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Development and Application of Monoclonal Antibodies for Specific Detection of Human Enteric Adenoviruses

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Monoclonal antibodies were prepared against enteric adenovirus by fusing P3-NS1/-Ag4-1 mouse myeloma cells with lymphocytes from BALB/c mice immunized with enteric adenovirus 40 (Ad40) G2297. Of the several putative clones secreting antibodies to adenovirus, five were found to react specifically to the enteric adenovirus. The specificity of two of these monoclones which recognize a single antigen of a molecular size of 17 kilodaltons was evaluated against 78 clinical isolates. One monoclone (5D8/2C2) reacted with both Ad40 and Ad41, and the other monoclone (2H6/C11) recognized Ad40 only in an enzyme-linked immunosorbent assay (ELISA). These ELISA results correlated well with those of the specific neutralization test or DNA restriction endonuclease analysis or both. The use of this rapid ELISA with these monoclones will find applications in the diagnosis of enteric adenovirus and should facilitate the epidemiologic studies of enteric adenovirus gastroenteritis.

Enteric adenoviruses are increasingly recognized as an important cause of acute infantile gastroenteritis in different parts of the world (1). Infection with enteric adenoviruses was detected by viral shedding in the stool or by seroconversion in 7.2% of hospitalized children in a 1-year prospective study (13). Endemic enteric adenovirus infection was recognized in 5.1% of inpatients with gastroenteritis by using electron microscopy on fecal specimens and by tissue culture inoculation over a period of 9 years (1). This subgroup of adenoviruses has a distinct set of antigenic determinants and fastidious tissue culture growth characteristics (11, 14). They have been classified into two distinct species, i.e., adenoviruses 40 and 41 (Ad40 and Ad41) on the basis of neutralization tests in monkey kidney cells and restriction endonuclease analysis of the DNA (3). In other studies, they were not differentiated by neutralization tests with different cells (HEK-293 cells or Chang conjunctival cells) and different hyperimmune sera and by the hemagglutination inhibition test and in solid-phase immune electron microscopy (1, 10, 15).

Enteric adenoviruses have been detected by different techniques. Detection by electron microscopy and by their failure to grow in conventional cell lines (standard HEK, HeLa or HEp-2 cell lines) that are known to support respiratory adenovirus provides presumptive diagnosis (1, 2). The neutralization of the virus with an anti-enteric adenovirus hyperimmune serum is slow and cumbersome but appears to be accurate and correlates with electropherotyping (1). The accuracy of the genus- or species-specific enzyme-linked immunosorbent assay (ELISA) requires a high-quality specific antiserum that is not readily available (13).

The purpose of this study was to develop monoclonal antibodies with specificity for human enteric Ad40. This report describes a simple, rapid, and specific means of diagnosing enteric adenovirus infection by using enteric adenovirus-specific monoclonal antibodies in an ELISA. With the development of these monoclonal antibodies, it has been possible to recognize Ad40 and Ad41 in fecal suspension and in tissue culture specimens.

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MATERIALS AND METHODS

Hybridoma production. Hybridoma cell lines were prepared by the fusion of mouse myeloma cells (P3-NS1/-Ag4-1) with lymphocytes from female BALB/c mice immunized with Ad40 G2297. The virus was propagated in HEK-293 cells, an Ad5 transformed line, and was partially purified by fluorocarbon extraction before immunization. Female BALB/c mice were given an intraperitoneal injection of virus mixed with an equal volume of Freund complete adjuvant, followed 4 weeks later by virus mixed with an equal volume of Freund incomplete adjuvant. A booster injection of the virus was given intravenously 3 days before the fusion. The monoclonal antibodies were developed by the standard technique (5). Several clones of viable hybrid cells were produced in the hypoxanthine-aminopterin-thymidine selection medium. After the selection of hybridomas by ELISA (see below), cells of interest were cloned by limiting dilution with a thymocyte feeder layer, retested for antibody production, and characterized (9).

Testing for antiadenovirus antibodies. The screening of hybridoma supernatants was done by an ELISA. Polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated overnight at 4°C with a hyperimmune rabbit antiserum to enteric adenovirus diluted in carbonate buffer (pH 9.6). The plates were washed three times with phosphate-buffered saline containing 0.05% Tween 20 (TPBS) and 100 μl of test adenovirus antigen in the form of crude cell culture harvest (1:2 dilution, frozen and thawed three times), or 10% fecal suspension was added and allowed to bind for 1 h at 37°C. The plates were washed three times with TPBS and incubated with 100 μl of the test hybridoma supernatants for 1 h at 37°C. The plates were again washed three times with TPBS and incubated with 100

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μl of goat anti-mouse immunoglobulin M and immunoglobulin G (heavy and light chains specific) conjugated to alkaline phosphatase (1:800 in TPBS) for 1 h at 37°C. With *p*-nitrophenyl phosphate as a substrate, the reaction products were measured at 400 nm approximately 30 min later. All assays were done in duplicate wells, and all tests were confirmed.

