CYTOMEGALOVIRUS IMMUNITY IN ALLOGENEIC MARROW GRAFTING

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IgM and IgG class antibodies to cytomegalovirus (CMV) late antigen were studied in 58 bone marrow transplant (BMT) recipients and their donors using a sensitive enzyme-linked immunosorbent assay (ELISA) and with standard virological and histopathological techniques. Patients who were CMV-seropositive before BMT had a significantly higher risk for active CMV infection after BMT than seronegative ones (23 of 29 vs. 3 of 26 patients; P<1×10⁻⁶). Transplantation of marrow from CMV-seropositive donors was associated with a higher incidence of active CMV infection after BMT than transplantation of marrow from seronegative donors (17 of 28 vs. 9 of 27 patients). Such transplantations also had a significantly higher incidence of grades II–IV acute graft-versus-host disease (23 of 29 vs. 11 of 27 patients; P=0.007).

Following BMT, the evolution of the IgG class CMV antibody response was influenced by the serological status of the marrow donor. First, a fall in IgG class CMV antibody titers during the first month after BMT was seen less often after transplantation of marrow from seropositive donors than after transplantation of marrow from seronegative donors. Second, recipients of marrow from CMV-seropositive donors who developed active CMV infection had an earlier IgG antibody response than those with seronegative marrow donors. These results suggest that the transfer of memory B and T cells occurs with the graft. Failure to mount a sustained IgM or IgG antibody response upon active CMV infection was associated with a fatal outcome.

Cytomegalovirus (CMV) infection occurs frequently following bone marrow transplantation (BMT). As a consequence of the profound immune deficiency that persists for many months after BMT, CMV infection is associated with a high rate of dissemination (1–3). In most studies, the complement fixation test has been used for the detection of CMV antibodies (2, 4, 5). Recently, enzyme-linked immunosorbent assays (ELISA) for the detection of CMV antibodies have become available. They are more sensitive than complement fixation and they are able to detect minute changes in the antibody response to CMV. This characteristic is especially relevant in severely immunocompromised patients. Using the ELISA technique, we studied a series of 58 BMT recipients and their donors for pretransplant IgM and IgG CMV antibodies, and for the post-transplant evolution of the CMV antibody response. Our results were correlated with the clinical course posttransplantation, with the emphasis on complications such as acute and chronic graft-versus-host disease (GVHD) and nonbacterial pneumonitis.

MATERIALS AND METHODS

Patients. Fifty-eight patients received bone marrow from their HLA-identical, MLR-nonreactive siblings. Fourteen of these patients with severe aplastic anemia were prepared for BMT with cyclophosphamide (50 mg/kg/day ×4) and total lymphoid irradiation (7.5 Gy in one session, n=11, and 20 Gy in 10 sessions, n=3). The 41 patients with acute leukemia in remission and the three patients with chronic granulocytic leukemia in chronic phase were prepared with cyclophosphamide (60 mg/kg/day ×2) and total-body irradiation (8 Gy in one session). In 26 of them, the lungs were shielded so as to receive only 6 Gy.

All patients received a median of 2.2×10⁸/kg nucleated bone marrow cells (range, 1.6–4.6×10⁸). They were nursed in laminar down-flow isolators and received selective antimicrobial modulation of the gut flora (6) from day 15 before BMT until day 60 after BMT. Transfusions of leukocyte-poor platelet concentrates, containing only 10–20 ml plasma, were given to maintain the platelet count above 10×10⁹/L (7). Leukocyte-poor red blood cells were administered to maintain the hemoglobin level above 5.0 mmol/L. Thirty-two patients received plasma infusions, mainly to treat hypoproteinemia. The plasma was obtained from healthy volunteer donors who had not been screened for CMV antibodies. The effect of the possible transfer of CMV antibodies was corrected for. All transfusion products were irradiated with 15 Gy prior to use.

