Comparative Recovery of Cytomegalovirus from Saliva, Mucolysed Induced Sputum, and Bronchoalveolar Lavage Fluid from Patients at Risk for or with Acquired Immunodeficiency Syndrome

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The recovery rates of cytomegalovirus from mucolysed induced sputum samples and bronchoalveolar lavage fluid obtained from individuals at risk for or with acquired immunodeficiency syndrome were compared. It was demonstrated that cytomegalovirus could be reliably recovered from mucolysed induced sputum, and such recovery was highly predictive of recovery from bronchoalveolar lavage samples obtained from the same individual.

Pulmonary infections are a major cause of morbidity and mortality among patients with human immunodeficiency virus type 1 infection. *Pneumocystis carinii* and cytomegalovirus (CMV) are the two most common agents identified in respiratory specimens obtained from such individuals (2, 9). Although fiber-optic bronchoscopy with transbronchial biopsy or bronchoalveolar lavage (BAL) or both have been the methods of choice for obtaining diagnostic specimens from this population, it has recently been shown that a less invasive procedure—sputum induction with hypertonic saline—yields specimens from which a diagnosis of *P. carinii* pneumonia can be successfully made (1, 7, 8). While the study by Ng et al. (7) showed that induced sputum was not useful for detecting bacterial and fungal pathogens, it was still important to know whether the procedure was suitable for the identification of viral pulmonary pathogens. In this paper we describe the sensitivity and specificity of induced sputum compared with BAL for the recovery of CMV in patients with pulmonary disease who were at risk for or who had acquired immunodeficiency syndrome.

The patients in this study either had acquired immunodeficiency syndrome or were in risk groups for human immunodeficiency virus type 1 infection, had respiratory symptoms suggestive of pulmonary infection, and were referred to the San Francisco General Hospital Chest Service for sputum induction. Details of patient selection and techniques for sputum induction, BAL, *P. carinii* identification, specimen processing, and culture for organisms other than viruses have been described elsewhere (1, 7). Saliva samples were obtained immediately before sputum induction by having patients chew rubber bands and expectorate into a sterile plastic container. None of the patients in this study were being treated with ganciclovir or foscarnet. Excluded from this study were 18 patients from whom other viruses (herpes simplex virus, adenoviruses, or coxsackievirus) were isolated and in whom CMV coinfection could not be excluded.

Saliva, mucolysed induced sputum, or BAL fluid were inoculated onto cultures of semicontinuous human embryonic lung, human foreskin fibroblasts, and primary cynomol-

gus monkey kidney cells. Cultures were incubated, given weekly medium changes (Eagle minimal essential medium), and read at weekly intervals until a cytopathic effect was observed or for 30 days. The presence of CMV in cultures exhibiting cytopathic effects consistent with CMV infection was confirmed by specific immunofluorescence staining with fluorescein-conjugated monoclonal antibodies to early and late antigens (Syva Corp., Palo Alto, Calif., or Bartels Immunodiagnostics, Bellevue, Wash. [4, 5]). None of the CMV isolates tested reacted with adenovirus group-specific antibodies (Cambridge BioScience, Worcester, Mass., and Whittaker Bioproducts, Walkersville, Md.).

Between August 1986 and July 1987, 443 induced sputum specimens from 362 patients were obtained and evaluated for CMV. CMV was recovered from 148 specimens (33%), *P. carinii* was identified in 231 (52%), and neither pathogen was identified in 155 (35%). In the 148 specimens from which CMV was recovered, it was the only potential pathogen identified in 57 (38%), whereas *P. carinii* was concomitantly present in 91 (61%). Thus, CMV was recovered from 39% (91 of 231) of induced sputum samples containing *P. carinii* compared with 27% (57 of 212) of those without *P. carinii* ($P = 0.007$).

Of 212 patients whose induced sputa were nondiagnostic for *P. carinii*, 111 had subsequent fiber-optic bronchoscopy performed and BAL samples obtained within 10 days (Table 1). There was no difference in time of appearance of cytopathic effect from induced sputum versus BAL. Recovery results were concordant in 85 (77%) of the paired specimens.

Depending on the criteria used, the sensitivity of recovering CMV from induced sputum versus BAL fluid ranged from 60 to 68%, and the specificity ranged from 85 to 100% (Table 2). Accordingly, the positive and negative predictive values for recovery of CMV from induced sputum versus BAL fluid ranged from 71 to 100 and 70 to 79%, respectively (Table 2).

There were 80 paired saliva-induced sputum specimens obtained. CMV was recovered from only 12 saliva specimens; CMV was also recovered from the paired induced sputum specimens of 10 of these patients. Of the 68 patients

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TABLE 1. Comparison of recovery of CMV from 111 paired induced sputum and BAL specimens

Result with induced sputum	No. of BAL specimens	
	CMV present	CMV absent
CMV present	24	10
CMV absent	16	61

for whom CMV was not recovered from saliva cultures, 19 had CMV recovered in their induced sputum cultures.

It is of interest that the sensitivity of induced sputum for the diagnosis of pneumocystis pneumonia in the same setting as this study was 75 to 78% (7). A comparable sensitivity for recovery of CMV from induced sputum versus BAL fluid was demonstrated in this study. These sensitivities are sufficiently high to be clinically useful, especially when one considers that induced sputum, in contrast to BAL fluid, is obtained in a noninvasive manner.

If cultures were performed by using the shell vial technique (3), results could be available within 24 h. Patients with a nondiagnostic induced specimen but who were suspected of having CMV pneumonia could then undergo bronchoscopy to obtain specimens for diagnosis in a timely manner.

Controversy exists as to the role of CMV as a cause of pneumonia in patients with acquired immunodeficiency syndrome (9). It has been argued that the vast majority of patients who have had CMV isolated from respiratory sites do not have disease attributable to this pathogen (S. E. Barton, D. A. Howkins, and B. G. Gazzard, Abstr. V Int. Conf. AIDS 1989, abstr. no. M.B.P. 115; H. R. Brodie, C. Broadus, W. Blumenfeld, P. C. Hopewell, A. Moss, and J. Mills, Abstr. Clin. Res. 1985, abstr. no. 396A; M. Youle, B. G. Gazzard, A. Chomas, and J. Lernstedt, Abstr. IV Int.

Conf. AIDS 1988, abstr. no. 3587). Nonetheless, there are convincing case reports of patients with progressive pulmonary symptoms from whom CMV was recovered (often with ancillary evidence of CMV infection, including the presence of intracellular viral inclusions) and who clinically responded to ganciclovir (6). If ganciclovir treatment is considered for progressive, undiagnosed pulmonary disease in patients at risk for or with acquired immunodeficiency syndrome, induced sputum and BAL fluid are useful specimens for documentation of CMV in the respiratory tract.

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TABLE 2. Value of induced sputum versus BAL fluid for the recovery of CMV^a

Qualitative value	No. positive/total no. of specimens (% positive)	
	BAL ^b	Induced sputum + BAL ^c
Sensitivity	24/40 (60)	34/50 (68)
Specificity	61/71 (85)	61/61 (100)
Predictive value, positive	24/34 (71)	50/50 (100)
Predictive value, negative	61/77 (79)	61/87 (70)

^a Values were calculated from the data in Table 1.

^b For these calculations, a true positive was defined as CMV isolated only from BAL fluid.

^c For these calculations, a true positive was defined as CMV isolated from either induced sputum or BAL fluid.