Delay in Platelet Recovery After Bone Marrow Transplantation: Impact of Cytomegalovirus Infection

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The effect of cytomegalovirus (CMV) infection on hematopoietic recovery after marrow-ablative chemoradiotherapy followed by autologous bone marrow transplantation (BMT) was studied in patients with non-Hodgkin's lymphoma of high-grade malignancy and in patients with acute leukemia. The recovery of platelets after autologous BMT occurred significantly quicker in CMV-negative patients than in CMV-positive patients (platelets > 50,000 per cubic millimeter after 21.5 vs. 40 days, respectively). No differences in the recovery of neutrophils were found between those with or without CMV infection. CMV-positive patients required significantly more transfusion support with thrombocyte concentrates than CMV-negative patients (three vs. six thrombocyte concentrates). In conclusion, CMV infections do not influence neutrophil recovery but do delay platelet recovery. As a consequence, patients with a CMV infection, whether primary, reactivated, or latent, require more thrombocyte concentrates, which increases the risk of transfusion-related infections.

Patients treated with marrow-ablative chemoradiotherapy followed by bone marrow transplantation (BMT) show a period of marrow aplasia with onset of hematopoietic recovery within three weeks after marrow infusion. The time to neutrophil recovery is similar in allogeneic, syngeneic, and autologous BMT, but there is some delay in the recovery of platelets after autologous BMT in patients with acute leukemia (AL). This delay in platelet recovery occurs less frequently after autologous BMT in patients with non-Hodgkin's lymphoma (NHL). In these patients, platelet recovery is the same as in allogeneic and syngeneic BMT. A delay in platelet recovery, however, has also been found incidentally after allogeneic and syngeneic BMT for AL and NHL, with no obvious explanation.

We have studied hematopoietic recovery after marrow-ablative chemoradiotherapy followed by autologous BMT in relation to cytomegalovirus (CMV) infection in patients with AL and NHL.

MATERIALS AND METHODS

Patients. Twenty-two patients were studied, whose follow-up was long enough for hematopoietic recovery (ie, granulocytes > 500 cells per cubic millimeter and platelets > 50,000 cells per cubic millimeter); 14 had NHL of high-grade malignancy and eight patients had AL. Details are summarized in Table 1. All were treated with cyclophosphamide [Cy] 60 mg/kg; days -4 and -3) and total body irradiation [TBI] 800 rad; 16 to 18 rad/min, SMev linear accelerator; day -1) followed by autologous BMT (ABMT) day 0). None received posttransplant cytoreductive treatment. All risks of the treatment protocol were fully explained to patients and relatives. Informed consent was obtained through forms approved by the Ethical Committee of the University Hospital Utrecht.

Conventional treatment before the ABMT procedure consisted of combination chemotherapy: mostly cyclophosphamide-hydroxydaunorubicin-oncovin-prednisone (CHOP) courses or comparable polychemotherapy for patients with NHL; induction and consolidation treatment with 6-thioguanine-cytosine arabinoside-daunorubicin (TAD) courses for patients with acute nonlymphocytic leukemia (ANLL); induction treatment with daunorubicin, oncovin, and prednisone; and consolidation treatment with cyclophosphamide and high-dose cytotoxic ara-aldesin derivatives for patients with acute lymphocytic leukemia (ALL). Patient 22 with acute undifferentiated leukemia (AUL) received L-asparaginase and two courses consisting of amsacrine and high-dose cytotoxic ara-aldesin derivatives because of failure to respond to treatment with the ALL scheme.

Patients were divided into two groups on the basis of their CMV status: 12 CMV-positive patients (11 with latent CMV infection, of whom three showed reactivation serologically, and one with primary CMV infection in the early posttransplant period) and ten CMV-negative patients, who were kept CMV-negative by a deliberate transfusion policy (CMV-negative donors for platelet transplants; leukocyte-free erythrocytes) as reported earlier. Except for one patient (no. 1) with primary CMV infection, the early posttransplant period until engraftment was uneventful. During bacteremia (gram-positive cocci), which occurred in five patients (three CMV-negative, two CMV-positive), no decline in the thrombocyte count was observed.

Bone marrow aspiration, cryopreservation, and reinfusion. Bone marrow was collected in all patients after confirmation of absent tumor involvement of the marrow by bone marrow biopsy from both iliac crests. Techniques for procurement and cryopreservation of the marrow were as described. The presence of committed stem cells in the cryopreserved marrow was monitored by colony assays for the granulocyte-monocyte series (CFU-GM). The cryopreserved marrow of the patients contained a mean of 1.9 x 10⁷ nucleated cells per kilogram and a mean of 10.8 x 10⁴ CFU-GM per kilogram (Table 1). The frozen marrow was transported in liquid nitrogen on the day of transplantation, rapidly thawed in a 37 °C water bath and, immediately infused intravenously without any manipulation.