All patients received methotrexate for the prophylaxis of acute GVHD. This complication was diagnosed and staged on the basis of clinical criteria (8). Therapy for acute GVHD consisted of corticosteroids (1–2 mg/kg/day, or 20 mg/kg/12 hr.
for 2 days). Eight patients with grades II–IV acute GVHD also received monoclonal antibody OKT3 (Ortho Pharmaceutical Co., Raritan, NJ) (9). Chronic GVHD was treated with corticosteroids (0.3–1.0 mg/kg/day) and azathioprine (1–2 mg/kg/day). Nonbacterial pneumonitis was defined as all forms of pneumonitis in which bacteria could not be identified as the causative agent. Thus, nonbacterial pneumonitis in this series comprises pneumonitis caused by CMV, Aspergillus fumigatus, or idiopathic interstitial pneumonitis.

Assessment of CMV infection. Serologic studies were performed once or twice prior to BMT, and at least at biweekly intervals after BMT for the first 100 days and at longer intervals thereafter. The titers of IgM and IgG antibodies against CMV late antigen were determined by ELISA, as described elsewhere (10). Sera were tested at serial dilutions ranging from 1:40 to 1:40960. Antibody titers $\geq 40$ were considered positive. Viral cultures of throat swabs, urine, and buccy coats were performed using standard techniques prior to BMT and at one to two weeks intervals during hospitalization after BMT. After discharge, viral cultures were performed only when clinically indicated. In biopsy and autopsy specimens, the characteristic cytopathogenic effects of CMV infection were studied. With respect to CMV infection, the patients were divided into four groups: no infection, primary infection, latent infection, and reactivation/reinfection (for definitions, see Table 1).

Only 2% of our blood donors have detectable IgM class CMV antibodies, so the IgM antibodies detected in the patients were considered not to have been passively transferred. The average titer of IgG class CMV antibodies in donor plasma is 2560. Consequently, IgG antibodies with titers less than 2560, in the period when plasma infusions were given, were not considered to be diagnostically significant. Based upon the serum half-life of IgG antibodies of about 28 days, rises in such antibody titers to values less than 2560 were considered diagnostically significant. Based upon the serum half-life of IgG antibodies of about 28 days, rises in such antibody titers to values less than 2560 were considered diagnostically significant. Of the 55 patients who were seronegative prior to BMT, only three (12%) developed active CMV infection. Two patients formed both IgM and IgG antibodies after BMT.

The above results suggest that the presence of CMV antibodies in the recipient prior to BMT significantly influences the incidence of active CMV infection after BMT (Fisher's exact test, $P<1\times 10^{-6}$, 2-sided). The presence of CMV antibodies prior to BMT did not influence the occurrence of the more severe forms (i.e., grades II–IV) of acute GVHD (Table 3). With respect to acute GVHD, 56 patients were evaluable. Thirty had CMV antibodies prior to BMT, of which 19 (63%) developed grades II–IV acute GVHD. Fifteen (58%) of the 26 seronegative recipients developed grades II–IV acute GVHD. The differences between those two groups were not significant.

Role of pretransplant CMV antibodies in the recipient. Of the 55 patients who were seronegative for CMV infection, 29 had IgG class CMV antibodies prior to BMT; four of these also had IgM class CMV antibodies, suggesting active infection at the time of BMT. Twenty-three patients (79%) had active CMV infection after BMT (Table 2). Nineteen showed significant rises in IgG antibody titers. In 14 of them, IgM antibodies were formed after BMT.

Of the 26 patients who were seronegative prior to BMT, only three (12%) developed active CMV infection. Two patients formed both IgM and IgG antibodies after BMT.

Table 2. Pretransplant CMV antibodies and active CMV infection

<table>
<thead>
<tr>
<th>Pre-BMT serology</th>
<th>Number of patients</th>
<th>Number of patients with active CMV infection after BMT</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Recipient Donor</td>
<td>Total Evaluate</td>
<td></td>
<td></td>
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<tr>
<td>+</td>
<td>+</td>
<td>19</td>
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<td>-</td>
<td>-</td>
<td>16</td>
<td>15</td>
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Table 3. Pretransplant CMV antibodies and grades II-IV acute GVHD

<table>
<thead>
<tr>
<th>Pre-BMT serology</th>
<th>Number of patients</th>
<th>Number of patients with grades II-IV acute GVHD</th>
<th>Percentage</th>
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</thead>
<tbody>
<tr>
<td>Recipient Donor</td>
<td>Total Evaluate</td>
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<tr>
<td>+</td>
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<td>19</td>
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Table 1. Definitions of types of CMV infection

