JOURNAL OF CLINICAL MICROBIOLOGY, June 1986, p. 1006–1008
0095-1137/86/061006-03$02.00/0

Vol. 23, No. 6

Rapid Detection of Cytomegalovirus in Bronchoalveolar Lavage Specimens by a Monoclonal Antibody Method

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Received 9 September 1985/Accepted 19 February 1986

Cytomegalovirus (CMV), a common cause of pneumonia in immunocompromised subjects, is conventionally diagnosed in the laboratory by tube cell culture assays or by detection of characteristic inclusions in histologic sections. Of 160 immunocompromised patients, CMV infection was diagnosed in 19 subjects by bronchoalveolar lavage (BAL), using a monoclonal antibody directed against an early nuclear antigen of the virus. Cytospin preparations from BAL and MRC-5 cell cultures inoculated with the BAL specimens yielded positive results for 6 (31.6%) and 18 (94%) of the 19 subjects, respectively, within hours of the bronchoscopic procedure, whereas conventional tube cell cultures were positive for 11 of the 19 subjects (57.9%) only after an average of 9.3 days. The monoclonal antibody method permitted easy and rapid detection of CMV in BAL specimens.

Cytomegalovirus (CMV) pneumonia is an important complication in immunocompromised patients (7, 9). Unfortunately, an open lung biopsy with subsequent examination of tissue sections for the presence of cytomegalic inclusion bodies or the detection of characteristic cytopathic effects (CPE) in cell cultures inoculated with the homogenized tissue has been required to establish the laboratory diagnosis of CMV infection.

Recent studies have used bronchoalveolar lavage (BAL) in the diagnosis of infection in immunocompromised subjects, including those with the acquired immune deficiency syndrome (8, 10). This technique has resulted in a high diagnostic yield and has been safely used in patients where transbronchoscopic biopsy procedures may be relatively contraindicated (10).

The diagnosis of CMV infection by bronchoscopy has traditionally used cell culture assays or the finding of intranuclear inclusions in lung cells or both (10). However, the conventional tube cell culture requires 7 to 21 days for the typical CPE to be recognized and thus results in significant delays in the laboratory diagnoses of these infections (11). Previous studies in our laboratory have indicated that detection of an early nuclear antigen of CMV with a monoclonal antibody by a fluorescence assay was a sensitive and specific method for rapid diagnosis of infections with this virus (2, 3). As an alternative to open lung biopsy procedure in some patients, we investigated this technology for the rapid diagnosis of CMV through the use of BAL specimens.

MATERIALS AND METHODS

BAL specimens. (i) Collection. Flexible fiberoptic bronchoscopy and BAL were performed in 160 immunocompromised patients as previously described (6). In brief, five 20-ml samples of normal saline were inserted into the suction port of a 5.2-mm fiberoptic bronchoscope (BF-B2; Olympus) with immediate return suction after the insertion of each sample.

(ii) Preparation of cytospin preparations. The cells in the lavage fluid were adjusted to a concentration of 2.0×10^5 cells per 0.2 ml of saline by using a standard hemacytometer. Cytospin preparations were made on glass slides by centrifugation at $500 \times g$ in a cytocentrifuge (Cytospin-2; Shandon Southern, Sewickley, Pa.).

(iii) Laboratory processing of BAL specimens. In addition to the cytospin preparations, the BAL specimens were processed for the diagnosis of bacterial, fungal, and viral infections according to a standard protocol also established for processing open-lung biopsy tissue (1).

Detection of cytomegalovirus. (i). Cytospin preparations. BAL cytospin preparations were fixed in cold acetone for 10 min and then stained by the indirect immunofluorescence technique with a monoclonal antibody (2H2.4) (Du Pont Co., Wilmington, Del.) to CMV by using goat anti-mouse immunoglobulin G-fluorescein isothiocyanate-labeled conjugate (Cooper Biomedical, Inc., West Chester, Pa.).

(ii) Shell vial cell culture assay. Human fibroblast (MRC-5) cells were seeded (50,000 cells per ml) into 1-dram (ca. 3.7 ml) shell vials containing a 12-mm cover slip and were maintained with 1 ml of Eagle minimal essential medium until used. Prior to inoculation, Eagle minimal essential medium was removed from the shell vial cell cultures and 0.2 ml of the BAL cell suspension was added to each of two vessels. The shell vials were closed with rubber stoppers, placed in carriers, and centrifuged at room temperature at $700 \times g$ for 1 h in a centrifuge (Sorvall RT6000; Du Pont). After centrifugation, 1.0 ml of Eagle minimal essential medium was added to the shell vials, and the cultures were incubated at 36°C. After 16 h of incubation, cover slips with infected monolayers were washed twice in phosphate-buffered saline, fixed in acetone, and stained with the CMV monoclonal antibody (8, 10).

