Cytomegalovirus Infection After Autologous Bone Marrow Transplantation: Occurrence of Cytomegalovirus Disease and Effect on Engraftment

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Epidemiologic and clinical characteristics of cytomegalovirus (CMV) infection and disease were analyzed retrospectively in 159 autologous marrow transplant recipients. The probability of CMV infection by day 100 after transplant was 22.5% in patients seronegative to CMV before transplant versus 61.1% in seropositive patients (P < .0001 by log rank test). Multivariate analysis identified positive pretransplant CMV serology as the only definable risk factor for CMV infection (relative risk 1.4, P < .0001). CMV pneumonia developed in 11 patients at a median time of 100 days after transplant and was fatal in nine cases. CMV pneumonia was associated with significantly decreased probability of survival by day 100 after transplant (relative risk of death of 16.7, P < .0001). In contrast to earlier reports, CMV infection had no significant effect on the rapidity of platelet or neutrophil recovery after transplant as assessed by time-dependent multivariate analysis. Because the incidence of severe CMV disease is not negligible after autologous marrow transplantation, preventive measures against CMV infection are warranted, as in allogeneic marrow transplantation.

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PATIENTS UNDERGOING allogeneic bone marrow transplantation are at risk for severe cytomegalovirus (CMV) infection. In the past, approximately two thirds of allograft recipients who were seropositive before transplant and one third of those who were seronegative before transplant developed CMV infection within the first 150 days after transplant.1 CMV pneumonia occurred in 30% of infected patients with a case-fatality rate above 80%.12 Fortunately, progress has recently been made in the prevention and treatment of CMV infection among allograft recipients, including prophylactic use of screened seronegative blood products or of acyclovir,3 and, in the case of pneumonia, therapeutic use of ganciclovir plus intravenous (IV) immunoglobulin (Ig).46 High-dose cytoreductive chemotherapy followed by autologous bone marrow transplantation is being explored widely as treatment for hematologic malignancies and certain solid tumors,38 but there has been only one report evaluating the significance of CMV infection in a large group of autograft recipients.5 In view of the progress made in the management of CMV infection among allogeneic marrow recipients, further study of the epidemiologic and clinical characteristics of CMV infection among autologous transplant recipients is warranted to define more precisely the need for preventive or therapeutic measures in these patients. Therefore, we undertook a retrospective analysis in a large series of patients who underwent autologous marrow transplantation to determine the clinical impact of CMV infection and of CMV disease. Earlier studies reported the occurrence of prolonged thrombocytopenia in a subset of autologous marrow recipients,10,11 and it has been suggested that CMV infection is a possible cause of delayed platelet recovery.9,12 Therefore, a major part of our analysis addressed the effect of CMV infection on engraftment.

Patients and Methods

All patients who received autologous bone marrow transplantation at the Fred Hutchinson Cancer Research Center (Seattle, WA) or the Swedish Hospital Medical Center (Seattle, WA) between January 1, 1980 and October 31, 1987 were analyzed retrospectively. If patients subsequently required a second transplant, only the first transplant course was evaluated. Patients were excluded from the final analysis if less than three viral cultures were taken from the surveillance cultures.

Virolgy. Routine virologic surveillance consisted of viral cultures from throat, urine, and blood. In most patients, this evaluation was done on one occasion before transplant and then once a week to day 100 after transplant. Additional cultures from these surveillance sites or from other sites were obtained if indicated clinically. In all patients who had an autograft, organ specimens were examined by virologic and histologic techniques for evidence of CMV infection.

Specimens for virus culture were inoculated onto monolayers of human foreskin fibroblasts and observed for 5 weeks. Positive cultures were identified by the appearance of typical cytopathic effects. In case of equivocal culture results, staining by indirect immunofluorescence with murine MoAbs (Syva Co, Palo Alto, CA) was used for confirmation of CMV. CMV was identified in tissue specimens by typical histology (intranuclear and/or cytoplasmic inclusions) or by specific immunofluorescence using murine MoAbs.
For serologic surveillance, serum samples were taken weekly up to day 100 after transplant and tested for IgG antibody to CMV by microtiter complement fixation. A titer of 1:8 or higher was considered positive.

CMV infection was defined as excretion of the virus, presence of CMV in tissue specimens, or a sustained fourfold or greater rise in CMV antibody level. CMV disease was defined as evidence of CMV in tissue specimens, and in the case of pneumonia in bronchoalveolar lavage specimens, associated with clinical symptoms and signs. Results of CMV serology and recovery of CMV in culture from other than tissue specimens were not used to define CMV disease.

