JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1983, p. 463–468
0095-1137/83/090463-06$02.00/0

Vol. 18, No. 3

Comparison of a Monoclonal Antibody with a Polyclonal Serum in an Enzyme-Linked Immunosorbent Assay for Detecting Adenovirus

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Received 28 December 1982/Accepted 6 May 1983

To develop and evaluate an enzyme-linked immunosorbent assay (ELISA) for detecting adenovirus antigens in which a group-specific monoclonal antibody to the adenovirus hexon is used, we studied two different ELISA test systems. The test system which was the most sensitive and specific was then compared in parallel tests with a similarly constructed ELISA in which a mouse polyclonal serum was used. Both the ELISA with the monoclonal antibody and that with the polyclonal serum detected purified hexon and 15 different adenovirus types with similar sensitivities. The two assays also showed no reaction with 23 heterologous viruses. Both tests detected adenovirus in stool and respiratory tract specimens tested for adenovirus by standard tissue culture techniques with similar sensitivities and specificities, but neither was sufficiently sensitive for routine testing of these types of clinical specimens. However, the ELISA with the monoclonal antibody proved to be a good test for the noncultivatable adenoviruses, detecting 12 of 12 stool samples that were positive by electron microscopy. The monoclonal antibody proved to be as sensitive and specific as the polyclonal serum and has the advantage that it can be produced in unlimited quantities and needs to be characterized only once.

Enzyme-linked immunosorbent assays (ELISAs) have been useful in detecting viral antigens in a number of infections, including hepatitis B (23, 24, 26), rotavirus diarrhea (18, 27), adenovirus diarrhea (7, 12, 20, 28), and some respiratory viral infections (8, 9, 11, 14, 19). The two major limitations affecting these ELISAs are the small quantity of antigen present in some clinical specimens and the lack of readily available, high-quality antiserum. The latter limitation might be overcome by using monoclonal antibodies. There are, however, few reports of direct comparisons of monoclonal antibody with polyclonal serum as a reagent in immunoassays for viral antigen detection (17, 25). Such studies will be important in determining the role which monoclonal antibodies should play in these immunoassays.

We took advantage of the availability of a well-characterized group-specific monoclonal antibody to the adenovirus hexon (5) to make such a comparison. We constructed an ELISA for the detection of adenovirus antigens in which this monoclonal antibody could be directly compared with a polyclonal serum from the same mouse strain that was the source of the hybridoma cells. This report summarizes our experience with several ELISA test systems in which different combinations of monoclonal antibody or polyclonal mouse serum plus polyclonal goat serum are used. We found the performance of the monoclonal antibody to be virtually identical to that of the polyclonal mouse serum.

MATERIALS AND METHODS

Purification of adenovirus hexon. Adenovirus type 2 (Ad2) hexon was purified by a modification of the procedure described by Boulanger and Puvion (2). The purified hexon gave one visible band on Ouchterlony gel diffusion against two polyclonal anti-adenovirus sera and one heavy band at 120,000 daltons and one faint band at 90,000 daltons with sodium dodecylsulfate-polyacrylamide gel electrophoresis (13). The protein concentration was determined with the Bio-Rad protein assay (3).

Anti-Ad2 hexon monoclonal antibody (2Hx-2). The group-specific monoclonal antibody against adenovirus hexon, provided by C. L. Cepko and P. A. Sharp of The Massachusetts Institute of Technology, Cambridge, Mass., was prepared as previously described (5). Immunoglobulin from the ascites fluid was purified by ammonium sulfate precipitation, once with 30% and then twice with 40% ammonium sulfate (10).

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TABLE 1. ELISA test systems for detecting adenovirus antigen

Test system	Capture antibody (dilution)	Detector antibody (dilution)	Conjugate (dilution)
G/2Hx-2	G (10^{-3})[a]	2Hx-2 (10^{-4})	GaM (10^{-3})
G/M	G (10^{-3})[a]	M (10^{-3})	GaM (10^{-3})
2Hx-2/G	2Hx-2 (10^{-4})[a]	G ($10^{-2.5}$)	RaG ($10^{-3.5}$)
M/G	M (10^{-3})[b]	G ($10^{-2.5}$)	RaG ($10^{-3.5}$)

[a] Diluted in carbonate buffer, pH 9.6.
[b] Diluted in phosphate buffer, pH 8.0.

