Low Incidence of Acute Graft-Versus-Host Disease by the Administration of Methotrexate and Cyclosporine in Japanese Leukemia Patients After Bone Marrow Transplantation From Human Leukocyte Antigen Compatible Siblings; Possible Role of Genetic Homogeneity

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Japanese patients with leukemia who received bone marrow from human leukocyte antigen (HLA)-compatible siblings had a low incidence of acute graft-versus-host disease (GVHD). Twenty-five (21%) of 120 patients developed moderate (grade II) to severe (grades III to IV) acute GVHD. Severe GVHD was only seen in patients older than 20 years of age. It is also notable that only 2 (5%) of 39 patients who received the combination of methotrexate and cyclosporine (MTX/CSP) for the prevention of GVHD developed grade II acute GVHD, and none developed grades III to IV acute GVHD. Thirteen (30%) of 44 patients receiving MTX alone and 10 (27%) of 37 patients receiving CSP alone developed grades II to IV acute GVHD. Multivariate life-table analysis indicated that the prophylaxis by MTX/CSP was the risk factor for the low incidence of grades II to IV acute GVHD. Compared with the reported incidence of acute GVHD in the patients of the United States, lower incidence of acute GVHD in Japanese BMT patients might be attributable to a lesser degree of genetic diversity in histocompatibility antigens among Japanese.

MATERIALS AND METHODS

From 1976 to December 1987, 120 leukemia patients received BMT from HLA genotypically compatible siblings in Nagoya BMT Group, Nagoya, Japan. The HLA compatibility was determined by HLA-A, B, C, and DR typing of the patients and their families. Mixed lymphocyte culture tests were also examined in 72 cases. Patient age ranged from 3 to 46 years (median, 25 years). Seventy-five patients were male and 45 were female. Type of leukemia and the clinical status when the patients received BMT are listed in Table 1. As preconditioning regimens for BMT, 60 patients received 60 mg/kg cyclophosphamide (CY) on each of two successive days followed by 9.6 to 13.2 Gy total body irradiation (TBI). Among these patients, 8 patients were younger than 9 years of age and 48 patients received BMT in remission of acute leukemia or chronic phase of CML. Sixty patients received 4 to 9 g/m² cytosine arabinoside (CA) in four divided doses in 2 to 3 days, 60 mg/kg CY in 2 days, and then 10 to 12 Gy TBI in four divided doses in 2 days. Among these patients, 11 patients were younger than 9 years of age and 47 patients received BMT in remission of acute leukemia or chronic phase of CML.

Three immunosuppressive regimens were used for the prevention of GVHD. From 1976 to 1985, 44 patients received MTX alone at a dose of 15 mg/m² intravenously on day 1, and 10 mg/m² on days 3, 7, and 11, and once weekly thereafter until day 102 according to the protocol described by Thomas et al.1 From 1983 to 1987, 37 patients were given CSP alone. CSP was begun on day 1 before the transplantation and given by 2-hour drip infusion at a dose of 1.5 to 2.5 mg/kg every 12 hours. After the patients had recovered from gastrointestinal damage, CSP was given orally in 1.5 to 2 times increased dose. Thereafter, the dose was decreased in three fourths with the interval of 3 to 8 weeks and discontinued in 6 months in the patients without both acute and chronic GVHD, and in 12 months...
LOW INCIDENCE OF ACUTE GVHD

The cumulative incidence of acute GVHD was analyzed by Kaplan-Meier estimate and its statistical significance was tested by generalized Wilcoxon test.

Results

Incidence of acute GVHD. Table 1 shows the number of patients who had different grades of acute GVHD in relation with patient’s age, prophylactic regimen of GVHD, type of leukemia, and clinical condition of transplantation. The number of cases that had died within 90 days after transplantation are given in parentheses.

The overall incidence of acute GVHD in 120 leukemia patients was 51%, and grade II to IV acute GVHD occurred in 21% of patients. Only 16% (3 of 19) patients under 9 years of age had acute GVHD, and no grades III to IV acute GVHD were observed.

All patients received oral unabsorbed antibiotics for sterilization of gut, and were isolated in laminar air flow rooms with the decontamination procedures.

