Use of Leukocyte-Depleted Platelets and Cytomegalovirus-Seronegative Red Blood Cells for Prevention of Primary Cytomegalovirus Infection After Marrow Transplant

By Raleigh A. Bowden, Sherrill J. Slichter, Merlin H. Sayers, Motomi Mori, Monica J. Cays, and Joel D. Meyers

Seventy-seven cytomegalovirus (CMV)-seronegative marrow transplant patients were randomized in a prospective controlled trial comparing the use of leukocyte-depleted platelets plus CMV-seronegative red blood cells with standard unscreened blood products for the prevention of primary CMV infection during the first 100 days after transplant. Eligible patients included CMV-seronegative patients undergoing autologous transplant or seronegative patients undergoing allogeneic transplant for aplastic anemia or nonhematologic malignancy who had seronegative marrow donors. Patients and marrow donors were serologically screened for CMV and randomized before conditioning for transplant and followed for CMV infection with weekly cultures of throat, urine, and blood and with weekly CMV serologies until day 100 after transplant. Leukocyte-depleted platelets were prepared by centrifugation, a procedure that removed greater than 99% of leukocytes. There were no CMV infections observed in 35 evaluable treatment patients compared with seven infections in 30 evaluable control patients ($P = .0013$). There was no statistically significant difference in the mean number of platelet concentrates in the treatment patients (164 concentrates) compared with the control patients (126 concentrates). Leukocyte-depleted platelets plus CMV-seronegative red blood cells are highly effective in preventing primary CMV infection after marrow transplant.

© 1991 by The American Society of Hematology.

From the Fred Hutchinson Cancer Research Center and the Departments of Pediatrics, Medicine, and Biostatistics, University of Washington School of Medicine; and the Puget Sound Blood Center, Seattle, WA.

Submitted October 22, 1990; accepted February 21, 1991.

Supported by HL 36444, HL 33775, CA 18029, and CA 15704 awarded by the National Institutes of Health.

Address reprint requests to Raleigh A. Bowden, MD, Program in Infectious Diseases, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1991 by The American Society of Hematology.

0006-4971/91/7801-0009$3.00/0


Cytomegalovirus (CMV) pneumonia remains a leading infectious cause of death after marrow transplant. CMV infection develops as a result of reactivation of latent virus in CMV-seropositive individuals or is acquired as a primary infection in CMV-seronegative individuals from either blood products or marrow from CMV-seropositive donors. Primary infection can be successfully prevented by the use of CMV-seronegative blood products in allogeneic marrow transplant patients with seronegative marrow donors as well as in other seronegative patients at risk for serious CMV infection. However, CMV-seronegative blood is not universally available and a need for an alternative means of protection from primary CMV infection occurs in communities in which: (1) insufficient CMV-seronegative blood donors are available; (2) a large number of marrow transplants, including autologous transplants are performed; (3) renal, cardiac, and liver transplants are performed; or (4) physicians wish to protect seronegative patients with malignancies who might be prospective marrow transplant patients from acquiring CMV from blood products.

Because the leukocyte fraction of blood products is thought to be the source of CMV, removal of this fraction from transfusions should reduce or eliminate the risk of transmission of CMV infection. Recent uncontrolled studies in CMV-seronegative marrow transplant patients using filtered red blood cells (RBCs) and CMV-seronegative platelets and in patients with hematologic malignancies using centrifuged platelets and filtered RBCs suggest that this approach may be effective. We chose to compare the use of leukocyte-depleted platelets plus CMV-seronegative RBCs with the use of unscreened blood products in a randomized trial for prevention of transfusion-associated primary CMV infection during the first 100 days after marrow transplant. The primary rationale for this combination was that the platelet needs of marrow transplant patients at our center place a much higher demand on blood bank resources than do RBC needs (ie, patients use a mean of 161 platelets concentrates and 19 RBC units/transplant). Therefore, the ability to avoid serologic screening of platelet transfusions offers the greatest benefit to the Blood Center. We also chose to perform this study primarily in autologous patients who, although they are at a lower risk for symptomatic CMV disease than patients undergoing allogeneic transplant, have a similar risk of CMV infection. If we could demonstrate protection in autologous patients, a rationale for similar studies in allogeneic patients who have been shown to be protected by seronegative blood products could be justified. Our ultimate goal was to examine the efficacy of leukocyte-depleted blood products for their potential as an alternative to the use of CMV-seronegative blood for prevention of primary CMV infection. Such an approach would provide protection for a greater number of patients, including those in communities and areas of the world with an insufficient number of seronegative donors.