Polyclonal antisera. Polyclonal mouse anti-Ad2 hexon serum (M) was prepared by intraperitoneally immunizing 10-week-old female BALB/*c* mice on days 0 and 42 with 15 μg of purified Ad2 hexon emulsified in incomplete Freund adjuvant. The sera collected at 3, 4, and 5 weeks after the last immunization were pooled. Immunoglobulin from the pooled serum was purified by three ammonium sulfate precipitations.

Polyclonal goat antiserum to adenovirus (G) (lot 41068; complement fixing titer, 1:128) was obtained from M. A. Bioproducts, Walkersville, Md. Goat anti-mouse immunoglobulin G (heavy and light chain) serum conjugated with peroxidase (GaM) (lot RD1101) was obtained from Litton Bionetics, Inc., Kensington, Md. Affinity-purified rabbit anti-goat immunoglobulin G (heavy and light chain) serum conjugated with peroxidase (RaG) (lot GJ18) was obtained from Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Md. When used as detector antibody, G was first absorbed with the monoclone 2Hx-2, covalently linked to Sepharose 4B (16). Two volumes of a 1:10 dilution of serum were incubated with one volume of the Sepharose for 1 h at 37°C.

Solid-phase ELISA. To develop the most sensitive and specific ELISA with 2Hx-2, we developed and evaluated two different test systems and compared them in parallel tests with two similarly constructed ELISAs with M (Table 1). Optimal dilutions were determined by checkerboard titrations, and the optimal buffer for the capture antibody was determined by checkerboard titrations. All four test systems had the following six steps: (i) capture antibody was passively adsorbed to flexible, 96-well, U-bottomed polyvinylchloride (PVC) microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) by incubation at 4°C for at least 16 h; (ii) capture antibody was aspirated, the PVC plate was washed three times, and antigen was added and incubated either overnight at room temperature or for 1.5 h at 37°C; (iii) antigen was aspirated, the PVC plate was washed three times, and detector antibody was added and incubated for 1.5 h at 37°C; (iv) detector antibody was aspirated, the PVC plate was washed three times, and conjugate was added and incubated for 1 h at 37°C; (v) the conjugate was aspirated, the PVC plate was washed three times, and substrate (0.4 mg of *O*-phenylene diamine per ml and 0.015% hydrogen peroxide) in citrate phosphate buffer (pH 5.5) was added and incubated at room temperature for 45 to 60 min; and (vi) the reaction was stopped with 3.5 M HCl and absorbance was read at 490 nm for the PVC plates with a Chromo Scan EIA Reader (Biotek Instruments, Inc., Burlington, Vt.).

For steps i through iv, 100 μl of the sample or reagent was added to the wells. For step v, 125 μl of the substrate solution was added to the wells. The PVC plates were washed with phosphate-buffered saline, at pH 7.2 (PBS), with 0.5% Tween 20 (PBS-T). The antigens and antibodies used in steps ii through iv were diluted in PBS with 0.5% gelatin and 0.15% Tween 20 (PBS-GT), and each sample was run in duplicate. All comparisons of monoclonal and polyclonal antibodies were made in tests run simultaneously.

Adenovirus strains and heterologous viruses. Fifteen adenovirus strains were obtained either from our reference virus collection or laboratory stock virus (Table 2). The following heterologous viruses, obtained from laboratory stock virus grown at 3^+ to 4^+ cytopathic effect and stored at −70°C, were also tested: parainfluenza virus types 1, 2, and 3; respiratory syncytial virus; influenza A/Victoria/3/75 (H3N2); influenza A/USSR/90/77 (H1N1) (high growth recombinant X-47); influenza B/Hong Kong/5/72; reovirus 1 and 2;