Detection of molecular identity of the antigens. The molecular identity of the antigens to which these monoclonal antibodies are directed was detected by using the Western blot enzyme immunobinding procedure. Ad40 G2297 (producing extensive viral cytopathic effect in HEK-293 cells) and HEK-293 cells were solubilized in 20 mM Tris hydrochloride (pH 8.0)–0.1 M NaCl–0.5% sodium deoxycholate–1% Triton X-100–0.1% sodium dodecyl sulfate (SDS) containing 1 mM phenylmethylsulfonyl fluoride and was subjected to SDS-polyacrylamide slab gel electrophoresis. The protein bands separated on the gel were electrophoretically transferred to the nitrocellulose paper. The antigens were localized by the sequential incubation of the blotted nitrocellulose strips with hybridoma supernatants or ascites fluid, peroxidase-conjugated goat anti-mouse gammaglobulin, and 4-chloro-*p*-naphthol.

SDS-polyacrylamide slab gel electrophoresis. The SDS-polyacrylamide gel electrophoresis of 10% polyacrylamide (acrylamide-bisacrylamide, 37.5:1) was performed at a constant current of 30 mA (6). The deoxycholate extract (1.2 ml) of enteric adenovirus or HEK-293 cells was mixed with 1 ml of sample buffer having 2% SDS and was run as a continuous band. The molecular weight markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were run simultaneously in a separate lane.

Western blot procedure. After SDS-polyacrylamide gel electrophoresis, the separated proteins were electrophoretically transferred onto nitrocellulose paper (12). Briefly, the gel was opposed to a sheet of nitrocellulose paper, and the transfer was conducted overnight at a constant 30 V. The nitrocellulose paper was dried between the folds of filter paper, cut into strips (0.3 cm wide), and stored at −70°C until use.

Detection of antigen on the nitrocellulose strips. The antigen was detected by using an enzyme immunobinding procedure (7). The blotted nitrocellulose strips were incubated in a buffered medium (50 mM Tris hydrochloride, 150 mM NaCl, 5 mM EDTA, 0.25% gelatin, 0.05% Nonidet P-40 [pH 7.4]) for 2 h with two changes of the buffer and then incubated for 2 h with the hybridoma supernatant-ascites fluid (1:50 in the incubation buffer). After being washed for 2 h with the incubation buffer with two changes, the strips were incubated for 2 h with peroxidase-conjugated goat anti-mouse immunoglobulin G (heavy and light chains specific) diluted (1:400) in incubation buffer containing no Nonidet P-40.

After being washed, the strips were incubated with 4-chloro-*p*-naphthol (0.6 mg/ml in 0.1 M Tris hydrochloride buffer [pH 7.4] containing 20% methanol and 0.01% H_2O_2). The strips were washed in water and dried. All incubations and washings were done at room temperature.

Specimen sources. Clinical isolates were obtained from B. Jeffries, H. W. Kim, C. D. Brandt, and R. H. Parrott of the Department of Virology, Children's Hospital National Medical Center, Washington, D.C., and A. H. Kidd of the National Institute of Virology, Republic of South Africa. Rabbit hyperimmune sera raised against enteric adenovirus were provided by H. E. Takiff and S. E. Straus of the National Institutes of Health, Bethesda, Md.

TABLE 1. Reactivity of two hybridomas with selected strains of adenovirus and rotavirus in the ELISA

Antigen	Known subgroup	Optical density[a] (×100) with hydridoma fluid	
		5D8/2C2	2H6/1E11
Ad12	A	−2	−6
Ad18	A	1	−7
Ad31	A	2	−8
Ad3	B	−6	−9
Ad7	B	−3	−8
Ad1	C	−4	−10
Ad2	C	−7	−8
Ad5	C	0	−8
Ad6	C	−2	−7
Ad15	D	0	0
Ad8	D	−4	−1
Ad4	E	−2	−9
Ad40 Dugan	F	97	88
Ad41 Tak	F	121	2
Rotavirus DS1 (human)	I	−3	−4
Rotavirus Wa (human)	II	−7	−5

[a] Enteric adenovirus-specific monoclonal antibodies used at 1:2 dilution.