The shell vial assay as described above was also used for the diagnosis of herpes simplex virus infections. Cover slips from two shell vials seeded with MRC-5 cells were stained 16 h postinoculation with fluorescent monoclonal antibodies (Syva Co., Palo Alto, Calif.) to herpes simplex virus type 1 and type 2 (5).

(iii) Conventional tube cell cultures. MRC-5 cells were inoculated with 0.2 ml of the BAL specimen, and the cultures were placed on a roller drum at 36°C and subse-

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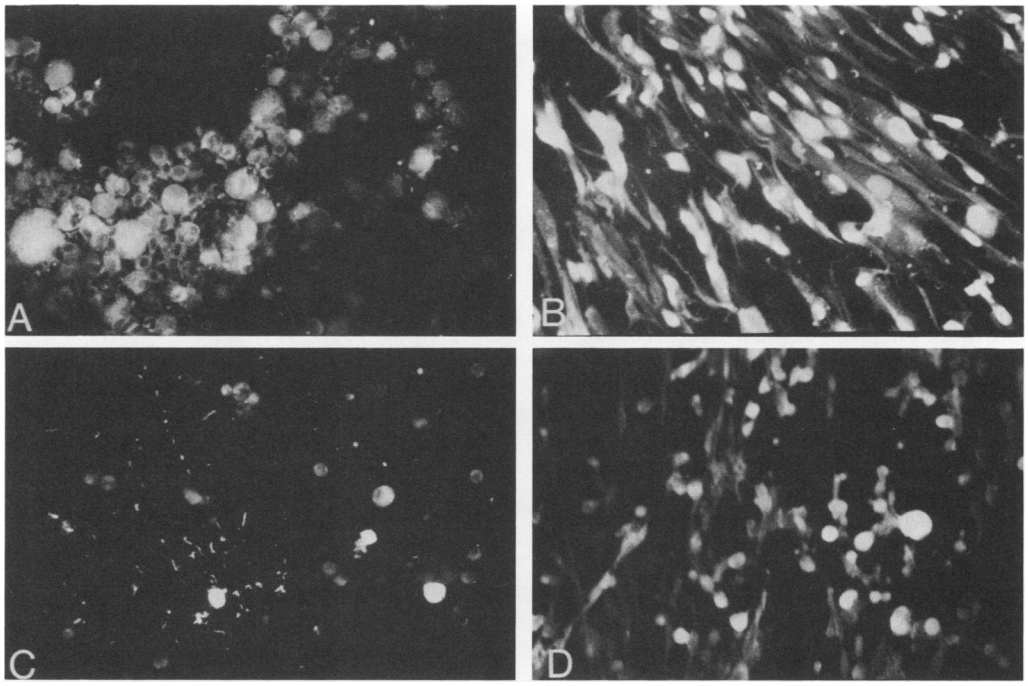

FIG. 1. Detection of CMV and herpes simplex virus type 1 by monoclonal antibodies and fluorescence methodology in BAL specimens. (A) Detection of CMV by cytospin preparation. (B) Detection of CMV by shell vial assay. (C) Detection of herpes simplex virus by cytospin preparation. (D) Detection of herpes simplex virus by shell vial assay.

quently examined at a magnification of ×125 for typical CPE produced by CMV.

RESULTS

The direct identification of the early nuclear antigen of CMV by monoclonal antibody was possible by using either the cytospin preparations of lavage cells (Fig. 1A), or the MRC fibroblast cells incubated with the BAL fluid (Fig. 1B). The fluorescence observed with the lavage cell cytospin preparations was less distinct and of lower intensity than that observed with the MRC fibroblast cells incubated with the BAL fluid. The detection of CMV antigen in lavage cell cytospin smears was obtained almost immediately after processing the specimen (<1 h); whereas the detection of the antigen in MRC fibroblast cells required an overnight incubation (16 h). With either method, however, the monoclonal antibody identified the presence of CMV in much less time than conventional culture techniques required for detection of CPE (an average of 9.3 days) (Table 1).

Of 160 immunocompromised subjects that underwent investigation for suspected opportunistic pulmonary infections during a 1.5-year period, CMV was detected in BAL specimens from 19 (11.9%) patients. The shell vial assay was positive for CMV 18 of 19 (94.7%) cases. Of the 19 patients with CMV infection, only 6 (31.6%) were positive after monoclonal antibody staining of cytospin preparations, and the virus was recovered in conventional tube cell cultures in 11 (57.9%) instances.