Supportive care. The patients were treated in single rooms with reversed isolation, and received selective decontamination of the alimentary tract with co-trimoxazole, colistin, amphotericin B, and nystatin suspension until granulocytes were above 500 per cubic millimeter. Platelets and leukocyte-free RBCs were administered in order to maintain platelet counts > 20,000 per cubic millimeter and hemoglobin (Hb) > 10 g/dL. No granulocyte transfusions were given. All (allogeneic) blood cells were irradiated (2,000 rad).

Evaluation of hematopoietic recovery. Peripheral blood cell counts and blood pictures were examined daily in all patients until neutrophil counts were > 500 cells per cubic millimeter and platelet counts were > 50,000 cells per cubic millimeter. Aspiration (or biopsy) of the marrow was done in all patients with AL at least once a month after BM transplantation and on indication in patients with NHL.

Statistical tests. Statistical analysis was performed with Wilcoxon's rank sum test. P values < .05 were regarded as significant.
RESULTS

Recovery of neutrophils (Fig 1). The recovery of neutrophils above 500 cells per cubic millimeter occurred in all patients after a median number of 19 days (range, 11 to 40). This recovery was the same in CMV-negative patients (median, 17 days) as in CMV-positive patients (median, 19 days).

Recovery of platelets (Fig 1). The recovery of platelets above 50,000 cells per cubic millimeter (self-sustaining) occurred in all patients after a median number of 27½ days (range, 13 to 59). This recovery was significantly better \( P < .01 \) in CMV-negative patients, with a median of 21½ days (range, 13 to 28) in CMV-negative patients and a median of 40 days (range, 24 to 59) in CMV-positive patients. A steady increase in platelets to above 100,000 cells per cubic millimeter occurred within 100 days in all except four patients. In three patients the level remained between 50,000 and 100,000 cells per cubic millimeter because of relapse or early death and the fourth patient remained slightly thrombocytopenic (between 80,000 and 100,000 cells per cubic millimeter) for almost 18 months with an otherwise normal hemogram. No explanation could be found for the slight thrombocytopenia.

Recovery of neutrophils in patients with NHL and patients with AL. In patients with NHL, the median number of days to reach neutrophil counts above 500 cells per cubic millimeter, was 20½ (range, 11 to 40). In patients with AL, the median number of days to reach neutrophil counts above 500 cells per cubic millimeter was 16½ (range, 13 to 19). These differences were not significant.

Recovery of platelets in patients with NHL and patients with AL. In patients with NHL, the median number of days to reach platelet counts (self-sustaining) above 50,000 cells per cubic millimeter was 24 (range, 13 to 42). In patients with AL, the median number of days to reach platelet counts above 50,000 cells per cubic millimeter was 42½ (range, 21 to 59). These differences were significant \( P < .01 \). Investigating patients with NHL, a significant better \( P = .01 \) recovery of platelets was also found in CMV-negative patients: after a median number of 20 days (range, 13 to 27) in CMV-negative patients vs 30 days (range, 21 to 42) in CMV-positive patients. Because only two of eight patients with AL were CMV-negative, patients with AL could not be studied separately. These two patients, however, achieved 50,000 platelets per cubic millimeter after 21 and 28 days, as with CMV-negative patients with NHL.

Correlation between recovery of neutrophils and platelets and other patient characteristics. No correlation was found between the number of nucleated cells or CFU-GM in
the bone marrow graft and recovery of platelets or neutrophils. In addition, no correlation was found between age of the patients, number of previous chemotherapeutic courses before BM collection, and time between bone marrow collection and BMT and recovery of platelets or neutrophils. Table 2 summarizes these data for recovery of platelets. The age of the patients was significantly different, but this held true only for the CMV status: CMV-positive patients were older than CMV-negative patients. Analyzing age and platelet recovery, irrespective of the CMV status, there was no correlation between age and recovery of platelets (Fig 2; correlation coefficient \( r = 0.036 \)).

**Number of platelet transfusions in the patients.** Transfusions with platelet concentrates were, on most occasions, from single donors, and seldom from random donors. Each concentrate (single or random) yielded about \( 3 \times 10^{10} \) platelets. CMV-negative patients received a median of three platelet concentrates (range, two to six) and CMV-positive patients received a median of six (range, two to 14). These differences were significant (\( P < 0.025 \)).

