the biological activities of parvovirus infection in newborn calves.

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