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Immune Reactivity in Relation to Cytomegalovirus Infection After Allogeneic Bone Marrow Transplantation


CTYOMEALGOVIRUS (CMV) infection is a frequent and clinically important infection following bone marrow transplantation (BMT). As a result of the profound immunodeficiency for at least six months following allogeneic BMT, CMV infection has a high rate of dissemination. In addition, CMV infection itself exerts an immunosuppressive effect, which has been demonstrated in otherwise immunocompetent individuals and in autologous BMT recipients. Schroff et al., who used complement fixation (CF) for serologic definition of active CMV infection, described a progressive decrease of the ratio between T4+ and T8+ T lymphocytes in allogeneic BMT recipients with primary CMV infection. Middeldorp et al. recently developed an enzyme-linked immunosorbent assay (ELISA) for the detection of CMV antibodies, which proved to be 20 to 5,000 times more sensitive than CF. We have studied the occurrence of CMV infection in allogeneic BMT recipients, using ELISA in addition to routine viral cultures and histomorphology, and related it to changes in peripheral blood T cell phenotypes.

PATIENTS AND METHODS

Patients

Fifty-eight patients received bone marrow from an HLA-identical, mixed lymphocyte reaction (MLR)-non-reactive sibling. Fourteen patients with severe aplastic anemia were prepared for BMT with cyclophosphamide (50 mg/kg/d four times) and total lymphoid irradiation (7.5 Gy once, n = 11; 2.0 Gy ten times, n = 3). The 41 patients with acute leukemia in remission, and three patients with chronic granulocytic leukemia in chronic phase were prepared with cyclophosphamide (60 mg/kg/d twice) and total body irradiation (8.0 Gy once). The patients received a median of 2.2 × 10^8/kg nucleated bone marrow cells (range, 1.6 to 4.6 × 10^8). They were nursed in laminar down-flow isolators and received selective anti-microbial modulation from day 15 pre-BMT until day 60 post-BMT. Transfusions of platelet concentrates, containing only 10 to 20 mL plasma, were given to maintain the platelet count above 10 × 10^9/L. Packed RBCs were administered to maintain the hemoglobin level above 5 mmol/L. Only leukocyte-depleted blood products, irradiated with 15 Gy, were given. All patients received methotrexate for prophylaxis of acute graft-versus-host disease (GVHD), which was diagnosed and staged on the basis of the clinical criteria, and confirmed histologically in the majority of patients. Therapy for acute GVHD consisted of corticosteroids (1 to 2 mg/kg/d, or 20 mg/kg/12 h for two days); to eight patients with grades II to IV acute GVHD, monoclonal antibody (MCA) OKT3 (Ortho Pharmaceutical Co, Raritan, NJ) was given as well. Chronic GVHD was treated with corticosteroids (0.3 to 1.0 mg/kg/d) and azathioprine (1 to 2 mg/kg/d).

Assessment of CMV Infection

Titers of IgG antibodies against CMV-late antigen were determined by ELISA as described elsewhere. Assays were performed prior to BMT in donor and recipient; then, in the recipient, at least at monthly intervals during the first six months post-BMT, and at larger intervals thereafter. Viral cultures of throat swabs, urine, and buffy coats were performed before BMT and at weekly intervals during hospitalization, using standard techniques. In biopsy and autopsy specimens, the charac-
teristic cytopathogenic effects were studied. Presence of no CMV infection was defined as no rise in titer of CMV antibodies, as well as negative cultures and/or biopsies. Primary CMV infection was defined as a pretransplant titer of <1:40 in the recipient, at least a fourfold rise in titer (minimum, 1:160) post-BMT, and/or positive cultures or histomorphology. Secondary CMV infection (ie, either reinfection with a new CMV strain or reactivation of endogenous CMV) was defined as a pretransplant titer of ≥1:40 in the recipient, at least a fourfold rise in titer post-BMT, and/or positive cultures or histomorphology. When plasma infusions were given that were likely to contain CMV antibodies, the rise in titer was required to be sustained for at least three weeks after the last plasma infusion for serologic definition of active CMV infection.

Analysis of T Cell Phenotypes

Heparinized venous blood samples were obtained twice a week from days 8 to 40 post-BMT, and with decreasing frequency thereafter. Results obtained during therapy with OKT3 and during the first week thereafter were excluded from analysis. T cell subset enumeration was performed using indirect immunofluorescence and flow cytometry as described previously. The following MCAs were used: as anti-T3, OKT31; as anti-T4, Leu 3a; and as anti-T8, FK18. Donor or recipient origin of T4+ and T8+ T cells was studied in recipients of a sex-mismatched graft using quinacrine staining for Y bodies in combination with T cell typing for T4 and T8 as described previously.