Engraftment. The recovery of neutrophil counts after transplant was examined by analyzing the number of days required to reach sustained counts of 0.1 x 10^9/L or greater, and of 1.0 x 10^9/L or greater. Recovery of platelet counts was evaluated by analyzing the number of days needed to reach self-sustaining counts of 20 x 10^9/L or greater, and of 50 x 10^9/L or greater, on at least 2 consecutive days without platelet transfusion support.

Statistical analyses. The probability of CMV infection or other events after transplant was evaluated by the Kaplan-Meier method with analysis by logrank test. Manifestations of CMV infection (eg, CMV excretion) other than pneumonia were evaluated for the first 100 days after transplant, during which time virologic surveillance was performed in all patients still in Seattle. For the analyses of CMV infection, patients were censored if they died or if they left Seattle before day 100 after transplant. CMV pneumonia was analyzed up to day 200 after transplant, based on the assumption that information about the occurrence of pneumonia was also complete after the patients had returned home from Seattle. Patients were only censored from the analysis of pneumonia if they died. The simultaneous influence of multiple variables on these events was assessed by the step-wise proportional hazards regression method. Possible associations occurring over time were modeled using time-dependent indicator variables. For example, the occurrence of CMV infection was modeled by a time-dependent variable set to 0 until such time as infection was documented, and then the variable took the value 1 for subsequent times. Because multiple variables were tested for their association with CMV infection and CMV disease, the probability of chance occurrences of statistical significance is increased over the nominal .05 significance level. For this reason, significance levels between .01 and .05 were considered only suggestive. All significance levels are two-sided.

RESULTS

One hundred eighty-four patients underwent autologous marrow transplantation during the study period. Twenty-five patients were excluded because of insufficient virology data. The clinical characteristics of the 159 remaining patients are summarized in Table 1. Seventeen patients received a polyspecific IV Ig for general infection prophylaxis. None of these patients had a sustained fourfold or greater rise in antibody titer to CMV after Ig infusions. Moreover, there was no significant difference in the occurrence of CMV infection or CMV disease between these 17 patients and the 142 patients without Ig infusions. No patient received prophylaxis with high-dose IV acyclovir; the use of low-dose acyclovir for prevention of herpes simplex virus infection was not analyzed. Four patients were given granulocyte transfusions for treatment of infection during periods of neutropenia. One of these patients, who was CMV seronegative and whose granulocyte donor was seronegative, developed CMV pneumonia on day 145 after transplant.

The median number of viral cultures per patient from all sites was 19 (range 3 to 68) in the first 100 days after transplant. The median number of CMV antibody tests per patient during the same time period after transplant was 11 (range 0 to 17). Four patients treated during the first 2 years of the study period had no serologic surveillance after transplant, but had the required number of viral cultures and therefore were included in the analysis.

CMV infection. Fifty patients (31.4%) developed CMV infection within the first 100 days after transplant. Of these 50 patients, 34 (68.0%) had CMV isolated in culture but no antibody rise, 7 (14.0%) had an increase in antibody only, and 8 (16.0%) had both CMV excretion and a fourfold or greater antibody rise. One patient (2.0%) had only tissue evidence of CMV infection, without excretion or detected antibody rise.

The site of virus recovery and the number of patients in whom CMV was detected by culture are listed in Table 2. The oropharynx was the most common site of excretion (68.0% of infected patients), followed by urinary excretion (52.0%).

Eighty-eight of 159 patients (55.3%) were seronegative for CMV before transplant, and 71 patients (44.7%) were seropositive. Fifteen patients (17.0%) in the former group and 35 patients (49.3%) in the latter group developed CMV infection within the first 100 days posttransplant. The
probability of manifestations of CMV infection by day 100 after transplant in the two serologic groups is shown in Table 3. Seropositive patients had a significantly higher probability of developing CMV infection during this period than seronegative patients (61.1% vs 22.5%, P < .0001) (Fig 1). CMV infection occurred in 14 of 37 (37.8%) patients transplanted for acute nonlymphocytic leukemia (ANL), 17 of 46 (37%) with acute lymphocytic leukemia (ALL), 0 of 2 with chronic myelogenous leukemia (CML), 3 of 14 (21.4%) with Hodgkin’s disease, and 15 of 50 (30%) with non-Hodgkin’s lymphoma.