<table>
<thead>
<tr>
<th>Type of CMV infection</th>
<th>Pretransplantation</th>
<th>Posttransplantation</th>
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<tr>
<td></td>
<td>Serology$^{a}$</td>
<td>Cultures: Histology$^{a}$</td>
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<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>and</td>
</tr>
<tr>
<td>Primary</td>
<td>-</td>
<td>and</td>
</tr>
<tr>
<td>Latent</td>
<td>-</td>
<td>and</td>
</tr>
<tr>
<td>Reactivation/reinfection</td>
<td>-</td>
<td>and</td>
</tr>
</tbody>
</table>

$^{a}$ = titer $<$40; + = titer $\geq$40; † = $\geq$4-fold rise in titer; ‡ = no rise in titer.

$^{b}$ = negative; + = positive.

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The CMV antibody status of marrow donors significantly influenced the occurrence of grades II–IV acute GVHD in the recipients (Table 3). Twenty-three of 29 patients (79%) with seropositive marrow donors developed grades II–IV acute GVHD, whereas only 11 of 27 patients (41%) with seronegative marrow donors did so (Fisher’s exact test, $p=0.007$, 2-sided).

**Course of the CMV antibody response.** As can be seen from Table 4, patients who received marrow from seronegative donors more frequently had a fall in their IgG class CMV antibodies during the first five weeks after BMT (90%) than those with a seropositive marrow donor (47%).

The CMV antibody status of the marrow donor seemed to influence the timing of the IgG antibody response in the patients. Of the 26 patients with active CMV infection post BMT, 17 had received marrow from a seropositive donor and nine from a seronegative one (Table 2). Five of those 17 patients did not show a serological response at all after BMT. Those results could be attributed to the early death (i.e., at day 44) of one patient, and to severe immune deficiency related to the occurrence of severe GVHD and/or the administration of monoclonal antibody OKT3 in four, who died at days 50, 61, 81, and 87, respectively. The remaining 12 patients had an IgG response after a median of 64 (range, 32–103) days. The IgG responses of the nine recipients of seronegative marrow occurred significantly later—i.e., at a median of 92 (range, 72–162) days—than those of the recipients with seropositive marrow donors ($P=0.01$; Wilcoxon’s two-sample test).

Inspection of the occurrence times for the IgM response to CMV revealed only moderate differences. Ten of the 16 patients who formed IgM class CMV antibodies had seropositive marrow donors. In those patients, IgM antibodies were formed after a median of 73 (range, 32–177) days. Six patients had seronegative marrow donors; their IgM response occurred at a median of 87 (range, 64–134) days.

**Relationship between antibody response and the clinical outcome of CMV infection.** Sixteen of the 26 patients who developed active CMV infection formed IgM class CMV antibodies, and 21 showed an IgG antibody response. All of the 10 patients who did not form IgM class CMV antibodies died (8 from nonbacterial pneumonitis, 1 from septicemia, and 1 from esophageal hemorrhage). In contrast, only three of 16 patients who formed IgM antibodies died (all from nonbacterial pneumonitis; in two of those patients, CMV could be demonstrated as the causative agent) (Fisher’s exact test, $P=0.0001$, 2-sided).

Failure to mount a significant rise in IgG titers at all (5 patients), or the occurrence of only a transient response (3 patients) also was associated with an adverse outcome. All eight patients died; seven from nonbacterial pneumonitis. In contrast, only six of the 18 patients who showed a sustained IgG antibody response died (4 of nonbacterial pneumonitis; 2 of leukemic relapse) (Fisher’s exact test, $P=0.004$, 2-sided).

**CMV infection and acute GVHD.** Acute GVHD developed in 40 of 55 patients who were evaluable for CMV infection; grade I in seven, grade II in 22, and grades III–IV in 11 patients. The median day of onset of clinical signs of acute GVHD was 26 days (range, 11–55 days) after BMT. Active CMV infection developed in 18 of the 40 patients with acute GVHD: as a primary infection in two, and as reactivation/reinfection in 16. In all instances, CMV infection became manifest after the development of clinical GVHD. The incidence of CMV infection in the patients with grades II–IV GVHD (15 of 33 patients) was similar to that in the patients with grades 0–I GVHD (11 of 22 patients).