The severity of acute GVHD was graded from grade 0 to IV according to the criteria described by Thomas et al. (1985-1987, 39 patients were given both MTX and CSP (MTX/CSP) according to the Seattle protocol with minor modifications. A dose of 10 mg/m² MTX was administered intravenously on day 1, and 6 mg/m² on days 3, 7, and 11. CSP was begun on day 1 before the transplantation at a dose of 1.5 mg/kg by 8-hour infusion (2-hour infusion in 10 cases) every 12 hours, continued with the same dose at least for 4 weeks, then given orally and decreased its doses as described in CSP alone prophylaxis. The trough and maximum levels of CSP in serum were measured at least once a week. When maximum serum level of CSP was over 800 ng/dL or serum creatinine level exceeded 1.8 mg/dL, CSP dose was decreased by two thirds. More than grade II acute GVHD was treated with prednisolone at a dose of 0.5 to 1 mg/kg/d at least for 2 weeks.

Unless the patients with acute GVHD had chronic GVHD. From 1985 to 1987, 39 patients were given both MTX and CSP (MTX/CSP) according to the Seattle protocol with minor modifications. A dose of 10 mg/m² MTX was administered intravenously on day 1, and 6 mg/m² on days 3, 7, and 11. CSP was begun on day 1 before the transplantation at a dose of 1.5 mg/kg by 8-hour infusion (2-hour infusion in 10 cases) every 12 hours, continued with the same dose at least for 4 weeks, then given orally and decreased its doses as described in CSP alone prophylaxis. The trough and maximum levels of CSP in serum were measured at least once a week. When maximum serum level of CSP was over 800 ng/dL or serum creatinine level exceeded 1.8 mg/dL, CSP dose was decreased by two thirds. More than grade II acute GVHD was treated with prednisolone at a dose of 0.5 to 1 mg/kg/d at least for 2 weeks.

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grade II GVHD was observed in only 5% (2 of 39) of patients, and no severe GVHD (grades III to IV) was noted. The number of patients with acute GVHD younger than 9 years of age or older than 10 years of age treated with different prophylactic regimens, that of different types of leukemias or risk of transplantation were also shown in Table 1.

The probability of the cumulative incidence of grades I to IV acute GVHD and that of grade II to IV acute GVHD in different groups of prophylactic regimens were analyzed by Kaplan-Meier life-table analysis (Fig 1). The incidence of grades II to IV GVHD by MTX/CSP was significantly lower than that of MTX alone (P = .01) or CSP alone (P = .01) by generalized Wilcoxon test.

To analyze the risk factors for the occurrence of grades I to IV acute GVHD or grades II to IV acute GVHD, Cox's multivariate life-table analysis was performed for patient age, GVHD prophylactic regimen, type of leukemia, and the clinical status at BMT (Table 2). Age was figured as a continuous variable.

Significant risk factors for the incidence of grades I to IV were found to be the age (P = .01). Multivariate analysis in stepwise fashion showed that the age was a significant variable, and no additional variables met the .05 significant level.

The factors for the occurrence of grades II to IV acute GVHD were also analyzed by the multivariate analysis, and it was clearly shown that the combination of MTX and CSP had significantly better prophylactic effect on acute GVHD than MTX alone (P = .02) or CSP alone (P = .03). Stepwise analysis confirmed that the MTX/CSP regimen was more effective for the prophylaxis of grades II to IV acute GVHD than other regimens (P = .03).

Incidence of chronic GVHD. The actual incidence of chronic GVHD in 104 patients who survived for more than 3 months after BMT were analyzed. Twenty-two percent (4 of 18) of patients younger than 9 years and 47% (40 of 86) of patients 10 years or older showed chronic GVHD. When the patients 10 years or older were analyzed in relation to the prophylactic regimens, the MTX group had 50% (15 of 30 patients) of chronic GVHD, CSP group 45% (15 of 33), and MTX/CSP group 43% (10 of 23).

