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

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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; Seprotech 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 team 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 top buffy 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...
cells were suspended with 2 ml of MEM. One milliliter was then 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.

**Inoculation of cell cultures.** Combined PMN and mononuclear cell fractions obtained by either the F-P/M or the Sepracell-MN method were suspended in 2 ml of MEM. Conventional tube cell cultures (MRC-5; Viromed, Minneapolis, Minn.) were inoculated with 1 ml; each of three shell vial cell cultures received 0.3 ml. Tube cell cultures were incubated at 35°C for 14 days and examined for the presence of cytopathic effects characteristic for CMV. Cover slips with MRC-5 monolayers in shell vials were stained at 16 h postinoculation with a monoclonal antibody specific for an early antigen of CMV (Du Pont, Boston, Mass.). Specific fluorescent foci due to CMV infection were detected by the indirect immunofluorescence test (13).

**Statistical analysis.** Data were analyzed by the Sign test.

**RESULTS**

Of 510 paired blood specimens, 76 (14.9%) yielded CMV; 66 (87%) were processed by F-P/M, and 65 (86%) were processed by the Sepracell-MN method. Of the 76 specimens, 72 (95%) samples positive for CMV were detected in shell vials, whereas only 42 (55%) isolates were recovered in conventional tube cell cultures (P < 0.01). Of the 72 CMV strains detected in shell vial cell cultures, 22 were obtained exclusively after the blood specimens were processed by the F-P/M (11) or Sepracell-MN method (11) (Table 1). Similarly, of the 42 CMV isolates recovered in tube cell cultures, 6 were positive only 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).

**DISCUSSION**

The long time required to process blood cultures by F-P/M for efficient recovery of mononuclear and PMN fractions has been justified by the 100% increase in detection of viremia compared with conventional buffy coat preparations, especially with neutropenic patients (7). The importance of selecting these cellular components of blood for recovery of CMV has been demonstrated in several studies (4, 5, 10, 12). Our laboratory has experienced a steady increase in the rate of requests for viral blood cultures, reflecting the significance associated with recovery of CMV from this source. Viremia in immunocompromised hosts has been associated with organ involvement and poor clinical outcome (3, 8, 14, 16). Because of the development of new anti-CMV therapy (2) and the use of rapid diagnostic techniques for CMV, it is now possible to initiate therapy early in the course of infection to provide the maximum opportunity for clinical improvement (C. V. Paya, P. E. Hermans, T. F. Smith, J. Rakela, R. H. Wiesner, R. A. F. Krom, V. E. Torres, S. Sterioff, and C. J. Wilkowski, Transplantation, in press).

Other alternatives to F-P/M have been evaluated. Plasmagel consists of a solution of sodium chloride and calcium chloride salts plus gelatin and is capable of providing recovery of both mononuclear and PMN cell fractions in a single step. This method was compared with LeucoPREP, which is used to separate monocytes from other blood components but required Macrodex as a second step to recover the PMN fraction. With 10 CMV isolates, both methods were positive in six instances; Plasmagel and LeucoPREP preparations were exclusively positive with three and one specimens, respectively (15). We evaluated another system that, like Plasmagel, was capable of separating both the mononuclear and PMN cell fractions in a single step.

In our experience, the Sepracell-MN system yielded the same rate of detection of CMV as F-P/M in both of the culture methods used in our study. Interestingly, 11 of the 72 CMV strains detected in the shell vial cell cultures were detected exclusively by either the Sepracell-MN or the F-P/M method. Similar data were obtained by detection of CMV with the tube cell culture. The reason for these results are unclear; they are possibly due to sampling variation. The main advantage of the Sepracell-MN method is the time savings and technical implementation compared with the F-P/M procedure. The cell culture system for the laboratory diagnosis of CMV infection, however, was a much more significant variable than the method used for the separation of blood leukocytes. Of the 76 total positive specimens, 72 (95%) were detected in shell vials, but only 42 (55%) of the CMV strains were recovered by detection of CPE in cell cultures. Schirm et al. compared the shell vial and conventional culture for detection of CMV from buffy coat preparations of 415 blood specimens. Their data agreed with ours in that 40 samples from 19 patients were positive in shell vials, whereas only 22 specimens were positive for CMV in

**TABLE 1. Detection of CMV from blood leukocytes by the cell culture method**

<table>
<thead>
<tr>
<th>Result with Sepracell-MN</th>
<th>No. of samples tested by shell vial with F-P/M that were:</th>
<th>No. of samples tested by conventional tube cell culture with F-P/M that were:</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>50</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>438</td>
</tr>
</tbody>
</table>

**TABLE 2. Detection of CMV in cell cultures according to blood leukocyte separation method**

<table>
<thead>
<tr>
<th>Result with shell vial</th>
<th>No. of samples tested with F-P/M and tube culture that were:</th>
<th>No. of samples tested with Sepracell-MN and tube culture that were:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>28</td>
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</tr>
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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


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) Fixed/Hands-on</th>
<th>Cost/specimen ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-P/M</td>
<td>6/510 (1.1)</td>
<td>8 (4–14)</td>
<td>19.3 (1–100)</td>
<td>110/38</td>
<td>3.50</td>
</tr>
<tr>
<td>Sepracell-MN</td>
<td>18/510 (3.5)</td>
<td>7.1 (4–14)</td>
<td>20.1 (1–100)</td>
<td>40/21</td>
<td>2.80</td>
</tr>
</tbody>
</table>

* IF, Immunofluorescent.