MATERIALS AND METHODS

Patients. All patients undergoing marrow transplant, and their donors, were tested for CMV antibody by both latex agglutination (CMVSCAN Card Test; Becton Dickinson and Co, Baltimore, MD) and enzyme immunoassay (EIA) (CMV STAT; Whittaker Bioproducts, Inc, Walkersville, MD) before transplant. Because of limited CMV seronegative blood resources in our center, CMV-seronegative blood is routinely provided only for patients undergoing allogeneic transplant for hematologic malignancy who have a significantly higher risk for CMV pneumonia compared with
patients undergoing autologous transplant or allogeneic transplant for aplastic anemia. Therefore, patients were eligible for the present study if they were CMV-seronegative and were undergoing either autologous marrow transplant or were undergoing allogeneic transplant for non-hematologic malignancy or aplastic anemia and had seronegative marrow donors confirmed by both serologic screening tests. Patients or marrow donors with discrepancies between the two serologic tests were retested by latex agglutination and two of three negative tests defined CMV-seronegative status. Patients were not eligible for this study if they had received more than 6 units of unscreened blood between the time of CMV antibody testing and the time of randomization.

Eligible patients were randomized before transplant to receive leukocyte-depleted platelets plus CMV-seronegative RBCs (treatment arm) or unscreened blood products (control arm). The randomization was stratified for the patient's underlying disease (allogeneic transplant patients with non-hematologic malignancy, autologous transplant patients). Patients remained on study from the time of randomization until CMV infection developed or day 100 after transplant, whichever occurred first. Approval was obtained from the Institutional Review Board for participation in this study. Patients and marrow donors were informed that samples for CMV cultures were obtained for research purposes only and that their privacy would be protected.

Preparation of blood products. The leukocyte-poor platelets were prepared at the Puget Sound Blood Center from units of unscreened whole blood. Approximately 50% of blood donors in Seattle are CMV seropositive. The blood was first centrifuged at 1,025g for 9 minutes, followed by a second spin at 3,000g for 20 minutes. The platelet pellet was then resuspended in 50 mL of platelet-poor plasma. For each transfusion, 4 to 6 platelet concentrates were pooled and subjected to a final centrifugation at 400g for 10 minutes, which removes greater than 99.8% of the leukocytes. A leukocyte and platelet count was performed on every transfusion of leukocyte-depleted product to insure quality of the product and so that subsequent development of CMV infection might be compared with the actual leukocyte counts of the units each patient received. This method also resulted in recovery of 86% of the original platelet count.

The CMV-seronegative RBCs were prepared as packed RBC units from blood donors found to be seronegative by latex agglutination.

Assessment of CMV infection. All patients were followed with weekly CMV cultures and serologies throughout the study period for evidence of CMV infection. Viral cultures of urine, throat, and blood buffy coat were inoculated onto complete monolayers of human foreskin fibroblasts and observed for 6 weeks by standard methods. Positive cultures were identified by typical cytopathic effect and confirmed by immunofluorescence after passage of the culture. When biopsy tissue was obtained, CMV was identified by typical viral inclusions, indirect immunofluorescent antibody staining for late CMV antigens with murine monoclonal antibodies (MoAbs; Genetic Systems Corporation, Seattle, WA), or by positive culture. Bronchoalveolar lavage was used to diagnose CMV pneumonia in patients with infiltrates on chest radiograph. We have previously shown that this method is 96% sensitive and 100% specific for the diagnosis of CMV pneumonia when compared with open lung biopsy.11 CMV antibody titers were determined by EIA. Sera with an optical density reading at a wavelength of 550 nm of greater than 0.99 were considered positive and significant seroconversion was defined as a value of 1.47 or greater, according to the manufacturer’s guidelines.

The endpoint of this study was the development of CMV infection. However, patients were also assessed for the occurrence of CMV disease. CMV infection was defined as development of a positive CMV culture, seroconversion, or identification of CMV in tissue (see above) greater than 21 days after transplant. Patients developing CMV infection during the first 21 days after transplant were considered not evaluable because these patients were presumed to have been infected at the time of transplant. Because patients in both study arms were expected to acquire passive antibody from either standard or leukocyte-depleted products, seroconversion was defined only when the development of a positive CMV titer persisted ≥6 weeks after discontinuation of blood support. CMV disease was defined as tissue evidence of invasive CMV infection with associated clinical symptoms.