**CMV infection and chronic GVHD.** Patients who survived longer than 125 days were considered to be evaluable for chronic GVHD. This complication developed in 10 of 20 patients without CMV infection, in both of two latently infected patients, and in nine of 19 patients with CMV reactivation/reinfection. In eight of the 21 patients, the chronic GVHD was extensive; four had CMV reactivation/reinfection, one had latent CMV infection, and three had no evidence of CMV infection.

**Interaction between acute GVHD, CMV infection, nonbacterial pneumonitis, and mortality.** An overview of the interaction between grades II–IV acute GVHD, active CMV infection, the occurrence of nonbacterial pneumonitis, and mortality is set out in Table 5. The occurrence of nonbacterial pneumonitis was significantly associated with grades II–IV GVHD (Fisher’s exact test, $P=0.004$, 2-sided), but not with CMV infection. Similarly, transplant-associated mortality was significantly associated with grades II–IV GVHD (Fisher’s exact test, $P=0.003$, 2-sided), but not with active CMV infection.

In this patient series, nonbacterial pneumonitis was associated with a high mortality. Of the 26 patients who developed nonbacterial pneumonitis, 21 died of this complication (81%). The mortality was highest among CMV-seropositive patients.

**Table 4.** IgG-class CMV antibody titers during the first five weeks after BMT

<table>
<thead>
<tr>
<th>Pre-BMT serology</th>
<th>Number of patients</th>
<th>Evolution of IgG class CMV antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recipient Donor</td>
<td>Total</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>19</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>12</td>
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</table>

*Four patients were excluded from analysis because they had received plasma infusions during the first five weeks following BMT.

**Table 5.** Interaction between acute GVHD, CMV infection, nonbacterial pneumonitis, and mortality

| Grades II–IV acute GVHD | Active CMV infection | Number of patients | Number of deaths
|-------------------------|----------------------|--------------------|-----------------
|                         | Total                | With NBp*          | Transplant-related |
|                         |                      |                    | Not related to transplant |
| +                       | +                    | 15                 | 9                 | 10             | 1             |
| +                       | −                    | 18                 | 9                 | 12             | 1             |
| −                       | +                    | 11                 | 3                 | 3              | 1             |
| −                       | −                    | 11                 | 2                 | 2              | 3             |
| Not evaluable           |                      | 3                  | 3                 | 3              | 0             |

* NBp* = nonbacterial pneumonitis.

**Transplant-related causes of death were:** nonbacterial pneumonitis (n=21), septicemia (n=3), chronic lung disease (n=2), GVHD (n=2), esophageal hemorrhage (n=1), and venoocclusive disease (n=1). Causes of death not related to transplant were: leukemic relapse (n=4), myocardial infarction (n=1), and cervical dislocation (n=1).
In 55 evaluable recipients of allogeneic marrow grafts, the overall incidence of active CMV infection, as determined with the sensitive ELISA technique for the detection of antibodies to CMV late antigen, was 47%. Primary infections were relatively rare (12% of the recipients who were seronegative prior to BMT), whereas reactivations/reinfections were common (79% of recipients who were seropositive prior to BMT). Other investigators (4, 11), using the less sensitive complement fixation test, reported a 50–60% incidence of CMV infection, with equal frequencies for primary infections and reactivations/reinfections. Our much higher incidence of reactivations/reinfections as compared with that of primary infections may have been caused by the greater sensitivity of the ELISA technique for detecting pretransplant antibody titers (10). Normal donor plasma frequently contains IgG class CMV antibodies—hence plasma infusions given for a prolonged period can severely hamper the interpretation of serological data, especially when sensitive techniques such as ELISA are used. That situation requires the application of strict exclusion criteria to avoid false positive classifications of CMV infection. The determination of IgM class CMV antibodies may be of value in these cases.