CMV pneumonia. CMV pneumonia occurred in 5 seronegative and 6 seropositive patients (Table 4). Five cases occurred among 74 patients (6.8%) who received 12 Gy TBI, 6 cases among 61 patients (9.8%) who received other TBI regimens, and no cases among 24 patients who received no TBI. No cases occurred among the 18 patients who received IV methotrexate after transplant (Table 1). In 7 patients CMV infection was detected a median of 42 days (range 22 to 144) before onset of pneumonia, whereas 4 patients had CMV infection and CMV pneumonia documented on the same day. Median onset of CMV pneumonia was at day 100 after transplant (range 55 to 164). The probability of developing CMV pneumonia by day 200 after transplant was 7.7% among pretransplant CMV seronegative and 11.3% among seropositive patients. This difference was not significant. Nine of the 11 patients (81.8%) died. Pneumonia was the primary cause of death in eight cases. In one case (UPN 1339), death was associated with progression of the underlying malignant disease, but CMV pneumonia was a concomitant cause. Treatment with antiviral agents or Ig infusions was given to five patients; CMV pneumonia was fatal in all five.

Risk factors for CMV infection and CMV disease. The factors as listed and grouped in Table 1 were evaluated by both univariate and multivariate analyses for their association with CMV infection or CMV disease within 100 days after transplant. Additionally, the effect of pretransplant TBI was examined by grouping patients who had received more than 12 Gy of fractionated TBI (51 patients), 12 Gy or less of fractionated TBI (76 patients), 10 Gy single-dose TBI (8 patients), or no TBI (24 patients). Preceding CMV excretion or fourfold or greater antibody rise was also examined in time-dependent analyses for their effect on CMV disease.

The only factors significantly associated with CMV infection by univariate analysis were pretransplant CMV serology (P < .0001) and TBI regimen (P = .049). Multivariate analysis showed that positive pretransplant CMV serology was the only independent risk factor for CMV infection (relative risk 1.4, 95% confidence interval [CI] 1.2, 1.6, P < .0001). There was no significant association between CMV disease and any of the factors analyzed, including preceding CMV excretion or antibody rise.

CMV infection and engraftment. The impact of CMV infection on engraftment within the first 100 days after transplant was first assessed by univariate analysis. Recovery of neutrophil counts was not significantly different between patients with and without CMV infection. The probability of reaching neutrophil counts of 0.1 × 10⁹/L by day 100 was 91.1% in CMV infected patients versus 84.9% in uninfected
patients. The median time required to attain these counts was 16 days after transplant in both groups. The probability of achieving neutrophil counts of $1.0 \times 10^9/L$ by day 100 after transplant was 76.6% in CMV infected patients versus 71.9% among uninfected patients, with a median time of 38 days versus 36 days (Fig 2).

Similar results were obtained for recovery of platelet counts. There was no difference in platelet recovery between patients with and without CMV infection. The probability of having platelet counts of $20 \times 10^9/L$ or greater by day 100 was 62.2% among CMV infected patients versus 73.9% among uninfected patients. The median time to reach these platelet counts was 41 days versus 38 days after transplant. Figure 3 shows the probability of recovery to sustained platelet counts of $50 \times 10^9/L$ or greater, which was 53.5% in CMV infected patients versus 63.9% in uninfected patients by day 100. The median time to attain these counts was 54 days versus 49 days after transplant.

The lack of a difference in neutrophil and platelet recovery associated with CMV infection was also found when the
subgroups of pretransplant CMV seronegative and CMV seropositive patients were analyzed separately.

**Other factors affecting engraftment.** To determine further which factors were predictive for engraftment up to day 100 after transplant, a time-dependent proportional hazards regression analysis was performed in which CMV infection was considered present only if it occurred before the event being evaluated. The factors that had a significant influence on engraftment are listed in Table 5. Acute nonlymphocytic leukemia as the underlying disease was associated with both delayed granulocyte recovery to counts of \(1.0 \times 10^9/L\) and delayed platelet recovery to counts of \(20 \times 10^9/L\) and \(50 \times 10^9/L\). Neither CMV infection nor CMV disease had a significant influence on neutrophil or platelet recovery in these analyses.

**Analysis of survival.** The probability of survival to day 100 after transplant was 83.3% for patients who developed CMV infection and 68.6% in patients without CMV infection (\(P = .08\) by log rank test). Proportional hazards regression analysis identified two factors associated with a decreased probability of survival within 100 days after transplant, namely, the occurrence of CMV pneumonia (relative risk of death of 16.7, 95% CI 5.1, 53.9, \(P < .0001\)) and pretransplant conditioning without TBI (relative risk of death of 2.7, 95% CI 1.3, 5.2, \(P = .008\)). In most instances, TBI was not included in the conditioning regimen in these patients because of the judgment that they had already received maximum tolerable radiotherapy. In contrast, acute lymphocytic leukemia as the underlying disease was associated with better survival (relative risk of death of 0.3, 95% CI 0.1, 0.9, \(P = .02\)).

