Comparison of Monoclonal Antibodies for Rapid Detection of Cytomegalovirus in Spin-Amplified Plate Cultures

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Cytomegalovirus (CMV) was detected in 56 of 275 specimens (20%); 50 of 56 (89%) were detected by conventional culture, and 37 (66%) were detected by rapid assay at 72 h with a commercial monoclonal antibody and a pooled monoclonal antibody. Although the two antibodies were equally sensitive at 72 h, the pooled antibody gave a brighter, more easily detected signal. Other viruses were isolated from 9 specimens (3.3%) by conventional culture. Use of rapid assays alone fails to detect slow-growing CMV and non-CMV viral pathogens.

Rapid, accurate tests for cytomegalovirus (CMV) are important for initiating appropriate antiviral therapy (3, 4, 9). Monoclonal antibody detection of an immediate early CMV protein in conjunction with centrifugation of the specimen onto permissive cells allows rapid identification of CMV (2, 5, 6, 10, 12). We have developed a spin-amplified rapid assay (SARA) using 48-well plates and indirect immunofluorescence with a pool of four mouse monoclonal antibodies against both early and late CMV proteins (UWMab) (7). The performance of the UWMab was compared with that of a commercial mouse monoclonal antibody to an immediate early CMV protein (IEMAb; Du Pont Co., Wilmington, Del.) in the SARA. These tests, in turn, were compared with conventional tube culture (CC) on all specimens except peripheral blood.

CC. Specimens submitted to the University of Washington Diagnostic Virology Laboratory for CMV isolation and rapid assay ($n = 135$) or for CMV rapid assay and viral screen ($n = 140$) were pretreated as described previously (1) and then inoculated within 12 h of collection into three culture systems: CC with human diploid fibroblasts, SARA with IEMAb for CMV antigen detection, and SARA with UWMab. Specimens for viral screening were also inoculated into two tubes each of primary monkey kidney cells and heteroploid cells (HL) and one tube of Buffalo green monkey cells. Tubes were incubated in Eagle minimal essential medium in 0.01 M Tricine buffer (EMEM) supplemented with 2% fetal bovine serum and antibiotics, with medium changes every 2 weeks. Tubes were observed daily for 5 weeks; CMV cytopathic effect was confirmed by indirect immunofluorescence (7).

SARA. SARAs were performed in 48-well plates (Costar, Cambridge, Mass.) seeded 1 day before inoculation with 2×10^5 human diploid fibroblast cells in 0.5 ml of EMEM. Four wells per specimen were inoculated in each of two replicate plates, of which one was used for staining after 24 h and the other was used for staining after 72 h. In addition, four wells of each plate were inoculated with CMV (AD-169), and four wells were inoculated with EMEM only. The plates were centrifuged at $700 \times g$ for 45 min and then incubated at 37°C in 5% CO_2 in 0.5 ml of EMEM with 10% fetal bovine serum.

At 24 and 72 h, two wells per specimen from one of the replicate plates were rinsed briefly with distilled water, fixed in methanol-acetone, and incubated for 30 min at 37°C with 0.15 ml of either UWMab (pooled supernatants from hybridoma cultures producing CMV-specific antibodies C6D1, D2B1, F2D3, and F8D3 [7]; optimal dilution, 1:8 to 1:16) or IEMAb diluted 1:25 in phosphate-buffered saline. After being washed, cells were incubated for 30 min at 37°C with 0.15 ml of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (heavy and light chains) (1:100 in phosphate-buffered saline; Organon Teknika Corp., West Chester, Pa.). Rhodamine counterstain (Difco Laboratories, Detroit, Mich.) was added, and cells were examined under $\times 16$ and $\times 40$ magnification.

Detection of CMV. Of 229 patients tested, 31 had multiple sites sampled and 198 submitted single specimens. Four patients had CMV isolated from both urine and throat. CMV was detected in 56 of 275 specimens (20%): 31 by both SARA and CC, 19 by CC alone, and 6 by SARA alone (Table 1). The sensitivity of the SARA was 62%, the specificity was 97%, the positive predictive value was 84%, and the negative predictive value was 88%. The six SARA-positive, CC-negative specimens were from bronchoalveolar lavage (BAL) samples ($n = 2$), urine ($n = 2$), throat ($n = 1$), or sputum ($n = 1$) (Table 1). The 19 SARA-negative, CC-positive specimens were from urine ($n = 7$), BAL samples ($n = 5$), throat ($n = 4$), lung ($n = 1$), sputum ($n = 1$), and bone marrow ($n = 1$). Specimens from the lower respiratory tract gave the highest yield for CMV: 33% of BAL specimens, 29% of sputum specimens, and 25% of lung specimens were positive (Table 1).

Time to cytopathic effect for the 19 specimens which were SARA negative and CC positive was significantly longer (10 to 39 days [median, 21 days]) than for the 31 specimens which were SARA positive and CC negative (2 to 28 days [median, 8 days]; $P < 0.01$, Wilcoxon rank sum test).