TABLE 2. Titration of different adenovirus types by ELISA test system G/2Hx-2

Adenovirus type	Adenovirus group by hemagglutination	$TCID_{50}$ per ml[a]	Absorbance at 490 nm for 10^{-2} dilution	Absorbance at 490 nm for 10^{-3} dilution	Endpoint dilution by ELISA
1	3A	$10^{6.1}$	>2.0	1.365	10^{-4}
2	3A	$10^{6.1}$	>2.0	1.214	$10^{-3.9}$
3	1A	$10^{6.0}$	1.556	0.336	$10^{-3.4}$
4	3A	$10^{6.5}$	>2.0	1.201	10^{-4}
5	3A	$10^{7.1}$	<2.0	1.500	$10^{-4.5}$
7	1A	$10^{5.6}$	>2.0	0.771	$10^{-3.7}$
8	2A	$10^{4.6}$	>2.0	0.500	$10^{-3.5}$
10	2B	$10^{5.1}$	1.053	0.133	$10^{-2.9}$
11	1A	$10^{6.4}$	1.711	0.409	$10^{-3.5}$
12	3B	$10^{5.8}$	1.500	0.336	$10^{-3.4}$
14	1B	$10^{5.1}$	>2.0	0.918	$10^{-3.9}$
19	2B	$10^{5.8}$	1.598	1.287	$10^{-4.0}$
21	1B	$10^{4.3}$	>2.0	1.381	$10^{-4.0}$
31	3B	$10^{3.8}$	>2.0	1.023	$10^{-4.0}$
35	1A	$10^{6.2}$	1.767	0.492	$10^{-3.6}$

[a] $TCID_{50}$, 50% Tissue culture infectious dose endpoint.

coxsackievirus A9, A16, B1, and B4–B6; echovirus 7, 9, and 11; poliovirus 1, 2, and 3; and herpes simplex virus types 1 and 2. Viruses other than adenovirus were diluted 1:5; and adenovirus strains were diluted 10^{-2}, 10^{-3}, and 10^{-4} in PBS-GT for testing in the ELISA.

Clinical specimens. All stool and respiratory specimens that were positive by standard tissue culture techniques for adenovirus between January 1980 and April 1982 were identified. For each positive specimen, two controls, matched for the type of specimen and the date of submission, were chosen randomly from the freezer log book. Only 9 of the 13 positive respiratory specimens and 24 of the 26 negative respiratory specimens identified had a sufficient amount of the original specimen to complete the testing. In addition, 12 stool specimens positive for noncultivable adenovirus by electron microscopy were tested.

The stool specimens were diluted approximately 1:10 in medium before inoculation onto tissue culture, and the remaining amount of specimen was stored at −70°C. The respiratory specimens (nasopharyngeal aspirates, throat swabs, and tracheal aspirates) were either received in transport media or rinsed from the specimen container with a minimal volume of sterile PBS. The specimens were inoculated onto tissue culture, and the remaining amount of specimen was stored at −70°C. Before being tested in our ELISA, the respiratory specimens were sonicated with a Branson probe sonicator.

For our ELISA, 100 μl of the thawed stool specimens was added to duplicate wells, and 50 μl of the respiratory specimens with 50 μl of the PBS-GT was added to the duplicate wells.

A specimen was considered positive if the mean of the absorbance for the two wells was greater than two times the mean of the background, or the mean of absorbance was greater than the mean of the background plus 3 standard deviations on two successive tests. The mean and standard deviation of the background was determined from the absorbance of six wells with negative stool or respiratory specimens, respectively.

RESULTS

Sensitivity and specificity. The sensitivities of the four test systems were similar (Fig. 1 and Table 3), but the absorbance levels of the background were not. The test systems in which G was used as capture antibody (test systems G/2Hx-2 and G/M, see Table 1) had lower levels of background absorbance.

We could detect all 15 adenovirus types with each of the four test systems that had a sensitivity similar to that of test system G/2Hx-2 (Table 2). When G was used as detector antibody (test system 2Hx-2/G and M/G), 6 of the 23 heterologous viruses gave false-positive readings. Of these six false-positive results, five were eliminated by absorbing G and RaG with 1% uninfected cell culture supernatant fluid. In contrast, no false-positive reactions occurred with the 23 heterologous viruses when G was used as capture antibody (test systems G/2Hx-2 and G/M). The two systems in which G was used as capture antibody had the best combination of sensitivity and specificity and were used for testing clinical specimens.

Clinical specimens. The test system with the monoclonal antibody (G/2Hx-2) had a sensitivity and specificity similar to those of the test with the polyclonal mouse serum (G/M) (Table 4). The results for the respiratory specimens were disappointing. The sensitivity and specificity were 11 and 96%, respectively, for G/2Hx-2 and 11 and 100%, respectively, for G/M. The results for the stool specimens, however, were as expected. For the cultivable stool adenoviruses, the sensitivity and specificity were 36 and 95%, respectively, for G/2Hx-2 and 18 and 95%, respectively, for G/M. The 12 stool specimens containing noncultivable adenoviruses identified by electron microscopy were tested only in test system G/2Hx-2. The sensitivity was 100%. Since the numbers of specimens tested are few, these figures for sensitivity and specificity are only rough estimates.

