Detection of Cytomegalovirus from Clinical Specimens in Centrifugation Culture by In Situ DNA Hybridization and Monoclonal Antibody Staining

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An in situ DNA hybridization kit for cytomegalovirus (CMV) was evaluated for the detection of CMV in centrifugation culture. Of 61 clinical specimens, 17 (27.8%) were positive for CMV by monoclonal antibody staining following centrifugation. Of the 17 positive specimens, 15 were detected by DNA hybridization (24.5%). However, the earliest that CMV could be detected by DNA hybridization was 58 h as compared with 16 h with monoclonal antibodies following centrifugation. DNA hybridization remains of great interest for the study and detection of CMV infection. However, current DNA hybridization techniques are not sufficiently rapid to replace the use of monoclonal antibodies in centrifugation culture.

Cytomegalovirus (CMV) disease remains a major clinical problem in the immunocompromised host and can cause severe manifestations in congenitally infected infants (4, 9, 19). Clinical diagnosis of CMV infection is difficult and is ultimately dependent on laboratory confirmation. Therefore, rapid laboratory diagnosis of CMV infection is necessary and desirable for appropriate patient management.

Recent developments in laboratory techniques have dramatically reduced the time needed to detect CMV from various specimen sources (2, 5-7, 13, 18). Among these techniques, hybridization remains of great interest for rapid and specific diagnosis of CMV infection. Both dot blot and in situ DNA hybridization can be performed in 5 to 48 h and have the potential advantage of being more sensitive than conventional cell cultures (10).

In this report we describe the evaluation of an in situ DNA hybridization kit using a biotinylated DNA probe (Enzo Biochem, Inc., New York, N.Y.) for the detection of CMV from clinical specimens in centrifugation culture. The sensitivity of the kit was compared with that of indirect immunofluorescence (IF) staining using a murine monoclonal antibody (MAb) for detection of CMV in centrifugation culture.

MATERIALS AND METHODS

A total of 61 specimens (19 bronchoalveolar lavages (BAL), 15 lung tissue specimens, 14 urine specimens, and 13 throat specimens) from 48 marrow transplant patients were inoculated into standard cell culture and centrifugation cell culture for detection of CMV. Specimens were inoculated at a volume of 0.25 ml into two tubes (16 by 125 mm) containing human foreskin fibroblast cell monolayers. Cultures were maintained for 4 weeks and examined twice weekly for the first 2 weeks and then once weekly for the next 2 weeks for CMV cytopathic effect. Specimens were also inoculated at a volume of 0.2 ml per vial into each of seven 1-dram (3.887-g) shell vials (Fisher Scientific Co., Seattle, Wash.) containing cover slip cultures of MRC-5 cells. Three cover slips were used for IF staining, and four cover slips were used for hybridization. MRC-5 cells were originally obtained from Viro Med Laboratories (Minneapolis, Minn.) in 175-cm²

flasks at a low passage number and subsequently passed into shell vials containing the cover slips in the laboratory. Centrifugation was performed at 700 × g for 40 min at 34°C as previously described (5, 6), after which 1.0 ml of Eagle minimal essential medium containing 2% fetal bovine serum and antibiotics was added to each vial. Cultures were then incubated at 36°C for 16 to 76 h, after which the shell vials were prepared for MAb staining and in situ DNA hybridization. Control cultures were infected with the CMV laboratory strain AD169 and used as positive controls. Uninfected monolayers were used as negative controls.

Indirect IF staining was performed in the shell vials with the CMV MAb 2H2.4 (Du Pont Diagnostics, Wilmington, Del.) (16). Two vials were stained at 16 h postinoculation (p.i.), and one vial was stained at 40 h p.i. A goat anti-mouse fluorescein isothiocyanate-conjugated antibody (Tago, Burlingame, Calif.) was used as the second step in the indirect IF assay. The procedure was the same as previously described (5). Cover slips were then examined with a Zeiss epifluorescence microscope at a magnification of ×250. Cover slip cultures showing distinctive apple-green fluorescence of the cell nucleus were considered positive for CMV.

In situ DNA hybridization was performed using the CMV Pathogene kit (Enzo). Cover slips for each specimen were hybridized at 16, 40, 58, and 76 h p.i. The hybridization procedure was as follows. Following acetone fixation for 10 min, cover slips were removed from the vials and placed cell side down into 1 drop of DNA probe solution on a glass slide, heated in a 92°C water bath for 4 min, followed by incubation at room temperature for 10 min. Cover slips were then treated with 4 drops of probe wash solution at room temperature for 10 min. The cover slips were then rinsed in wash buffer for 10 to 15 s (provided with the kit), after which 3 drops of detection complex was added to the cover slips, and they were incubated at room temperature for 15 min. Cover slips were again washed as described above, after which each cover slip was treated with 4 drops of a chromogen substrate solution containing aminoethylcarbazole-H₂O₂-acetate. The cover slips were then incubated at room temperature for 10 to 15 min and rinsed with distilled water. The cover slips were mounted with distilled water onto a glass slide and viewed under a light microscope. Dark-red

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TABLE 1. Detection of CMV by IF MAb staining and in situ DNA hybridization in centrifugation culture

Specimen source	No. of specimens	No. positive by method at time (h)				Standard cell culture
		Indirect IF		In situ DNA hybridization ^a		
		16	40	58	76	
BAL	19	6 ^b	7	2	6	5 ^c
Lung tissue	15	5	5	2	5	5
Urine	14	4	4	0	3	4
Throat	13	1	1	0	1	1

^a There was no detection of CMV by DNA hybridization at 16 and 40 h.