**DISCUSSION**

Hematopoietic recovery after marrow-ablative chemoradiotherapy followed by ABMT has been clearly demonstrated. The recovery of neutrophils is about the same in autologous, syngeneic, and allogeneic BMT.\(^1,2,8\) Prolonged thrombocytopenia has been reported in autologous BMT in patients with AL,\(^3,5\) which occurred less frequently in patients with NHL.\(^4,6,7\) Some patients with NHL did show prolonged thrombocytopenia.\(^8\) Some delay in platelet recovery, however, has also been reported after allogeneic and syngeneic BMT.\(^1,8,13\) Of importance is the fact that all patients observed in these studies received the marrow-ablative high-dose chemoradiotherapy. In these studies, as in our study, no relationship has been found between the number of nucleated marrow cells in the graft and the recovery of neutrophils and platelets. In addition, there has been no correlation between the recovery of platelets and number of CFU-GM in the graft.\(^11,14\) In fact, no explanation has been found for the disproportionate delay in platelet recovery, ie, platelet autoantibodies or absence of megakaryocytes in marrow biopsies after ABMT.\(^4,5\) After allogeneic BMT, graft-v-host disease may be a contributing factor, but mainly after established engraftment.\(^15,16\)

In this ABMT study, no differences were found in the recovery of neutrophils in patients with NHL or with AL, either in those with or without CMV infection. Recovery of platelets, however, was delayed in patients with AL, as compared to patients with NHL after ABMT, as reported previously.\(^1,7\) Considering the CMV status of the patients, significant differences were found in platelet recovery between those with and those without CMV infections. These differences in platelet recovery were also found in analyzing separately patients with NHL (\( P < 0.01 \)), and the two CMV-negative patients with AL showed comparable platelet recovery to CMV-negative patients with NHL. Because the patients with CMV infections were older than those without it, age may have been a factor. However, no correlation was found between age and platelet recovery. In this study, no platelet autoantibodies or differences in megakaryocyte content in marrow biopsies after BMT were found to explain the differences in platelet recovery. In addition, all patients received the same drugs for selective decontamination of the alimentary tract, eg, co-trimoxazole, after BMT. Therefore, CMV infection plays an important role in the recovery of platelets after (autologous) BMT.

The mechanisms by which CMV infection causes a delay in platelet recovery are unknown. Thrombocytopenia (and granulocytopenia) are associated with various viral infections, such as CMV\(^17,18\) in humans, but marrow aspirates during viral illnesses do not usually show a decrease in megakaryocytes.\(^19\) Aplastic anaemia has been found after infection with non-A non-B hepatitis virus and Epstein-Barr virus.\(^20,21\) A direct cytotoxic effect for bone marrow erythroid progenitor cells by human parvovirus\(^19\) and for megakaryo-

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**Table 2. Patient Characteristics in Relation to CMV Status**

<table>
<thead>
<tr>
<th>Patients</th>
<th>CMV-Negative</th>
<th>CMV-Positive</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Age (yr) (median, range)</td>
<td>26 (19–35)</td>
<td>37 (15–53)</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>No. of chemotherapeutic courses before BM collection (median, range)</td>
<td>3 (1–6)</td>
<td>3 (2–13)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of nucleated cells ((x 10^6/kg)) in the graft (median, range)</td>
<td>1.8 (1.3–2.5)</td>
<td>1.8 (1.2–2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of CFU-GM ((x 10^3/kg)) in the graft, (median, range)</td>
<td>11.5 (5–24)</td>
<td>8 (2–24)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of days after BMT to reach ( &gt;50,000 ) platelets/mm(^3)</td>
<td>21.5 (13–28)</td>
<td>40 (24–59)</td>
<td>( P &lt; 0.01 )</td>
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Myelosuppressive agents used in the courses in both patient groups were: doxorubicin (daunorubicin), cyclophosphamide, cytosine arabinoside, and 6-thioguanine. In addition, amsacrine was given in one CMV-positive patient and methotrexate in one CMV-negative patient. NS, not significant.
cytes or their progenitors in murine CMV has been demonstrated. Viral structures in megakaryocytes in human congenital CMV infections and decreased numbers of megakaryocytes and cytopathic changes in megakaryocytes after live measles vaccination are reported. An indirect effect on hematopoiesis by viral infections, by generating suppressor lymphocytes, antibodies, or interferons may also be possible. Future investigations may reveal mechanisms of this platelet inhibition by CMV infections.

Apart from the high morbidity and mortality rates, the additive immunosuppression and increased graft injury caused by CMV infections after allotransplantation, prolonged thrombocytopenia contributes to the morbidity and requirement of more transfusions, with a higher risk for transfusion-related infections. There is a need for a transfusion policy which prevents transmission of CMV by selecting donors based on their CMV status.

REFERENCES
30. Verdonck LF, de Gast GC: Is cytomegalovirus infection a major cause of T cell alterations after (autologous) bone marrow transplantation? Lancet 1:932, 1983
Delay in platelet recovery after bone marrow transplantation: impact of cytomegalovirus infection

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