DISCUSSION

It is noteworthy that the incidence of acute GVHD seems to be lower in Japan as seen in our study than that reported in the United States. Because the regimens for the prophylaxis of acute GVHD, patient age, and diseases are similar to those reported from the Seattle BMT team, it seems justified to compare the incidence of acute GVHD in HLA-compati-

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Table 2. Multivariable Analysis of Acute GVHD by Cox's Proportional Hazard Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lower Risk</th>
<th>Higher Risk</th>
<th>Hazard Ratio</th>
<th>P Value</th>
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<tr>
<td>Grade I—IV GVHD*</td>
<td></td>
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<td>Age</td>
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<td>MTX/CSP</td>
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<td>GVHD prophylaxis</td>
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<tr>
<td>Grade II—IV GVHD†</td>
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<td>GVHD prophylaxis</td>
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<td>Status of disease</td>
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</table>

Hazard ratio of higher risk group compared with lower risk group. Age was figured as a continuous variable.

*Beta value was 0.03.
†Beta value was 0.01.
ble BMT between their data and our own. The combination of MTX and CSP resulted in 5% of grades II to IV acute GVHD in Nagoya versus 33% in Seattle.4 CSP alone gave 31% of grades II to IV acute GVHD in Nagoya versus 54% in Seattle.5 MTX alone gave 27% of grades II to IV acute GVHD in Nagoya versus 56% in Seattle.6 It is notable that the patients younger than 19 years of age and the patients, regardless of age, who received either CSP or MTX/CSP showed no severe grades III to IV acute GVHD in Nagoya. It is unlikely that the difference is due to other factors, such as the diagnostic criteria of acute GVHD, patient's status at BMT, preconditioning methods, GVHD prophylaxis regimens, or gut-sterilization methods. Other reports from the United States, Europe, and the International Bone Marrow Registry also indicate that the prophylaxis of GVHD with MTX or CSP has resulted in a 37% to 45% incidence of grades II to IV GVHD.8,9 Low incidence of acute GVHD among Japanese was also observed in patients with severe aplastic anemia (AA) who received marrow from HLA compatible siblings. AA patients with the prophylaxis of GVHD by MTX or CSP developed grades II to IV acute GVHD in only 13% in the Nagoya BMT group15 and 34% in Seattle.16

Thus, even considering the difficulty of comparison of the clinical results from different centers, there still seems to remain the difference in the incidence of acute GVHD in HLA-compatible BMT between Nagoya and Seattle. This difference may be attributable to the degree of genetic diversities among Japanese in Japan and whites in the United States. Takahashi and Juji17 calculated the chance for patients to find HLA-compatible unrelated donors from the data of the International HLA Workshop. The estimated size of donor pool in which 80% of patients can find at least one HLA-A, B, and DR compatible donor was 50,000 in Japanese, whereas it was almost 1,000,000 in whites in the United States. Human minor histocompatibility antigens that relate to acute GVHD or rejection in HLA-compatible BMT are now under investigation.18 The data on the diversity of the HL A system may provide indirect evidence for the differences in the genetic background between these two populations, ie, a possible diversity of the polymorphism of the minor histocompatibility antigen system. Although the data on HLA-DP typing were not available, the probability of recombination between HLA-DQ and HLA-DP within families may also contribute to the difference in the occurrence of acute GVHD in HLA-A, B, C, and D/DR compatible related BMT, as suggested by Pawelec et al.19

One other, although probably a minor, reason why there is such a difference may be the isolation of all our patients in laminar airflow rooms. A beneficial effect of a protective environment for GVHD has already been reported from Seattle in the patients with AA.20

Although the incidence of acute GVHD was reduced by the prophylaxis with MTX/CSP, there still developed substantial chronic GVHD in these patients, and the incidence of chronic GVHD between three prophylactic regimens may not differ. An increased number of patients was required for the statistical analysis by multivariate life-table analysis. Similar data were obtained in leukemia and AA patients in Seattle.4,16 These results may suggest that the mechanism of chronic GVHD differs from that of acute GVHD, which involves the recognition of minor histocompatibility antigens.

ACKNOWLEDGMENT

The authors thank the staff members of the hematology and pediatric services of Nagoya BMT Group and Aichi Red Cross Blood Center.

REFERENCES

15. Kojima S, Matsuyama K, Kodera Y, Hirabayashi N, Mori-
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