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PRODUCTION OF MONOCLONAL ANTIBODIES TO ROTAVIRUS SUITABLE FOR DETECTION OF ROTAVIRUS IN STOOL SAMPLES BY ONE-STEP EIA

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Summary. - Two stable hybridoma cell clones producing high amounts of monoclonal antibodies (MAb) to rotavirus have been selected. The MAbs were shown to recognize different epitopes of the major inner capsid rotavirus protein VP 6. The two types of MAb in question cross-reacted with human and animal group A rotaviruses. Due to their high affinity and good binding capacity to microtiter plates a simultaneous EIA was developed for detection of rotavirus in stool samples. The sensitivity and specificity of MAb one-step EIA was compared with an approved polyclonal sandwich EIA by testing 1309 stool samples.

Key words: rotavirus; monoclonal antibody; EIA

Introduction

Rotavirus is the major cause of acute gastroenteritis in infants and young children. Similar diseases were found in several animal species. For detection of rotavirus in faecal samples enzyme-linked immunosorbent assay (ELISA) techniques are used due to high sensitivity, specificity, simplicity and the possibility to test a great number of samples. Commercial EIA kits for rotavirus use polyclonal antisera ("Rotazym", Abott, U.S.A.; „Rotavirus-EIA-Kitt", Dakopatts, Denmark) or a combination of polyclonal and monoclonal antibodies („Pathfinder", Kallestad Laboratories, U.S.A.; „Enzygnost", Behring, F.R.G.). The tests last for several hours. Several washing and incubation steps are needed when using polyclonal antibodies and it is impossible to standardize the test. MAbs overcome this drawback (Pothier, 1986) especially those with cross-reactivity encompassing human and animal rotaviruses of all 9 group A serotypes (Nakagomi, 1990). Such MAbs are directed against the major inner

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capsid protein VP 6 which is present in all rotaviruses. In this report we describe isolation and characterization of MAbs to rotavirus suitable for rapid one-step EIA rotavirus detection in stool specimens.

Materials and Methods

Viruses. The human rotavirus Wa (serotype 1), DS-1 (serotype 2), a human isolate R 326, the simian rotavirus SA 11 (serotype 3), and the bovine rotavirus NCDV (serotype 6) were grown in MA 104 cells (Schumacher, 1985).

Preparation and characterization of MAbs. Balb/c mice were injected several times at 4 weeks intervals by i.p. route with 0.5 ml of human rotavirus isolate R 326 mixed with an equal volume of Freund's complete or incomplete adjuvants. Since four days prior to fusion the mice were given an i. p. injection of 0.4 ml R 326 at daily intervals. The myeloma cell line FO was cultured in RPMI-1640 medium containing 15 % foetal calf serum. The isolated murine spleen cells were fused with FO myeloma cells in the presence of polyethylenglycol 4000. The hybridomas producing suitable MAbs were selected from about 20 distinct ones showing antibody production (Tölle, 1989). The immunoglobulin subclasses of the selected monoclonal antibodies were determined by EIA using class-specific antisera to mouse immunoglobulins (Mono AB-ID EIA-Kit Zymed, Conco, F.R.G.). The antibodies in culture fluids were determined by sandwich EIA (Grunert, 1987). The hybridoma clones were used for production of mouse ascitic fluid. Immunoglobulins from ascitic fluid were precipitated with ammonium sulphate and purified on a DEAE-Sepharose 6 B column (Peters, 1985). One monoclonal antibody was labelled with peroxidase as described by Wilson and Nakane (1978).

Western blot analysis was used for determining the protein specificity of MAbs. Rotavirus structural proteins were separated in 10 % PAA gel under reducing and nonreducing conditions and blotted to NC paper. Thereafter, the antigen-antibody reaction was performed and the protein was stained with diaminobenzidine.

