Cytomegalovirus Infection Causes Delayed Platelet Recovery After Bone Marrow Transplantation

By Leo F. Verdonck, Gijsbert C. de Gast, Hans G. van Heuhten, Karel Nieuwenhuis, and Adriana W. Dekker

The pathogenic effect of cytomegalovirus (CMV) infection on the hematopoietic recovery after bone marrow transplantation (BMT) was retrospectively studied in 87 recipients of (nonpurged) autologous BMT and in 56 recipients of allogeneic BMT from HLA-identical siblings. Indications for autologous BMT were lymphomas or acute leukemias and for allogeneic BMT various malignancies or aplastic anemia. Patients were divided for the study in two groups, CMV-positive and CMV-negative on the basis of the CMV status before transplantation. Recipients of allogeneic BMT had a variety of diseases. Patient characteristics are given in Table 1. All patients were treated with protocols approved by the Local Investigational Review Board and gave informed consent.

Studies in mice with murine CMV infection have shown that CMV is direct cytotoxic for megakaryocytes or their progenitors and CMV-infected marrow grafts are unable to reconstitute lethally irradiated mice. In humans, CMV infection has been associated with graft failure as well.

We have previously reported in a preliminary study that CMV infection causes delayed platelet recovery after autologous BMT, and this observation was confirmed by another study. However, a recent report could not demonstrate any effect of CMV infection on platelet recovery after autologous BMT. To elucidate these conflicting data we now report the results of the effect of CMV infection on the hematopoietic recovery after marrow transplant in a greater cohort of autologous and allogeneic recipients of BMT.

MATERIALS AND METHODS

Patients. One hundred and forty-three patients, 87 recipients of (nonpurged) autologous BMT and 56 recipients of allogeneic BMT from HLA-identical siblings were studied between March 1982 and June 1990. Patients were divided into two groups: CMV-positive versus CMV-negative on basis of their CMV status before transplantation. Recipients of autologous BMT had hematologic malignancies, and recipients of allogeneic BMT had a variety of diseases. Patient characteristics are given in Table 1. All patients were treated with protocols approved by the Local Investigational Review Board and gave informed consent.

The conditioning regimens used to prepare patients for marrow transplantation, marrow harvesting and manipulation, and the supportive care posttransplant have been described previously. In brief, autologous marrow grafts were cryopreserved without in vitro treatment, ie, nonpurged marrow grafts, and allogeneic marrow grafts were depleted of T cells. The majority of recipients received a combination of cyclophosphamide and total body irradiation or cyclophosphamide and busulfan. All blood products were irradiated (3,000 cGy); leukocyte-poor red blood cell transfusions were given from random donors. Platelet concentrates were given from random donors when patients were CMV-positive; however, CMV-negative patients received platelet concentrates from CMV-negative donors throughout the study period. Patients did not receive granulocyte concentrates or plasma transfusions.

Evaluation of hematopoietic recovery. Recovery of cell counts was evaluated by analyzing the number of days needed to reach granulocytes greater than 500 cells/μL and platelets greater than 50,000 cells/μL on at least 2 consecutive days and without platelet transfusion support. Sixteen patients did not achieve the criteria for hematopoietic recovery because of early death or leukemic relapse. These patients were included and were censored at the time of death or relapse.

Virology. Serologic studies and CMV cultures were performed before BMT in patients and marrow donors and after BMT in patients once weekly during admission at the hospital and 2, 3, 6, and 12 months after discharge. Titers of IgM and IgG antibodies were determined by enzyme-linked immunosorbent assay. CMV cultures were obtained from throat and urine specimens (and buffy coat for allogeneic BMT recipients). Moreover, CMV cultures and serology were repeated if patients developed signs or symptoms of infection. Cultures were analyzed by typical cytopathic effects in the infected cell cultures and by indirect immunofluorescence with murine monoclonal antibodies. In addition, CMV cultures were obtained, when indicated, from biopsy (and autopsy) specimens and analyzed by immunofluorescence and by DNA analysis. Reactivation of CMV infection is defined as a fourfold or greater increase in IgG antibody titer, presence of IgM antibodies, or if
CMV INFECTION DELAYS PLATELET RECOVERY AFTER BMT

CMV-positive patients and 18 of 32 (56%) evaluable CMV-negative patients developed acute graft-versus-host disease (GVHD) grade I or grade II; none of the patients developed greater than grade II acute GVHD. Twelve allogeneic BMT recipients developed grade II acute GVHD before they met the criteria for hematopoietic recovery.

CMV-negative patients, supported by the transfusion policy as outlined above, remained CMV-negative during the study period. None of the BMT recipients with latent CMV infection excreted CMV at the time of BMT. Twenty-one (of 24) allogeneic BMT recipients with latent infection were evaluable (alive ≥ 30 days posttransplant) for reactivation of CMV infection, and 19 of 21 (90%) had reactivation as demonstrated by CMV cultures. Virus excretion occurred after a median of 64 (range 30 to 83) days postplant. Forty (of 44) autologous BMT recipients with latent infection were evaluable for reactivation. Eight of 40 (20%) had reactivation by CMV cultures (five patients; virus excretion after a median of 60 [range 30 to 85] days with or without antibody increase) or by CMV serology (three patients; without CMV excretion). CMV-pneumonia developed in only 1 of 44 (2%) CMV-positive autologous BMT recipients, but in 6 of 22 (27%) CMV-positive allogeneic BMT recipients (including four patients with preceding acute GVHD). All patients with CMV pneumonia died, despite early treatment with the antiviral agent DHPG and/or CMV-Ig.