### Table 5. Multivariate Analysis of Factors Affecting Time to Neutrophil and Platelet Recovery in the First 100 Days After Transplant

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Relative Risk†</th>
<th>95% Confidence Interval</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Neutrophil counts (&gt;0.1 \times 10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>0.6</td>
<td>0.4, 0.8</td>
<td>.005</td>
</tr>
<tr>
<td>4-HC marrow transplant</td>
<td>0.3</td>
<td>0.1, 0.7</td>
<td>.006</td>
</tr>
<tr>
<td>No TBI</td>
<td>1.9</td>
<td>1.1, 3.2</td>
<td>.02</td>
</tr>
<tr>
<td>Other chemotherapy</td>
<td>1.7</td>
<td>1.0, 2.8</td>
<td>.04</td>
</tr>
<tr>
<td>2. Neutrophil counts (&gt;1.0 \times 10^9/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANL</td>
<td>0.4</td>
<td>0.3, 0.7</td>
<td>.0009</td>
</tr>
<tr>
<td>No ex vivo marrow treatment</td>
<td>1.5</td>
<td>1.0, 2.2</td>
<td>.05</td>
</tr>
<tr>
<td>3. Platelet counts (&gt;20 \times 10^9/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ANL</td>
<td>0.2</td>
<td>0.1, 0.4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TBI (&gt;12) Gy</td>
<td>2.1</td>
<td>1.4, 3.2</td>
<td>.0006</td>
</tr>
<tr>
<td>Other chemotherapy</td>
<td>0.5</td>
<td>0.3, 0.9</td>
<td>.03</td>
</tr>
<tr>
<td>4. Platelet counts (&gt;50 \times 10^9/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ANL</td>
<td>0.3</td>
<td>0.1, 0.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TBI (&gt;12) Gy</td>
<td>2.1</td>
<td>1.4, 3.3</td>
<td>.0008</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this study of autologous marrow recipients, CMV infection was less frequent than observed among allogeneic transplant recipients, but the probability of developing CMV infection, and more importantly severe CMV disease, was not negligible. CMV infection occurred in 31% of the study population. The probability of CMV infection in the first 100 days after transplant was threefold higher among pretransplant seropositive patients than in seronegative patients. Positive pretransplant serology was the only indentifiable risk factor for CMV infection by multivariate analysis. By contrast, an earlier study from our institution that used similar study criteria reported a 51% incidence of CMV infection in the first 150 days after transplant among 545 allograft recipients. A similar incidence of infection was described in another large group of allogeneic transplant recipients.

The lower incidence of CMV infection after autologous marrow transplantation may be attributable to several factors. The most likely is the lack of graft-versus-host disease (GVHD). After allogeneic marrow transplant, the occurrence of acute GVHD increases the risk of CMV infection significantly among both CMV seronegative and seropositive patients. The probability of CMV infection observed in autograft recipients in this study is comparable with that among allograft recipients who do not develop acute GVHD. Additional factors may include the less common use of posttransplant immunosuppressive therapy and the lack of marrow donor-related transmission of CMV infection after autologous marrow transplantation.

CMV pneumonia developed in 11 patients and was associated with fatal outcome in 9 cases. The probability of CMV pneumonia by day 200 after transplant was 7.7% in seronegative patients and 11.3% in seropositive patients. These results contrast with earlier reports of autologous transplant recipients in which the actuarial incidence of CMV pneumonia varied from 2% to 4%. Partially responsible for the differences between this and previous studies of CMV pneumonia after marrow autografting is the apparently later onset of CMV pneumonia (median onset day 100) compared with allograft recipients, although late cases of CMV pneumonia also occur in allograft recipients with chronic GVHD. However, the use of different conditioning regimens may also be partially responsible for the differences between this and previous studies of CMV pneumonia after marrow autografting. In one study that evaluated pediatric patients, TBI was not used for conditioning, and CMV pneumonia developed in only 5 of 165 (3%) children. In another report, 52 of 143 patients (36%) did not receive TBI; CMV pneumonia occurred in only 3 patients, 2 of whom were given TBI before transplant.

In our study, most patients received TBI (135 patients or 85%). All 11 cases of CMV pneumonia occurred among the 135 patients who received TBI conditioning.
Multivariate analysis did not identify definable risk factors for CMV pneumonia including TBI. However, the small number of patients and poor survival in some categories (ie, patients who did not receive TBI) may have reduced the likelihood of detecting statistically significant risk factors.