Monoclonal one-step EIA for rotavirus detection in faecal samples was made on microtitre plates precoated either with 100 μl/well of MAb IVE-II C 6 or with purified control ascitic fluid without MAbs. In carbonate-bicarbonate buffer pH 9.6 after adsorption for 2 hr at 37 °C and overnight incubation at 4 °C, 50 μl peroxidase labelled monoclonal antibody IVE-IV F 4 diluted in buffer and 50 μl stool suspension were given simultaneously to 2 wells of the precoated plates. The mixture was shaken for 25 min at room temperature. After washing, 100 μl of the substrate OPD/H_2O_2 was added and incubated for 10 min at room temperature in darkness. The reaction was stopped by adding of 2 mol/l H_2SO_4 and the absorbance was determined. The sample was considered positive if extinction of 0.2 was noted at 492 nm and the P/N ratio was ≥ 2.

Stool specimens. A total of 1309 samples were collected from children (aged from 6 months to 3 years) who were seen for acute diarrheal illness. The samples were obtained from 1989 to 1990. 10 % (wt/vol) suspensions of the stool samples were examined.

Results

Characterization of monoclonal antibodies

Two stable hybridoma clones producing specific antibody to rotavirus were selected (Tab. 1). The clones showed a high stable antibody production, rapid growth and stability during storage in liquid nitrogen. The hybridoma cells grew in all standard media requiring no time for adaptation. The cells had to be subcultured every 3-4 days, the suspension being diluted 1:3. These hybri-

Table 1. Designation of monoclonal antibodies

Register number	Hybridoma clone	Mab	Mouse Ig subclass	Dilution in sandwich EIA
ZIM-0478	II C6	IVE-II C6	IgG 1	1:2000
ZIM-0479	IV F4	IVE-IV F4	IgG 2b	1:5000

doma clones produced MAbs for at least 10 passages without recloning. The western blot analysis showed that the antibody IVE-IV F 4 was directed against the major inner capsid protein VP 6 (42 kD protein). The antibody IVE-II C 6,

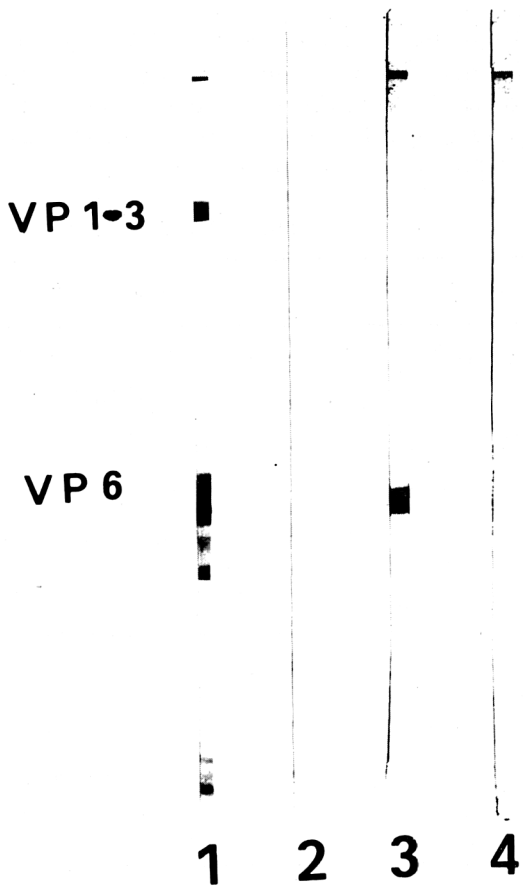

Fig. 1
Western blot analysis
lane 1: positive control serum
lane 2: negative control serum
lane 3: MAb IVE-IV F 4
lane 4: MAb IVE-II C 6

however, did not react with the VP 6 protein or any other viral proteins, which may be attributable to possible denaturation of viral proteins (Fig. 1).

Both monoclonal IgG antibodies cross-reacted with the human rotavirus strains Wa, DS-1 and the isolate R 326, the simian rotavirus SA 11 and the bovine rotavirus NCDV.