The reactivation of latent CMV infection before hematopoietic recovery (see below) was achieved occurred in 9 of 21 evaluable allotransplant recipients and in 4 of 37 evaluable autotransplant recipients.

Hematopoietic recovery in patients with lymphomas treated with autologous BMT. The recovery of granulocytes greater than 500 cells/μL occurred after a median number of 21 days (range, 11 to 33) in CMV-negative patients and after a median number of 24 days (range, 14 to 46) in CMV-positive patients. This difference was not significant. The recovery of platelets greater than 50,000 cells/μL occurred significantly faster in CMV-negative patients (Fig 1); the time to reach this platelet count occurred after a median number of 24 days (range, 13 to 34) in CMV-negative patients versus a median number of 36 days (range, 17 to 87) in CMV-positive patients (P = .0002).

RESULTS

Patient characteristics and CMV-virology. The clinical characteristics for CMV-negative and CMV-positive patients are summarized in Table 1. Median age of CMV-negative autologous BMT recipients was slightly higher; otherwise, characteristics were not different for both groups. Especially similar were the number of nucleated cells and committed stem cells in the graft, determined after cryopreservation and thawing for autologous marrow grafting and after in vitro T-cell depletion for allogeneic marrow grafting. After allogeneic BMT, 14 of 21 (67%) evaluable CMV-negative patients and our transfusion policy: all CMV-seronegative patients with autologous BMT recipients was slightly higher; otherwise, characteristics were not different for both groups.

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<th>Table 1. Patient Characteristics</th>
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<td>No. of recipients of autologous BMT</td>
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<td>Median age, y (range) (mean ± SD)</td>
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<td>Median no. of nucleated cells (&gt;10^3/kg) in cryopreserved graft (range)</td>
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<td>No. of recipients of allogeneic BMT</td>
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*Includes patients with multiple myeloma (4), metastatic melanoma (3), severe aplastic anemia (2), Hodgkin’s disease (1), myelodysplastic syndrome (2).

CMV cultures became positive in patients with IgG antibodies (latent infection). Because of the unequivocal results of CMV-seronegative patients with our transfusion policy: all CMV-seronegative patients remained CMV-negative by culture and serology,11,12 routine CMV cultures were cancelled in CMV-negative autologous BMT recipients since September 1988. Because of this policy, routine CMV cultures were not performed in 12 CMV-seronegative autologous BMT recipients. However, because of fever, CMV cultures were performed in seven of these patients and were shown to be negative (as did CMV serologic studies during follow-up).

Statistical analysis. The probability of achieving granulocytes greater than 500 cells/μL and platelets greater than 50,000 cells/μL after BMT was evaluated by the Kaplan-Meier method23 with analysis by logrank test.23

Fig 1. Recovery of platelets greater than 50,000 cells/μL after autologous BMT in CMV-negative and CMV-positive patients with lymphomas.
Hematopoietic recovery in patients with acute leukemias treated with autologous BMT. The recovery of granulocytes greater than 500 cells/μL occurred after a median number of 37 days (range, 12 to 45) in CMV-negative patients and after a median number of 27 days (range, 13 to 54) in CMV-positive patients. This difference was not significant. The recovery of platelets greater than 50,000 cells/μL occurred after a median number of 67 days (range, 18 to 132) in CMV-negative patients and after a median number of 51 days (range, 33 to 244) in CMV-positive patients. This difference was not significant. However, the number of patients is rather small (13 vs 21) in this subgroup.

Furthermore, we analyzed separately patients with AML and patients with ALL in relation to their CMV status and, although the number of patients in each category became rather small, the hematopoietic recovery was not influenced by CMV infection in both diseases (data not shown). In analyzing the hematopoietic recovery for the disease, irrespective of CMV status, no significant differences were observed between patients with AML or with ALL (data not shown).

Hematopoietic recovery in patients treated with allogeneic BMT. The recovery of granulocytes greater than 500 cells/μL occurred after a median number of 22 days (range, 12 to 38) in CMV-negative patients and after a median number of 24 days (range, 16 to 40) in CMV-positive patients. This difference was not significant. The recovery of platelets greater than 50,000 cells/μL occurred significantly faster in CMV-negative patients (Fig 2), and the time to reach this platelet count occurred after a median number of 27 days (range, 15 to 165) in CMV-negative patients versus a median number of 41 days (range, 19 to 291) in CMV-positive patients \( (P=0.007) \). To eliminate a (negative) GVHD effect on the hematopoietic recovery, we excluded in this analysis patients who developed acute GVHD grade II before they met the criteria for hematopoietic recovery. This happened in eight CMV-negative patients and in four CMV-positive patients. The recovery of granulocytes greater than 500 cells/μL was not significantly different in this subgroup (patients with no or grade I acute GVHD): 21 days (range, 12 to 33) in CMV-negative patients and 24 days (range, 19 to 40) in CMV-positive patients. However, the difference between CMV-negative and CMV-positive patients for the recovery of platelets remained \( (P=0.01) \): platelets greater than 50,000 cells/μL occurred after a median number of 24 days (range, 15 to 165) in CMV-negative patients and after a median number of 42 days (range, 21 to 291) in CMV-positive patients (Fig 3).