RESULTS

GVHD

Acute GVHD developed in 41 of the 58 patients; it was mild (grade I) in seven, and moderate (grade II) to severe (grades III or IV) in 34 patients. Two patients could not be evaluated because they died within one month post-BMT, while still at risk for acute GVHD. Of 40 patients who survived for more than 125 days, 19 developed chronic GVHD (14 mild, five severe).

CMV Infection

Eight patients were excluded from analysis. Three patients with negative serology, cultures, and autopsies died within one month; this was considered too early for proper analysis. Five patients with negative cultures and autopsies were excluded because they had received plasma infusions during their entire posttransplant period, thus rendering interpretation of serologic data impossible. Of the 50 evaluable patients, 28 developed active CMV infection. This was diagnosed on the basis of positive cultures and serology in eight, on the basis of serology solely, in 16, and in the remaining four, on positive cultures or histomorphology only.

Role of Pretransplant CMV Antibodies

The incidence of CMV infection post-BMT in relation to the pretransplant serologic status of donor and recipient is given in Table 1. A remarkably high incidence of infection was seen in seropositive recipients (23 of 25) as compared with seronegative ones (five of 25; $\chi^2$, 26.30; $P < .0001$). The antibody status of the donor did not influence the incidence of CMV infection post-BMT (17 of 26 with seropositive donors developed active CMV infection v 1 of 24 with seronegative donors; $\chi^2$, 1.94; $P = .16$), but did influence the timing of the antibody response in patients who were already seropositive pre-BMT. The fourfold or greater rise in antibody titer occurred at a median of 76 days (range, 29 to 119 days) post-BMT in patients with seropositive donors and at a median of 104 days (range, 43 to 162 days) in those with seronegative donors ($P < .01$, Wilcoxon's rank test). Peak CMV antibody titers in both groups were similar (data not shown). The exposure to blood products (packed RBCs, platelets, and plasma) in the four different donor–recipient combinations did not differ significantly (data not shown). None of the patients received granulocyte transfusions.

Next, the incidence of acute GVHD in relation to the pretransplant serologic status of donor and recipient was studied (Table 2).

<table>
<thead>
<tr>
<th>Pre-BMT Serology</th>
<th>No. of Patients</th>
<th>No. of Patients With CMV Infection</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Recipient</td>
<td>Donor</td>
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<tr>
<td>+</td>
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<td>16</td>
<td>15</td>
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Table 2. Pretransplant CMV Antibodies and the Development of Acute GVHD

<table>
<thead>
<tr>
<th>Pre-BMT Serology</th>
<th>No. of Patients With Grades II to IV GVHD</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>Recipient Donor</td>
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<tr>
<td>+ +</td>
<td>18</td>
<td>14</td>
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<tr>
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<td>- +</td>
<td>11</td>
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</table>

Seropositive donors induced grades II to IV acute GVHD more often than seronegative ones (23 of 29 vs 11 of 27, respectively; $\chi^2$, 8.72; $P = .003$). The antibody status of the recipient did not influence the occurrence of grades II to IV acute GVHD (19 of 30 seropositive recipients developed grades II to IV acute GVHD vs 15 of 26 seronegative ones; $\chi^2$, 0.19; $P = .67$).

**T Lymphocyte Repopulation**

To study the pattern of T cell repopulation following BMT in relation to CMV infection, the data were pooled per time interval and with regard to CMV infection as indicated in the legend to Fig 1. Only three patients with primary CMV infection could be studied here; they showed the same repopulation pattern as seronegative patients. Because of this small