The supernatant fluids from the initial tissue culture passage for 12 of the 15 false-negative specimens were available for testing in both ELISA test systems. Of the 12, 10 were positive in both systems. One additional isolate, when passaged one more time, was then detectable in both ELISA tests. The one remaining negative specimen could not be regrown, and thus we could not determine whether this specimen had insufficient titer of adenovirus or if that strain of adenovirus did not react in these test systems. There was insufficient original material from the two specimens which gave false-positive results to make another attempt to isolate adenovirus or to look for adenovirus-like particles by electron microscopy.

DISCUSSION

The monoclonal antibody 2Hx-2 proved to be as good a reagent for detecting adenovirus as was M in similar ELISA tests. This conclusion held for the detection of purified hexon, 15 different adenovirus strains, and adenovirus antigen in clinical specimens. The level of background absorbance was low for both ELISA tests; neither gave false-positive tests with 23 heterologous viruses, and both gave a low rate of false-positive reactions when used to test clinical specimens.

Both ELISA tests detected as little as 1 ng of purified hexon per ml. This sensitivity was similar to that reported by other groups (7, 12). However, the capacity of these tests to detect adenovirus antigen in clinical respiratory specimens was not as good as that reported by others (60 to 65% of that obtained with isolation [9, 15] and 100% of that obtained with the fluorescent-

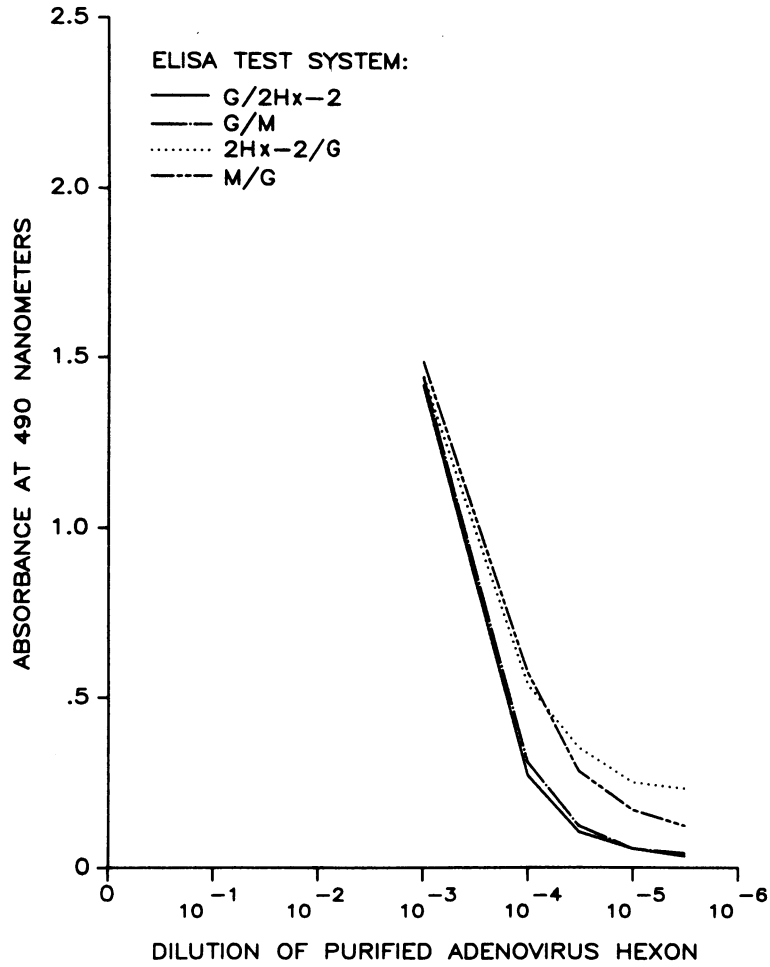

FIG. 1. One of two titrations of purified Ad2 hexon tested in four different ELISA test systems: (i) G/2Hx-2 with G as capture antibody, 2Hx-2 as detector antibody, and GaM as the conjugate; (ii) G/M with G as capture antibody, M as detector antibody, and GaM as the conjugate; (iii) 2Hx-2/G with 2Hx-2 as capture antibody, G as detector antibody, and RaG as the conjugate; (iv) M/G with M as the capture antibody, G as the detector antibody and RaG as the conjugate. The mean plus 3 standard deviations of background absorbance was 0.034 for G/2Hx-2, 0.040 for G/M, 0.129 for M/G, and 0.248 for 2Hx-2.