DISCUSSION

This study indicates that CMV infection causes delayed platelet recovery after autologous and allogeneic marrow transplantation. The effect of CMV infection on platelet recovery is present in autologous BMT for patients with lymphomas, but not for patients with acute leukemias, and in allogeneic BMT CMV affects all patients. CMV infection has no effect on the recovery of granulocytes in autologous or in allogeneic BMT. Autologous BMT recipients with acute leukemias have a much slower recovery of granulocytes and platelets than autologous BMT recipients with lymphomas (and with CMV infection).

We have previously reported that CMV infection causes delayed platelet recovery after autologous BMT, but the number of patients was small and patients with lymphomas and with acute leukemias could not be analyzed separately. In patients with acute leukemias, delayed platelet recovery and, to a lesser extent, also granulocytic recovery, has been reported after autologous (nonpurged) BMT. These data are confirmed in this study, and in addition we show that the hematopoietic recovery is not affected by CMV infection. The reason for the extreme delayed hematopoietic recovery after autologous BMT for patients with acute leukemias is unknown at the moment, but can probably be ascribed to the original stem cell disorder, continuing in remission.

The negative effect of CMV infection on the platelet recovery was pronounced in allogeneic BMT recipients. All allograft recipients received T-cell–depleted marrow grafts and CMV-positive and CMV-negative patients had similar numbers of nucleated marrow cells and committed stem cells in the graft. Because acute GVHD can cause prolonged pancytopenia, especially isolated thrombocytopenia after BMT, we excluded in our analysis patients who developed acute GVHD beyond grade I before they met the criteria for hematopoietic recovery after BMT. The negative impact of CMV infection on the recovery of platelets held true in this analysis.
Our observation that CMV infection causes delayed platelet recovery after autologous BMT was confirmed by Wingard et al., although confined to CMV-positive patients with reactivation of CMV infection. However, a very recent report by Reusser et al. could not demonstrate any effect of CMV infection, including CMV reactivation, on the recovery of platelets after autologous BMT. A major drawback of both studies was that blood products used for transfusion support were not screened for CMV status. This drawback was reflected by the fact that a high proportion of initially CMV-negative patients became CMV-positive (50%, and 23%, respectively). Thus, the conclusion drawn by Reusser et al., that CMV infection is not associated with delayed platelet recovery, is questionable. Furthermore, the majority of the autologous BMT recipients in both studies had acute leukemias. Because a delayed platelet recovery after autologous BMT is ubiquitous in these patients, the negative effect of CMV infection is probably camouflaged. The observed association between reactivation of CMV infection and delayed platelet recovery was not applicable in our study because almost all autologous BMT recipients achieved hematopoietic recovery before CMV reactivation occurred. Obviously, latent CMV infection itself is responsible for delayed platelet recovery in the early phase posttransplant.

The importance of a deliberate transfusion policy that effectively prevents CMV transmission to CMV-negative recipients was reported previously by our group and confirmed later in a large study. In the present study, none of the CMV-negative autologous or allogeneic BMT recipients acquired a (primary) CMV infection (and risk for CMV pneumonia) during the first 6 months after BMT. Although CMV pneumonia occurred in only 1 of 44 (2%) CMV-positive autologous BMT recipients and in 6 of 22 (27%) CMV-positive allogeneic BMT recipients, the risk of CMV pneumonia in autologous BMT is not negligible, as was demonstrated by Reusser et al., who observed an incidence of about 10%.

The mechanisms by which CMV infection causes a delay in platelet recovery are gradually being elucidated: CMV infection can be directly cytotoxic for megakaryocytes or their progenitors and also to hematopoietic stem cells in the mouse model, and pursuing to human studies in vitro, the marrow progenitor cell can be infected by CMV and disturb hematopoiesis, indirect impairment of hematopoiesis is also possible through infection of marrow stromal cells, including perturbation of growth factor production.

In conclusion, CMV infection causes delayed platelet recovery after BMT. However, in autologous BMT the underlying disease is more determinant for hematopoiesis after transplantation. The delayed platelet recovery requires more transfusions. A transfusion policy that prevents transmission of CMV to CMV-negative BMT recipients will have a major impact on morbidity and mortality and on expenses of treatment.

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Cytomegalovirus infection causes delayed platelet recovery after bone marrow transplantation

LF Verdonck, GC de Gast, HG van Heugten, HK Nieuwenhuis and AW Dekker