^b Both cover slips from one specimen were unreadable at 16 h.

^c One BAL specimen positive by IF staining was negative in standard culture and negative by the DNA hybridization assay. Another BAL specimen was contaminated in standard culture but positive by both IF staining and the DNA hybridization assay.

inclusionlike deposits in the cells indicated a positive DNA hybridization reaction.

RESULTS

CMV was detected in 17 (28%; 7 BAL, 5 lung tissue specimens, 4 urine specimens, and 1 throat specimen) of 61 specimens. All 17 positive specimens were first detected in centrifugation culture by the MAb; 16 specimens were positive at 16 h p.i., with an additional specimen positive at 40 h p.i. Cover slips from one BAL specimen were unreadable at 16 h p.i. by IF staining but were positive at 40 h p.i. CMV was detected by in situ DNA hybridization in 15 of 61 (24.6%) specimens following centrifugation. However, the first positive results were not obtainable until 58 h p.i. Of the 17 positive specimens, 4 were positive by DNA hybridization at 58 h p.i. (2 BAL and 2 lung tissue); 11 additional specimens were positive by DNA hybridization at 76 h p.i. (Table 1). All 4 specimens positive by hybridization at 58 h were also positive at 76 h. Of the two positive specimens not detected by DNA hybridization, one specimen (urine) had 16 positive foci by IF staining at 16 h p.i. and CMV was isolated in standard culture on day 21. The other specimen (BAL) had four positive foci by IF staining at 16 h p.i. but was not positive in standard culture. One additional BAL specimen was contaminated in standard culture but positive by both IF and hybridization. CMV was isolated from 15 of 61 specimens (25%) in standard culture.

Compared with IF staining in centrifugation culture, the in situ DNA hybridization assay had a sensitivity of 88% and a specificity of 100%. However, these results were only achieved at 76 h p.i.

DISCUSSION

Hybridization techniques have been described for direct detection of CMV (2, 8, 11, 13, 18). While the use of DNA hybridization for CMV detection offers the theoretical advantages of being more sensitive with greater specificity than cell culture systems, hybridization techniques also require greater technical expertise and more expensive reagents than do current techniques being used in diagnostic virology laboratories. Therefore, the implementation of hybridization techniques for routine detection of CMV has yet not occurred in diagnostic laboratories (10, 14).

Two recent studies have evaluated the CMV Pathogene DNA hybridization kit for detection of CMV in centrifugation culture (J. W. Gibson, M. J. Rosenstrauss, K. M. Darcy,

and W. L. Drew, *Int. Symp. Med. Virol.* abstr. no. 5, 1987; M. Rosenstrauss, J. Victor, L. Clarke, and B. Daidone, *Abstr. Annu. Meet. Am. Soc. Microbiol.* 1988, C64, p. 342). Both compared DNA hybridization in centrifugation culture to standard cell culture for detection of CMV. Both studies showed that in situ DNA hybridization was nearly as sensitive and specific for the detection of CMV as virus isolation in standard culture, with a sensitivity of 92.3% and a specificity of 94.9% in the study of Gibson et al. and a sensitivity of 90.5% and a specificity of 100% in the study of Rosenstrauss et al. In these studies, CMV could be detected 3 to 4 days p.i. by DNA hybridization in centrifugation culture. Sorbello et al. (17) showed that IF staining using a MAb in centrifugation culture was more rapid than in situ DNA hybridization following centrifugation using three different multiplicities of infection of the CMV laboratory strain AD169 and two CMV-positive urine samples. CMV AD169 was detected by IF staining at 18 h p.i. at multiplicities of infection of 0.1, 1.0, and 10 infectious particles per cell, whereas DNA hybridization took 5, 4, and 1 day p.i., respectively, to detect CMV. Both urine samples were found positive by IF staining at 18 h p.i. One urine sample was found positive at 2 days and the other at 3 days following DNA hybridization.

Scott et al. (15) also used the CMV strain AD169 in a recent study and showed that CMV infection could be detected in centrifugation culture with an early CMV MAb by IF staining at 24 h p.i., whereas it was 5 days p.i. before their DNA probe assay could detect CMV infection (15).

In this study, 15 of the 17 positive specimens were detected by both assays (88%). The hybridization assay failed to detect two of the CMV-positive specimens. One or both of these specimens may have been positive if cover slips were hybridized at later time points than our study design allowed. However, 16 of the 17 (94%) positive specimens were detected by IF staining with MAb 42 h before—and specimen 17 18 h before—the first positive specimens were detected by hybridization. This is a considerable time delay in detecting CMV and would inhibit effective patient management. Furthermore, while the ultimate sensitivity of the hybridization assay was 88% at 76 h p.i., the sensitivity of this technique was nil compared with that of the MAb assay at 16 and 40 h p.i.

Currently, the use of centrifugation culture followed by staining of the cell monolayer with specific MAbs reactive with the immediate-early nuclear protein of CMV is the most effective rapid method for the detection of CMV infection (1, 3, 5, 11, 14). As shown in this study and in the studies of Sorbello et al. (17) and Scott et al. (15), DNA hybridization following centrifugation is as specific and nearly as sensitive as centrifugation culture using MAbs. However, substantial time is required before sufficient CMV DNA replication occurs to allow detection, and currently this method is not sufficiently rapid to replace the use of MAbs in centrifugation culture. However, use of the polymerase chain reaction may eventually change this situation (12).

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