Comparison of monoclonal antibody reagents. CMV was detected after 24 h by SARA in 19 (7.3%) and 22 (8.4%) of the specimens with UWMab and IEMAb, respectively (Table 2). Eighteen of these specimens subsequently had CMV identified by CC. Two of the three specimens which were positive only with IEMAb were also positive by CC; one was not. At 72 h, 37 specimens were positive with both IEMAb and UWMab.

The two antibody reagents had very different staining

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TABLE 1. CMV detection by SARA and CC with specimens from different sites^a

Result (SARA/CC)	No. of specimens from:						
	Urine	BAL	TS/NP	Lung	Sput	Marr	Other
-/+	7	5	4	1	1	1	0
+/-	2	2	1	0	1	0	0
+/+	17	12	1	1	0	0	0
-/-	104	38	34	6	5	3	23
% Positive ^b	20.0	33.3	15.0	25.0	28.5	25.0	0

^a Results of the CMV detection assays (SARA at 72 h and CC at 5 weeks) were tallied by specimen site: urine, BAL, throat or nasal swabs (TS/NP), lung biopsies (Lung), sputum (Sput), bone marrow (Marr), and other sources (pleural fluid, autopsy tissues, and rectal swabs). The highest yields were from BAL and sputum specimens (33 and 28.5% positive, respectively).

^b Overall percent positive, 20.8%.

characteristics. UWMAb produced a very bright signal with heavy, often punctate nuclear staining. IEMAb gave a faint signal with an ovoid or kidney-shaped appearance (Fig. 1). Scoring for extent of staining from 1+ (barely discernible) to 4+ (very bright) revealed an average score at 72 h of 1.7 for IEMAb and 3.2 for UWMAb. The brighter signal with UWMAb may have been due to the presence of antibodies against CMV structural proteins in the pool. The relative ease of screening multiple cultures with the UWMAb pool was a significant advantage for readers. On the other hand, dilution of antibodies to immediate early proteins by addition of the late-protein antibodies in the pool may have resulted in the three false-negative results at 24 h.

Non-CMV isolates. Nine specimens (3.3%) were positive for viruses other than CMV, including six that were positive for herpes simplex virus from human diploid fibroblasts (three from throat or nasal swabs and one each from sputum,

TABLE 2. Comparison of CMV isolation in SARA after 24 and 72 h and CC

Result (SARA/CC)	No. of specimens detected at:			
	24 h		72 h	
	UWMAb	IEMAb	UWMAb	IEMAb
+/+	18	20	31	31
-/+	32	30 ^a	19	19
-/-	220	219	213	213
+/-	1	2 ^a	6	6
T-C ^{b/-}	4	4	6	6

^a The three specimens which were positive only by IEMAb at 24 h were positive with UWMAb at 72 h.

^b Of 275 specimens submitted, 271 were evaluated by SARA after 24 h, and 4 were toxic or contaminated (T-C). Wells from these four specimens and from two additional specimens were also contaminated or toxic at 72 h. Five of these six specimens and an additional six specimens were passaged in CC (within the first 2 weeks) to overcome toxic effects.

BAL fluid, and tracheal aspirate). Herpes simplex virus was isolated from the throat of a patient whose urine and sputum cultures were both positive for CMV. A tracheal aspirate and urine specimen from another patient were positive for adenovirus from primary monkey kidney, Buffalo green monkey, and HL cells. These cell lines also grew coxsackievirus B from another urine specimen.

CMV recovery rates by CC and SARA. Our CMV recovery rate (20.4%) from CC after 5 weeks of observation was higher than those reported with 2 to 3 weeks of culture (5, 6, 10, 11, 13, 14). Of our 50 positive specimens, 15 (30%) were positive by 7 days, 32 (62%) were positive by 14 days, 40 (80%) were positive by 21 days, and 47 (94%) were positive by 28 days. At 2 weeks of observation, our recovery rate was 11.6%; longer surveillance clearly results in higher recovery. The higher recovery of CMV in CC also accounts for the

FIG. 1. Comparison of staining patterns with two monoclonal antibody preparations against CMV. Cells in replicate plates were inoculated with a urine specimen, incubated for 72 h, and stained with UWMAb (A) or IEMAb (B). Both were read as positive, but the well stained with UWMAb gave a brighter, more easily detected stain. Photomicrographs were taken at a magnification of $\times 16$ with a 20-s exposure with a Zeiss standard 16 binocular microscope with epifluorescence attachments.

lower relative recovery of CMV by SARA. When results were calculated with 2 weeks of CC as a cutoff for positive CC, 36 specimens were positive: 31 (86%) by CC and 32 (89%) by SARA.

Rapid CMV assays are important diagnostic tools. The antibody reagents used in these tests should be selected for maximum sensitivity with multiple isolates (8) and ease of detection (14). However, even the best of rapid assays cannot match the sensitivity of CC for slower-growing or low-titer CMV and cannot detect other viruses of potential significance. We recommend that more prolonged culture protocols be used in conjunction with rapid assays to diagnose CMV infection.

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