The effect of TBI in the conditioning regimen is also of interest because of observations in allograft recipients: Patients receiving allogeneic transplants for severe aplastic anemia who are not conditioned with TBI have a substantially lower risk of CMV pneumonia compared with allograft recipients with hematologic malignancy or severe aplastic anemia whose preparative regimen includes TBI. The difference in risk remains even after adjustment for the occurrence of acute GVHD in the analysis. However, twins undergoing transplant for hematologic malignancy who receive TBI conditioning have a risk of CMV pneumonia that appears to be even lower than that observed after marrow autografting. Further observations will be necessary to clarify the influence of TBI and perhaps of ex vivo marrow treatment on the risk of CMV pneumonia after marrow autografting.

Prolonged thrombocytopenia has been reported after autologous transplant for hematologic malignancy. One study of 22 autograft recipients suggested that CMV seropositive patients had a significant delay in platelet recovery after transplant when compared with seronegative patients. In a larger study by Wingard et al, the effect of CMV infection on engraftment was analyzed separately in the groups of CMV seropositive and seronegative patients. In that study, there was a significant delay in platelet recovery among seropositive patients with CMV infection compared with seronegative patients without CMV infection. No effect was found among seronegative patients, nor was a clear effect found on neutrophil engraftment. However, patients in that study were routinely monitored for CMV infection only to day 50 after transplant. Virologic surveillance beyond day 50 was incomplete. Because engraftment was evaluated up to day 150 after transplant, the lack of information on CMV infection from day 50 to day 150 may have influenced that analysis. Moreover, that analysis was not time-dependent, thereby potentially including CMV infections that developed after platelet or neutrophil engraftment had occurred.

In our study, we analyzed the effect of multiple factors on platelet and neutrophil recovery during the first 100 days after transplant in a time-dependent analysis. CMV infection was only considered to have occurred if it developed before engraftment. No effect of CMV infection was found on either platelet or neutrophil recovery. The same observations were made when CMV seronegative and seropositive patients were analyzed separately. The apparent discrepancy between these data and the results of Wingard et al may conceivably be related in part to differences in treatment modalities in the two studies. For example, whereas ex vivo marrow purging with 4-hydroperoxycyclophosphamide (4-HC) was performed in only 11 of our 159 patients (7%), it was performed in 114 of 143 patients (80%) in the Wingard et al study. An effect of CMV infection on engraftment may be more pronounced or become more apparent after marrow purging, although it is not clear why delayed engraftment among patients with CMV infection would occur exclusively among patients who were seropositive before transplant and not among those who were seronegative as in that study. Moreover, although we did find that marrow purging with 4-HC was associated with a delay in recovery to a neutrophil count of 0.1 × 10⁹/L and that ex vivo marrow treatment of any type (ie, 4-HC or MoAbs) was associated with delay in recovery to a neutrophil count of 1.0 × 10⁹/L, these treatments did not affect platelet recovery and the effect was independent of CMV infection in multivariate analysis (Table 5). Acute nonlymphocytic leukemia, as the underlying disease, was the only factor that delayed both platelet and neutrophil recovery. Pretransplant conditioning with a TBI dose of ≥12 Gy favored more rapid platelet recovery. The results of these analyses suggest that CMV infection is not associated with delayed platelet or neutrophil engraftment after autologous marrow transplant.

In conclusion, although the probability of CMV infection after transplant was lower in autologous than in allogeneic transplant recipients, the probability of CMV pneumonia was not negligible. Multivariate analysis showed that the occurrence of CMV pneumonia was associated with significantly decreased probability of survival. Although not analyzed in this study, gastrointestinal infection due to CMV also occurs in autograft recipients and is an additional risk. We conclude that preventive measures against CMV infection are warranted in autologous marrow recipients as in patients undergoing allogeneic transplantation. Protection of CMV seronegative autologous marrow recipients by use of CMV-seronegative blood products is effective and can be provided at low or no cost. Leukocyte depletion is another alternative. Seropositive patients might benefit from prophylaxis with antiviral agents such as acyclovir, which is already given at lower doses for prevention of herpes simplex virus infection in many centers, or foscarnet, both aimed at preventing reactivation of latent virus. Studies to determine the efficacy and safety of prophylactic measures against CMV infection in autologous marrow recipients are needed, particularly if factors (eg, conditioning regimen) that characterize those autograft recipients at especially high risk of CMV disease can be identified in future studies.

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