Using the blocking EIA test (Peters, 1985) the MAbs IVE-II C 6 and IVE-IV F 4 were shown to react with topographically non-overlapping epitopes. The IVE-II C 6 antibody proved to possess excellent binding properties to microtitre plates. Therefore, it was used as detector antibody with high activity and affinity. For detecting rotavirus with the one-step EIA only a short incubation time was necessary. Peroxidase labelled MAb IVE-IV F 4 was used in high dilutions up to 1:5000. A one-step EIA for detection of rotavirus in human stool samples was established with both MAbs (Tab. 2).

Comparison of polyclonal and monoclonal EIA in testing faecal samples from children

From 1989 to 1990 a total of 1309 stool samples from children were tested with both, the new monoclonal one-step EIA and the approved polyclonal sandwich EIA (Schumacher, 1985). The results obtained in 1301 cases (99.3 %) were identical. The 8 cases showing discordant results were additionaly tested by electron microscopy (Tab. 3).

Table 2. The limits of the MAb- and PAb-EIA

	MAb-EIA (R 326)	PAb-EIA (SA 11)
R 326 dilution	1:3000	1:6000
protein	3.2 ng/ml	16 ng/ml
SA 11 dilution	1:1000	1:70
protein	10 ng/ml	140 ng/ml

Table 3. Comparison of polyclonal and monoclonal EIA

No of specimens	Mab-EIA	PAb-EIA	EM
1077	0	0	
224	+	+	
7	0	+	2+
1	+	0	0
Sensitivity	99.1 %	100.0 %	
Specificity	99.9 %	99.5 %	

Discussion

Several reports have been published concerning MAbs to rotavirus group antigen and their use for diagnostic purposes (Beards, 1984; Cukor, 1984; Herrmann, 1985; Kinsley, 1986), but informations about properties of the antibody producing hybridoma cell lines (requirements to media, multiplication rate, culture conditions, stability) are scanty. Myeloma cell lines P3-X63-Ag8, 653; NS-1; NS-0, are known to require very good culture conditions. The monoclonal antibodies to rotavirus described so far (Greenberg, 1983; Taniguchi, 1984; Coulson, 1985; Grunert, 1987) were used in low dilutions and required long incubation times. Thus it was evident that either the content of antibodies in the preparations was low or their affinity was not good. Our two selected stable hybridoma cell clones (II C 6 and IV F 4) which produced specific antibodies to rotavirus proved to grow in all standard media requiring no adaptation time and were shown to be high MAb producers.

In order to get a highly sensitive and specific monoclonal EIA test system, one type of MAbs antibodies to the rotavirus group antigen is necessary to use as capture antibody fixed to the solid phase and, in addition, an another enzyme labelled MAb reacting with another antigenic site of the group specific antigen as detector antibody. The two antibodies produced by our hybridoma cell clones meet these requirements. They recognize different epitopes located on the group antigen and they have cross-reactive properties. It was possible to put simultaneously the detector antibody (IVE-IV F 4) and the stool sample to the microtitre plate precoated with capture antibody (IVE-II C 6), only one incubation step is necessary. In general, duration of the test depends upon the affinity of capture and detector antibodies. The test reported by Porthier *et al.* (1986) required 30 min at 37 °C and the EIA from Röhm Pharma, F.R.G. 60 min at room temperature. In our one-step EIA the incubation time was reduced to 25 min at room temperature, due to the very high affinity of the antibodies (produced by our hybridoma cells) and the good binding properties to the solid phase. The evident advantages of this monoclonal one-step EIA consists in its simple and rapid performance combined with high sensitivity and specificity in comparison with an adequate polyclonal EIA. The tests can be standardized by using only MAbs of high affinity. Reactions of the selected antibodies with the bovine rotavirus NCDV suggest the suitability of described test system for detecting also animal rotaviruses in faecal samples.

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