antibody test [FA] [20]). The reason for this discrepancy is not clear. It could not be attributed to adenovirus strains which did not react in these two ELISAs, because for 11 of the false-negative specimens, the virus isolated in tissue culture was positive in both ELISA tests. The difference between our results and the better results from other groups may reflect differences in the type, quality, and possibly preparation of clinical specimens.

TABLE 3. Sensitivity of four ELISA test systems for detecting purified Ad2 hexon

ELISA test system	Lowest concn (ng/ml) of purified Ad2 hexon detectable by ELISA[a]	Mean (range) of background absorbance
2Hx-2/G	0.7	0.159–0.229
M/G	0.2	0.040–0.101
G/2Hx2	0.3	0.016–0.020
G/M	0.4	0.024–0.030

[a] Interpolated lowest concentration which gave an absorbance reading of 3 standard deviations above the mean of the background absorbance.

Our ELISA appeared to be a good test for detecting the noncultivable adenoviruses, as was expected on the basis of other reports (7, 12, 18, 28). The low sensitivity of the test for detecting cultivable adenoviruses is consistent with the finding that adenoviruses not associated with gastroenteritis and positive in stool cultures are often not seen by electron microscopy (4), presumably because of a lower level of virus in the stools.

TABLE 4. Results of ELISA for detecting adenovirus in clinical specimens

Clinical specimen	No. tested	No. positive for adenovirus in system:			No. positive/no. of tissue culture supernatants tested[a]
		G/2Hx-2	G/M	Both	
Stool positive for adenovirus[b]	11	4	2	2	5/5
Stool negative for adenovirus[b]	22	1	1	1	
Stool positive for adenovirus by electron microscopy	12	12			
NP[c] aspirate/throat swab positive for adenovirus[b]	9	1	1	1	5/6
NP aspirate/throat swab negative for adenovirus[b]	24	1	0	0	

[a] Only tissue culture supernatants from those clinical specimens negative for adenovirus by ELISA (false negatives) were tested.
[b] Tested by standard tissue culture techniques.
[c] NP, Nasopharyngeal.

We expected that the ELISA in which the monoclonal antibody was used as capture antibody (test system 2Hx-2/G) would be more sensitive than the other three test systems, in which polyclonal antibody was used as capture antibody, because the wells were coated with immunoglobulin containing a higher proportion of antibodies of the desired specificity. Instead, test system 2Hx-2/G had a sensitivity similar to those of the other three test systems, with high levels of background absorbance and nonspecific reactions. The nonspecific reactions resulted from antibodies in G reacting with antigens nonspecifically sticking to the plastic. By using the more specific antibody as detector antibody rather than capture antibody, we improved the specificity of the test without changing the sensitivity.

Monoclonal antibodies have been used to detect a variety of antigens for clinical diagnostic work (21), including influenza virus by FA (17), hepatitis B virus by radioimmunoassay (25), herpes simplex virus by FA (1), chlamydia by FA (22), and cytomegalovirus by FA (6). The sensitivity and specificity of tests with monoclonal antibodies have proven to be better than those done by radioimmunoassay with polyclonal serum (25) and not as good as those done by FA with polyclonal serum (17). In this study the monoclonal antibody proved to be as good as the polyclonal serum. It combined the broad reactivity needed to detect the many adenovirus types with good specificity. Although not sufficiently sensitive for respiratory specimens, this ELISA appears to be a good test for the nonculturable adenoviruses associated with gastroenteritis. Moreover, this reagent can be made in unlimited quantities and needs to be characterized only once. Clearly, there will be a role for monoclonal antibodies in solid-phase immunoassays in the future.

ACKNOWLEDGMENTS

We thank C. P. Cepko and P. A. Sharp of The Massachusetts Institute of Technology, Cambridge, Mass., for providing the monoclonal antibody and R. M. Hendry of Children's Hospital Medical Center, Boston, Mass., for his advice on developing the ELISAs.

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