Statistics. The incidence of CMV infection was estimated by the Kaplan and Meier method, with comparison of the incidence curves by log-rank test.12 Analysis for differences in age and number of blood products between patient groups was performed by Wilcoxon rank sum test and analysis of other fixed demographic variables was performed using either Fisher’s exact or χ2 tests.

RESULTS

Patients. Seventy-seven patients were randomized. The majority were patients undergoing autologous transplant for hematologic malignancies (see Table 1). Five patients randomized to the treatment arm and seven patients to the control arm were either never transplanted or were found to be ineligible before transplant because of change in donor CMV serostatus, leaving 35 treatment and 30 control patients in the final analysis. There were no significant differences between the demographic characteristics of patients in the leukocyte-depleted and control groups. Because most patients received autologous transplants, they were given no post-grafting immunosuppression. Twelve patients in the leukocyte-depleted group and eight patients in the control groups received post-grafting intra-

<table>
<thead>
<tr>
<th>Table 1. Demographic Characteristics</th>
<th>Leukocyte-Depleted Blood</th>
<th>Standard Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>Patients excluded before transplant</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Total evaluable patients</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>20:20</td>
<td>15:22</td>
</tr>
<tr>
<td>Median age (y) (range)</td>
<td>27 (4-50)</td>
<td>26 (2-56)</td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>ANL</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>AA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Transplant type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allogeneic</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Autologous</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBI + CTX</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>CTX + TBI + McAb</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>CTX + TBI/BU + 4HC marrow</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>TBI + other chemotherapy</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>BU + CTX</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphocytic leukemia; ANL, acute non-lymphocytic leukemia; AA, aplastic anemia; TBI, total body irradiation; CTX, cytoxan; McAb, and T-cell MoAb; BU, busulfan; HC, hydroxyccyclophosphamide.
CMV infection. There were no CMV infections in the leukocyte-depleted group compared with seven infections in the control group. The probability of developing CMV infection is shown in Fig 1 and was significantly greater for patients receiving standard unscreened blood ($P = .0013$ by log-rank test). The manifestations of CMV infection, including the site and time after transplant that the culture became positive, is shown in Table 2. The median onset of infection was 59 days after transplant. The patient with CMV pneumonia was diagnosed by bronchoalveolar lavage 100 days after transplant and the patient with enteritis was diagnosed by endoscopic biopsy of esophagus and duodenum 60 days after transplant. There were no CMV infections in either group in the first 21 days after transplant.

Delivery of blood products. Patients in the leukocyte-depleted group received an average of 25 units of RBCs (range, 4 to 97 units) and 165 platelets concentrates (range, 6 to 568 concentrates) compared with 20 units of RBCs (range, 7 to 55 units) and 130 platelet concentrates (range, 28 to 428 concentrates) in the control group. The differences were not significant. Standard platelet concentrates in our laboratory have $1.2 \times 10^6$ leukocytes/concentrate. The method of leukocyte-depletion used in this study resulted in platelet concentrates with a median residual leukocyte count of $4 \times 10^4$/platelet concentrate (range, 1 to $10 \times 10^4$ leukocytes/concentrate). Patients in the control group who became infected with CMV received an average of 22.5 RBC units (range, 14 to 37 units) and 123 platelet concentrates (range, 40 to 244 concentrates), which was not statistically different from the numbers of units or concentrates for uninfected patients in the control or treatment groups.

**DISCUSSION**

This study has demonstrated that leukocyte-depleted platelet concentrates and CMV-seronegative RBCs are effective in preventing transfusion-acquired CMV infection in seronegative marrow transplant patients when compared with standard blood products ($P = .0013$). It supports the observations of Verdonck et al, who administered filtered RBCs and CMV-seronegative platelets in an uncontrolled trial to CMV-seronegative allogeneic and autologous marrow transplant patients and found no CMV infections; and the study of de Graan-Hentzen et al, who retrospectively found no CMV infections in 59 seronegative cancer patients administered centrifuged platelets and filtered RBCs. A randomized controlled trial of filtered RBCs in seronegative infants born to seronegative mothers found no infections in 30 infants in the filtered arm compared with nine infections in 42 infants receiving unfiltered RBCs. A major difference between the present study and previous studies was the number of blood products administered. Our patients received an average of 25 units of RBCs (range, 4 to 97 units) and 164 platelet concentrates (range, 6 to 568 concentrates), compared with an average of 8 RBC units (range, 6 to 18 units) and 6 platelet concentrates (mean, 3 to 31 concentrates) in the study by Verdonck et al, a mean of 15 units of RBCs (range, 0 to 38 units) and 54 platelet concentrates (range, 0 to 207 concentrates) in the de Graan-Hentzen study, and a median volume of only 32.5
mL of RBCs in the study by Gilbert et al in infants. In addition to being a controlled trial, the present study represents a substantial increase in the number of units or total volume of transfusion products over previous studies and provides strong evidence that leukocyte depletion can effectively prevent transmission of CMV infection.