TABLE 2. Reactivity of enteric adenovirus-specific monoclonal antibodies with various adenovirus antigens in the ELISA

Antigen species (no. of samples tested)	Mean (range) optical density (×100) for monoclonal antibody[a]	
	5D8/2C2	2H6/1E11
Ad40 (14)	55.79 (29.5–99)	50.81 (27.5–92)
Ad41 (64)	95.94 (19–141)	−3.30 (−21–12.5)

[a] Enteric adenovirus-specific monoclonal antibodies used at 1:2 dilution.

RESULTS

Specificity of the monoclonal antibodies. Several hybridomas were found to secrete antibodies as tested by the ELISA. Of these monoclones, five (5D8/2C2, 2H6/1E11, 5B7/2G8, 2A2/1D12, and 2C7/2E2) reacted specifically to the fastidious enteric adenovirus. The monoclone 5D8/2C2 recognized both prototypes Ad40 Dugan and Ad41 Tak, and the other four monoclones recognized only Ad40. None of these monoclones cross-reacted with previously established adenovirus types, subgroups A through E, or rotavirus subgroups I and II (Table 1).

The two monoclones 5D8/2C2 and 2H6/1E11 were further evaluated against 78 clinical isolates in the form of fecal suspensions or tissue cultures which were found to be enteric adenovirus by a specific neutralization test or by DNA restriction site analysis or both (4, 8). The monoclone 5D8/2C2 identified Ad40 or Ad41 in each of the 78 isolates. The monoclone 2H6/1E11 reacted with Ad40 only (Table 2). The extent to which low-titer fecal samples can be accurately assayed has yet to be determined. These results correlated completely with those of the neutralization test and restriction endonuclease analysis.

Each of the monoclones was found to be of the immunoglobulin G class. None of the monoclones neutralized Ad40 or Ad41 in HEK-293 cells.

Molecular identity of the antigens. The monoclones 5D8/2C2 and 2H6/1E11 reacted with a single antigen of a molecular size of approximately 17 kilodaltons in the Western blot immunobinding procedure (Fig. 1).

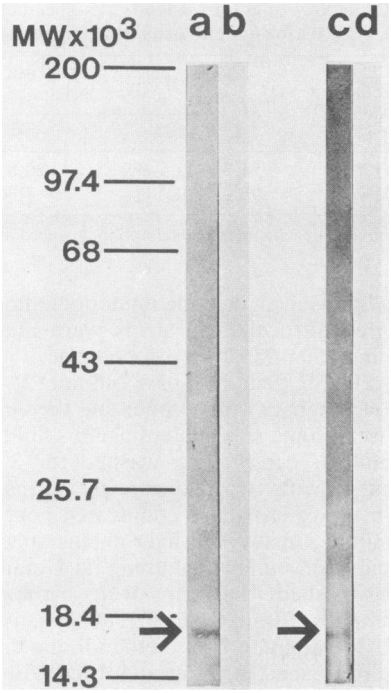

FIG. 1. Molecular identity of the antigen demonstrated by the monoclonal antibodies. 5D8/2C2 (lane a) and 2H6/1E11 (lane c) subclones reacted against the single antigen with a molecular weight (MW) of approximately 17,000 in the nitrocellulose blot of solubilized viral (Ad40 G2297) extract. These monoclones did not react with any antigen in the blot of solubilized host cell extract (lanes b and d).

By immune electron microscopy, none of these monoclones showed a reaction with Ad40 G2297.

DISCUSSION

Antigenic analysis revealed that two monoclones (5D8/2C2 and 2H6/1E11) were able to recognize specifically Ad40 or Ad41 or both. Both of these monoclones reacted with the single antigen of a molecular size of 18 kilodaltons in the Western blot procedure. This antigen seems to be polypeptide VII, the DNA core protein of the virion, as has been described by Wadell et al. (the apparent molecular size of polypeptide VII is 17.2 kilodaltons [14]). The lack of reactivity of the monoclones with adenovirus by immune electron microscopy also indicates that they are directed against the interior DNA core protein and not against the protein present on the surface of the virus. Since these monoclones show different reactions with Ad40 and Ad41, it is likely that they are against the different antigenic determinants. These monoclones confirm the close antigenic relationship as well as the distinctiveness of enteric adenoviruses 40 and 41.

The ELISA using these enteric adenovirus-specific monoclonal antibodies as detected antibodies was specific for the detection of Ad40 or Ad41 or both. This assay is easy to perform and can be completed within 4 h by using precoated plates. This rapid and specific ELISA using enteric adenovirus-specific monoclonal antibodies should prove to be a valuable epidemiological tool for studying viral gastroenteritis. Such studies should also elucidate the sensitivity of this ELISA.

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