Detection of Cytomegalovirus from Blood Leukocytes Separated by Sepracell-MN and Ficoll-Paque/Macrodex Methods

CARLOS V. PAYA,1 ARLO D. WOLD,2 AND THOMAS F. SMITH2*

Department of Internal Medicine and Infectious Diseases1 and Section of Clinical Microbiology,2 Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

Received 6 May 1988/Accepted 15 July 1988

Two density gradient separation techniques for separation of blood leukocytes were compared for the laboratory diagnosis of cytomegalovirus (CMV) viremia. Of 510 blood specimens processed by both methods, 76 (14.9%) yielded CMV. Of the 76 positive specimens, 66 (87%) and 65 (86%) were processed by the Ficoll-Paque/Macrodex (F-P/M; Macrodex is dextran 70 in normal saline; Pharmacia, Piscataway, N.J.) and Sepracell-MN methods, respectively. Of the 76 CMV-positive blood specimens, 72 (95%) were detected in shell vial cell cultures, whereas only 42 (55%) were detected in conventional tube cell cultures. The time for recognition of specific cytopathic effects due to CMV in tube cell cultures (8.0 versus 7.1 days), the number of fluorescent foci in each positive shell vial culture (19.3 versus 20.1), and the costs of the reagents ($3.50 versus $2.80) were similar and independent of the leukocyte separation method (F-P/M versus Sepracell-MN). Recovery of CMV from heparinized blood (F-P/M method) was similar to that from EDTA-anticoagulated blood (Sepracell-MN method). The Sepracell-MN method is a rapid and sensitive method for detection of CMV from blood specimens and is recommended as a replacement for the more tedious and time-consuming F-P/M procedure.

Cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in immunocompromised patients (6). Viremia occurs commonly in these patients and may indicate systemic disease associated with a poor prognosis (3, 8, 14, 16). CMV is localized mainly within the polymorphonuclear (PMN) and mononuclear blood cell fractions (4, 5, 10, 12); therefore, laboratory diagnosis of CMV viremia was greatly enhanced by application of density gradient separation techniques for isolation of these leukocyte fractions rather than the traditional buffy coat method (7). Recently, a colloidal silica medium (Sepracell-MN; Sepratech Corp., Oklahoma City, Okla.) has been shown to separate both the PMN and mononuclear fractions of blood in a single step in about one-half of the time required by the Ficoll-Paque/Macrodex (F-P/M) (Macrodex is dextran 70 in normal saline; Pharmacia, Piscataway, N.J.) procedure. Importantly, efficient and selective processing of leukocyte fractions of blood specimens and rapid laboratory methods for diagnosis of CMV infections are required for prompt implementation of antiviral chemotherapy for medical management of patients with viremia. We compared the recovery of CMV in conventional tube and shell vial cell cultures from 510 blood specimens processed by the F-P/M and Sepracell-MN methods.

MATERIALS AND METHODS

Study design. A prospective evaluation of two different preparatory blood specimen processing methods for detection of CMV in cell cultures was conducted over an 8-month period (July 1987 to February 1988). Blood samples were obtained from patients at Mayo Clinic and affiliated hospitals according to a study protocol approved by our Institutional Review Board.

Sample collection. With any request for a viral blood culture, a venipuncture technique obtained the standard 5 ml of blood in a heparinized tube and an extra 5-ml sample in an EDTA-coated tube. Both blood samples were immediately transported to the clinical microbiology laboratory.

Sample processing. Leukocyte fractions of blood specimens were separated by F-P/M (heparinized specimen) or Sepracell-MN (EDTA-treated specimen) as follows. For the F-P/M separation method, 5 ml of heparinized blood was mixed with 5 ml of sterile saline in a sterile plastic tube and mixed. Nine milliliters of this solution was overlaid on 3 ml of Ficoll-Paque solution. These were centrifuged for 30 min at 500 × g. The topuffy layer (lymphocytes) was removed, transferred into 5 ml of Eagle minimal essential medium (MEM), and kept at 4°C. The Ficoll-Paque layer was discarded. The packed erythrocyte volume was measured and adjusted to a ratio of 2:1 with 6% dextran, and 3 ml of sterile saline was added. After mixing, the tube was left at room temperature for 60 min, after which the top layer of PMN cells was removed from the centrifuge tube and added to the lymphocyte fraction in MEM. The cells were then centrifuged (700 × g) and washed twice with 5 ml of MEM and suspended in 2 ml of MEM. One milliliter was inoculated into a tube cell culture, and the rest was divided into three 0.3-ml aliquots and inoculated into three shell vial cell cultures.