CMV can be transmitted by peripheral blood leukocytes (12, 13). Consequently, the donor bone marrow and transfused blood products are potentially important sources of CMV. In our center, all platelet and red cell transfusions are depleted of leukocytes (7). Furthermore, none of the BMT recipients in this series received granulocyte transfusions. This transfusion policy may have prevented transmission of CMV, because only 12% of seronegative patients developed CMV infection, which was fatal for those patients. Even when donors only have latent CMV infection, the marrow graft might serve as a vector for CMV infection. We found a large, but not significant difference in incidence of active CMV infection between patients with seropositive donors (61%) and those with seronegative marrow donors (33%).

The source of CMV in previously seropositive patients who develop active CMV infection is difficult to ascertain. Winston et al. (14), using restriction endonuclease analysis of viral DNA, showed that posttransplant infections can be due to pretransplant CMV isolates. Based on the low number of primary infections in seronegative recipients, and on the similar exposure to blood products in seropositive and seronegative recipients (data not shown), we suggest that reactivation of latent CMV, rather than reinfection with a newly acquired CMV strain, may be responsible for the majority of those infections.

Primary CMV infections may be clinically more important than reactivations or reinfections, as reported in renal transplant recipients (15, 16). However, because of the small number of primarily infected patients in our study, we cannot draw firm conclusions on this point. Transient IgG antibody responses and the inability to form IgM antibodies are associated with an adverse outcome of CMV infection. Other groups (2, 11, 17) have reported an unfavorable outcome of CMV infection in BMT recipients with a negative complement-fixation antibody test. Obviously, the lack of a sustained antibody response in these patients (of which the majority suffered from grades II–IV acute GVHD) indicates a state of extreme immune deficiency. Recently, the administration of i.v. immunoglobulin preparations or CMV hyperimmune globulin has been shown to modify or even prevent clinical symptoms of CMV infection in BMT recipients (18, 19). For that reason, passive immunization of BMT recipients is now increasingly being applied.

Active CMV infection was not associated with increased transplant-associated mortality. In our patient series, transplant-associated mortality was often preceded by grades II–IV acute GVHD. That complication was significantly associated with nonbacterial pneumonitis, which constituted the main cause of death. CMV was isolated from the lungs of only a minority of the patients with nonbacterial pneumonitis.

The earlier rise in the titers of IgG class CMV antibodies in the recipients with seropositive marrow donors, as compared with that of recipients with seronegative marrow donors, is consistent with an anamnestic response of donor-derived memory B and T cells that may be due to the transfer of functional B cells or plasma cells with the bone marrow graft, as has been documented by Wahren et al (20). Transfer of CMV with the graft also may have been contributed to the earlier IgG responses in recipients of seropositive donor marrow. Finally, recipient-derived memory B cells may also play a role; these cells seem to survive chemoradiotherapy for a limited period (20).

The analysis of CMV antibodies using a highly sensitive technique seems to be of clinical importance in the pretransplant workup of bone marrow recipients and donors. Seropositive recipients constitute a high-risk group for active CMV infection. Although the occurrence of such active infections followed the increased immunosuppression used for treating acute GVHD in many patients, immunosuppression with corticosteroids per se did not cause an increased incidence of active CMV infections. Quinnan et al. (21) have shown that cytotoxic T lymphocytes play an important role in recovery from CMV infection in BMT recipients. Therefore, one should avoid overly aggressive immunosuppressive therapy for acute GVHD in these patients. Anti-T-cell monoclonal antibodies in particular, although beneficial in the treatment of acute GVHD, may result in systemic CMV infection (9).

Recently, Lönnqvist et al. (22) have reported an increased incidence of chronic GVHD in patients who previously had developed CMV infection. In contrast, we found an equal incidence of chronic GVHD among patients with and without CMV infection. This discrepancy might be explained by differences between the patient populations, since half of Lönnqvist’s patients were younger than 15 years of age. Increasing recipient age is also associated with chronic GVHD, so the relatively advanced age of our patients might have masked this effect of CMV infection.

Transplantation of marrow from CMV-seropositive donors was associated with an increased risk for grades II–IV acute GVHD. In mice, CMV infection greatly increases the severity of GVHD (23). Because acute GVHD typically develops during the first month after BMT and CMV infection mostly becomes manifest during the second, we suggest that donor-derived memory T cells, reacting either in the recipient with the virus or with minor histocompatibility antigens that crossreact with CMV, become triggered and cause acute GVHD.

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REFERENCES


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