CPE for herpes simplex virus (two patients) and influenza virus type A (H3N2) (one patient) developed in conventional tube cell cultures inoculated with BAL specimens. Both the BAL cytospin preparations and the shell vial assays revealed fluorescent foci specific for herpes simplex virus type 1 by monoclonal antibody staining (Fig. 1C and D). Influenza virus (H3N2) was recovered from a BAL of another patient. Importantly, all three of the BAL cytospin preparations and shell vial assays were negative for CMV.

DISCUSSION

Bronchoalveolar lavage specimens have recently been demonstrated to be of value for the laboratory diagnosis of *Pneumocystis carinii* infections in immunocompromised patients (8, 10). We have also used this specimen source for the detection of CMV, another major pathogen causing pneumo-

TABLE 1. Detection of CMV in BAL specimens

Patient	Age (yr) (sex)	Monoclonal antibody reaction		Tube cell culture reaction
		Cytospin preparation	Shell vials	
1	75 (F)	+	+	−
2	53 (M)	+	+	+ (6)[a]
3	57 (M)	−	+	+ (3)
4	65 (M)	−	−	+ (7)
5	68 (M)	−	+	−
6	48 (F)	−	+	−
7	42 (M)	+	+	−
8	36 (M)	−	+	+ (14)
9	48 (M)	−	+	+ (10)
10	43 (F)	−	+	+ (10)
11	51 (M)	−	+	−
12	20 (F)	−	+	+ (9)
13	28 (F)	−	+	SC[b]
14	48 (M)	−	+	−
15	49 (F)	−	+	+ (14)
16	74 (M)	+	+	SC
17	42 (M)	+	+	+ (11)
18	61 (M)	−	+	+ (7)
19	54 (F)	+	+	+ (11)

[a] Days required for detection by CPE are given in parentheses.
[b] SC, Specimen contaminated.

nia in this patient population. However, recognition of CMV by characteristic CPE in infected tube cell cultures in the laboratory required an average of 8 days in a previous series and 9.3 days in the present study (2).

To provide a rapid laboratory diagnosis of this viral infection, we used two different procedures. First, we stained cytospin preparations of the BAL specimens with monoclonal antibody in a fluorescence assay. The procedure could be performed within 1 h after the specimen was collected, but CMV fluorescent foci were detected in only 6 of 19, or 31.6% of the specimens. Intact cells, homogenously deposited by centrifugation onto glass slides for cytospin preparations, were necessary for optimal interpretation of specific fluorescence. Thus, the ideal cytospin preparation would be comprised of individual intact cells arranged as a monolayer on the glass surface. In practice, this rarely occurred; ruptured cells and debris made interpretation of fluorescence especially difficult. Alternatively, inoculation of samples of BAL suspension into shell vials containing monolayers of fibroblast cells, followed by staining with monoclonal antibodies and the fluorescence assay 16 h postinoculation, proved the most sensitive method (94.7% of patients with CMV were diagnosed this way). Definitive detection of CMV infection with this method was made owing to the specific fluorescence and morphology of the nucleus of the cell.

Previous studies in our laboratory have indicated that monoclonal antibodies in the shell vial assay have detected the presence of CMV with the same specificity, but with much greater sensitivity, when compared with conventional cell culture methods. In an examination of 770 urine, blood, and lung specimens, 124 (16%) were positive by immunofluorescence, whereas only 88 (11%) were positive by the standard tube cell culture assay (3). Thus, the detection of CMV by the fluorescence assay 16 h postinoculation in our present study with BAL specimens (18 of 19 detected in shell vials, 11 of 19 recovered in conventional tube cell culture) is consistent with these results. Bronchoalveolar lavage specimens from two patients (no. 13 and 16 in Table 1) produced microbial contamination in conventional tube cell cultures before CMV CPE could be detected; however, both specimens were detected by the shell vial assay performed 16 h postinoculation. We therefore recommend the use of this procedure, in preference to either cytospin or BAL specimens inoculated into conventional tube cell cultures, for the most rapid results with the greatest sensitivity for the laboratory diagnosis of CMV infections.

The combined use of BAL and the monoclonal antibody method to detect CMV permits identification of the virus within hours of the bronchoscopic evaluation of the patient. Bronchoalveolar lavage is a technique of increasing utility in the evaluation of the immunocompromised patient, and the ability to provide the clinician with a rapid diagnostic method for CMV will parallel the currently available rapid diagnostic methods available for other opportunistic organisms such as *P. carinii*. Since herpes simplex virus and influenza virus were also detected from BAL specimens submitted to our laboratory, the cytospin technique and particularly the shell vial assay may have applications for the rapid and specific diagnosis of these and perhaps other viruses involved in lower respiratory tract disease. Rapid and specific diagnostic methods are essential for the adequate evaluation of this patient population and for the appropriate assessment of current and experimental therapy.

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