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**Fig 1.** Absolute numbers of T3+, T4+, and T8+ T lymphocytes, and T4-T8 ratios in relation to active CMV infection. Vertical bars indicate mean + 1 SD. O—O, No CMV infection (n = 12); □—□, primary CMV infection (n = 3); ●—●, secondary CMV infection (n = 13). Shaded areas represent normal ranges as determined in 170 control persons. Data from the following time intervals were pooled: days 8 to 15, 16 to 25, 26 to 35, 36 to 45, 46 to 55, 56 to 75, 76 to 100, 101 to 125, 126 to 150, 151 to 200, 201 to 300, and 301 to 500. Differences between patients without CMV infection and those with secondary infection were significant ($P < .05$, using Student’s $t$ test) from day 45 onward for the T4-T8 ratios, and from day 45 onward with the exception of days 101 to 125 for the absolute numbers of T3+ and T8+ T lymphocytes.
number, only a comparison between seronegative patients (n = 12) and those with secondary infection (either reinfection or reactivation; n = 13) was made. Up to 45 days post-BMT, the two groups showed similar repopulation patterns, i.e., an increase in T₃⁺ T cells to subnormal levels, a very slow increase of T₄⁺ T cells, and a return of T₈⁺ T cells to the normal range. As a result, the T₄-T₈ ratio decreased to values <1.0. After day 45, however, differences became apparent (Fig 1). In seronegative patients, T₃⁺ T cells gradually increased to the lower limit of the normal range, and T₈⁺ T cells remained at about the same, i.e., normal, level. Patients with secondary CMV infection, however, showed a brisk increase in T₃⁺ T cells to the normal range, and in T₈⁺ T cells to supranormal numbers. Both patient groups showed a slow increase in T₄⁺ T cells, which reached the lower limit of the normal range only after 300 days. Thus, patients with secondary CMV infection could be distinguished by abnormally high numbers of circulating T₈⁺ T cells and, consequently, a significantly lower T₄-T₈ ratio. Since this could implicate a recipient-derived immunologic reaction, the donor or recipient origin of T₄⁺ and T₈⁺ T cells was studied in recipients of sex-mismatched marrow. Six patients with secondary CMV infection and four seronegative patients were studied between two and six months of post-BMT. In all patients, the Y⁺ fraction of T₄⁺ and T₈⁺ T cells was within the normal range of that of their donors (males, >40%; females, <5%).

The pretransplant antibody status of the donor did not appear to influence the T cell repopulation pattern. Of the 13 patients with secondary CMV infection studied, three had seronegative donors. These patients showed the same (timing of the) strong increase of T₈⁺ T cells as the ten patients with seropositive marrow donors.

DISCUSSION

A remarkable finding in this survey of CMV infection in allogeneic BMT recipients is the extremely high rate of active infection in patients who were seropositive before BMT (92%) as compared with seronegative ones (20%). Since both groups did not differ significantly with respect to the amount of blood products transfused, which may transmit CMV,¹⁵ and to seropositivity of their marrow donors, we assume that the high rate of CMV infection in the seropositive BMT recipients is caused by reactivation of endogenous CMV. However, infection with a totally different strain of CMV is also conceivable. At present, we are not able to distinguish between these two possibilities. The low infection incidence (20%) in seronegative recipients indicates that the latter possibility may be relatively uncommon.

In mice, GVH reactions have been shown to enhance CMV infection.¹⁶ Our results are not in agreement with this finding; pretransplant seropositivity of the recipient correlates with CMV infection post-BMT in the majority of cases, and the incidence of significant (ie, grades II to IV) acute GVHD in seropositive and seronegative recipients is similar (Table 2). Rather, it might be related to the slow immunologic recovery and the immunosuppressive therapy (methotrexate as GVHD prophylaxis) in allogeneic BMT recipients, since after autologous marrow grafting, only a 33% incidence of secondary CMV infection has been reported.¹² Interestingly, seropositive donors induced grades II to IV acute GVHD more often than seronegative ones; at present, we do not have an explanation for this phenomenon.

The earlier rise in CMV antibody titer seen in seropositive recipients of seropositive marrow, as compared with seropositive recipients of seronegative marrow, might be caused by memory B cells present in the graft. In contrast, this earlier response is not reflected in the repopulation of the peripheral blood with donor T cells (data not shown). The latter appears to be mainly influenced by the presence of CMV contracted by the recipient prior to BMT, resulting in an increase in circulating T₈⁺ T cells. This is in accordance with findings in autologous BMT recipients.⁵
CONCLUSIONS

In CMV-seropositive recipients of an allogeneic BMT, an extremely high incidence (92%) of active infection (either reactivation or reinfection) post-BMT is seen as compared with seronegative BMT recipients (20%). This is associated with an abnormal peripheral blood T cell repopulation pattern, characterized by an excess of T8+ T cells. On the average, if these patients receive marrow from a seropositive donor, an earlier rise in CMV antibody titer is seen than after transplantation of seronegative marrow. CMV-seropositive marrow donors induce grades II to IV acute GVHD more often than seronegative ones.

ACKNOWLEDGMENT

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REFERENCES