For the Sepracell-MN method, 5 ml of blood from the EDTA tube was mixed with 6.7 ml of Sepracell-MN medium in a 15-ml centrifuge tube. These were centrifuged at 1,500 × g for 20 min, after which the first cell layer (mononuclear cells) was placed in a 15-ml centrifuge tube containing 5 ml of phosphate-buffered saline. The next cell layer (PMN cells above the packed erythrocytes) was collected and added to the mixture of phosphate-buffered saline and mononuclear cells. This specimen was then washed with phosphate-buffered saline and centrifuged at 700 × g for 10 min, the phosphate-buffered saline was aspirated, and the sedimented
of the 42 were fluorescent foci cytopathic divided were cells with tube after were vial cell with MRC-5 monolayers higher immunofluorescence indirect testing was detected by the F-P/M method, whereas the Sepracell-MN method, similar to the time costs (19.3 (three CMV strains recovered by the F-P/M method, whereas 75% of CMV) were positive in F-P/M-processed specimens and 5 were isolated exclusively in leukocytes separated by the Sepracell-MN method (Table 1). Detection of CMV was better in shell vials than in tube cell cultures, regardless of the separation method. Of the 66 CMV strains obtained with the F-P/M method, 60 (91%) were detected in shell vials and 36 (60%) were detected in tube cell cultures (Table 2; P < 0.01). Similarly, after separation of leukocytes by the Sepracell-MN method, 60 (92%) and 37 (57%) of the 65 CMV strains were obtained in shell vials or tube cell cultures, respectively (Table 2; P < 0.01). Toxicity to the MRC-5 culture tube monolayer was higher in samples processed by the Sepracell-MN method (3.5 versus 1.1%). However, the time for recognition of specific cytopathic effects due to CMV in tube cell cultures (8.0 versus 7.1 days), the number of fluorescent foci in each positive shell vial culture (19.3 versus 20.1) (three cover slips), and the costs of the reagents ($3.50 versus $2.80) were similar and independent of the leukocyte separation method (F-P/M versus Sepracell-MN). Leukocyte separation by the Sepracell-MN method required less than one-half of the processing time required by the F-P/M technique (Table 3).
TABLE 3. Comparison of cell culture variables, processing time, and cost of reagents with regard to blood leukocyte separation method

<table>
<thead>
<tr>
<th>Separation method</th>
<th>No. of specimens showing toxicity/total (%) with tube cell culture</th>
<th>Mean (range) CMV recovery time (days) in tube cell culture</th>
<th>Mean no. (range) of positive IF foci/positive shell vial specimen</th>
<th>Processing time (min)</th>
<th>Cost/specimen ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-P/M</td>
<td>6/510 (1.1)</td>
<td>8–14</td>
<td>19.3 (1–100)</td>
<td>110</td>
<td>3.50</td>
</tr>
<tr>
<td>Sepracell-MN</td>
<td>18/510 (3.5)</td>
<td>7.1–14</td>
<td>20.1 (1–100)</td>
<td>40</td>
<td>2.80</td>
</tr>
</tbody>
</table>

*IF, Immunofluorescent.

conventional tube cell cultures (9,11). Thus, these and past studies in our laboratory show that shell vials are more sensitive than tube cell cultures for the laboratory diagnosis of CMV infections but that maximum detection of this virus requires both systems (9).

In view of the reported toxicity of heparin for CMV, it was interesting to find that the detection rate of the virus did not vary significantly in blood specimens collected for F-P/M (heparin) or Sepracell-MN (EDTA) (1). Apparently, the intracellular association of CMV in blood protects the virus from inhibition due to heparin, which can be demonstrated in vitro.

The Sepracell-MN method is a rapid and sensitive method for detection of CMV from blood specimens and is recommended as a replacement for the more tedious and time-consuming F-P/M procedure.

LITERATURE CITED