It is not known which type or how many leukocytes in blood from seropositive individuals carry CMV. Previous studies infecting mononuclear blood cells with clinical strains of CMV have shown that monocytes, T cells, B cells, and natural killer cells express immediate early proteins of CMV, including as many as 2% to 3% of peripheral blood mononuclear cells. Schrier et al has also shown that 2.4% of CD4 helper and 0.8% of CD8 suppressor/cytotoxic T cells express immediate early proteins in healthy CMV-seropositive blood donors, for a frequency of 1/500 cells. The present study suggests that a majority of these cells do not result in productive infection. Because patients in the leukocyte-depleted arm presumably received half of their platelet transfusions from seropositive donors, or they received approximately 80 platelet concentrates from seropositive donors in the first 100 days after transplant, they would have been exposed to approximately $3.2 \times 10^8$ potentially infectious cells of which 0.2% should contain CMV proteins. The fact that we observed no infections in the leukocyte-depleted group suggests that we have reduced the number of leukocytes below the critical threshold for transmission of infection.

A variety of techniques are available for leukocyte depletion of blood products. While frozen deglycerolized RBCs have been shown to be effective, they are not practical on a large scale. Previously described cotton filters result in a 1.5 to 2 log reduction in leukocytes and have been shown to be effective in uncontrolled studies in cancer or marrow transplant patients and in a controlled trial in newborns. However, they require relatively large volumes of fluid, which may present problems for fluid-restricted patients. Centrifugation for preparation of leukocyte-depleted platelets was used in the present study and others and is a relatively easy technique also resulting in a 1.5 to 2 log reduction without a clinically significant platelet loss. More recently, and since the design of this study, newer platelet and RBC filters have been developed that can be used "in line" at the bedside that result in a 3 log or greater reduction in leukocytes that show promising preliminary results in the prevention of transfusion-associated CMV infection after marrow transplant. Because allogeneic transplant patients are at significantly increased risk for morbidity and mortality compared with patients undergoing autologous transplant, this approach could ultimately have an more important impact on the ability to deliver CMV-safe products to allogeneic patients. Data suggests that the risk of CMV infection is directly related to the exposure number to increased numbers of seropositive donors, while the severity of symptomatic CMV disease increases with the degree of immunosuppression, supporting the inclusion of allogeneic patients in the present study where the endpoint of the study is CMV infection. While the results of the present study and one other study including allogeneic patients appear promising as a means of preventing transfusion-associated CMV infection, further studies in large numbers of allogeneic patients are warranted, however, because of their increased risk of CMV disease compared with patients undergoing autologous transplant.

In conclusion, we have shown in a randomized controlled trial that the use of leukocyte-depleted platelets is an effective means for the prevention of transfusion-associated CMV infection. Presumably leukocyte depletion will also be applied to RBCs, which were not tested in this study, in part, because the RBC filters available at the time of this study were not technically easy to use in patients receiving such large volumes of RBC support. With recent technical advances that include the development of more efficient platelet and RBC filters that can now be used at the bedside, leukocyte depletion of both RBCs and platelets is now possible. This approach may obviate the need for CMV serologic screening of blood products for patients at risk for serious disease associated with primary CMV infection acquired from blood products. However, further studies are needed in patients undergoing allogeneic transplant who are at higher risk for CMV infection and disease before this can be assumed. This approach may also increase the availability of "CMV-safe" blood products for a variety of patients at risk for transfusion-associated CMV infection.

REFERENCES

6. Verdonck LF, de Graan-Hentzen YCE, Dekker AW, Muddle GC, de Gast GC: Cytomegalovirus seronegative platelets and leukocyte-poor red blood cells from random donors can prevent primary cytomegalovirus infection after bone marrow transplantation. Bone Marrow Transplant 2:73, 1987
Cytomegalovirus infection in patients with hematologic malignancies by intensive white cell depletion of blood products. Transfusion 29:757, 1989


Use of leukocyte-depleted platelets and cytomegalovirus-seronegative red blood cells for prevention of primary cytomegalovirus infection after marrow transplant

RA Bowden, SJ Slichter, MH Sayers, M Mori, MJ Cays and JD Meyers