Induction of Cutaneous Graft-Versus-Host Disease by Administration of Cyclosporine to Patients Undergoing Autologous Bone Marrow Transplantation for Acute Myeloid Leukemia

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Cutaneous graft-versus-host disease (GVHD) has been reported after administration of cyclosporine (CSP) after autologous bone marrow transplantation (ABMT) with unpurged marrow in patients with lymphoma. To determine whether GVHD can be induced after ABMT with chemopurged marrow in acute myeloid leukemia (AML), we administered intravenous CSP for 28 days (beginning on the day of ABMT) to 19 patients with AML (12 in first remission [CR1], six in CR2, and one in CR3) who received busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg) and ABMT with 4-hydroperoxycyclophosphamide (4HC)-treated marrow. In this dose-escalation trial, CSP daily doses were 1 mg/kg in seven patients, 2.5 mg/kg in eight patients, or 3.75 mg/kg in four patients. Skin biopsies were obtained weekly after ABMT or on appearance of rash and were graded for GVH changes. Overall, 15 of 19 patients (79%) had cutaneous histopathologic grade 2 GVHD at a median of 33 days (range, 14 to 49) after ABMT; in 10, cutaneous manifestations were present at time of positive biopsy. The frequency, time to onset, and duration of GVHD were similar among the three CSP dosage groups. No patients had hepatic or gastrointestinal dysfunction attributable to GVHD or required specific therapy for GVHD. Positive biopsies for GVHD were seen in seven of eight patients who received full-course, full-dose CSP and 8 of 11 patients who had CSP discontinued or dosage reduced because of renal insufficiency. Three patients (one with positive biopsy) died with ABMT-related complications. Seven patients (four CR1, three CR2) relapsed with AML at a median of 411 days (range, 178 to 549) after ABMT; six of seven had positive biopsies for cutaneous GVHD. Nine patients (seven CR1, one CR2, and one CR3) are alive without relapse at a median of 501+ days (range, 252+ to 811+) after ABMT; eight of nine had cutaneous GVHD. Short-course CSP can induce autologous GVHD in recipients of chemopurged marrow autografts for AML, but randomized prospective trials are needed to determine whether this immunologic reaction is associated with alterations in leukemic relapse rate and disease-free survival after ABMT in AML.

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autologous GVHD in groups of patients administered increasing CSP doses after ABMT.

MATERIALS AND METHODS

Patients. Nineteen consecutive patients (seven male, 12 female) referred to The Johns Hopkins Oncology Center with AML in remission were enrolled in this study (Table 1). Their median age was 34 years (range, 18 to 50). In all patients the diagnosis and histopathologic classification of AML was confirmed by review of diagnostic BM aspirates, and remission status was confirmed by review of BM aspirates immediately before marrow collection. The French-American-British (FAB) histopathologic types were: M1, seven patients; M2, four patients; M3, two patients; M4, five patients; and M7, one patient. At the time of ABMT, 12 patients were in first complete remission (CR1), six patients were in CR2, and one patient was in CR3. The median duration of CR1 was 10 months (range, 9 to 22) for those patients receiving ABMT in CR2 or CR3, and the median duration of current CR (1, 2, or 3) at the time of autologous marrow collection was 4.2 months (range, 2.2 to 10.2). No patients had extramedullary leukemia at the time of ABMT. All treatment protocols were approved by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions, and all patients gave written informed consent to participate in this study and in the ABMT procedure.

ABMT procedure. Marrow was collected from patients during their current remission; a median of 4.1 × 10^8 nucleated marrow cells/kg (range, 2.9 to 6.7 × 10^9) was obtained. The erthrocyte-free marrow mononuclear cell fraction was obtained by density-gradient centrifugation on Ficoll-diatrizoate, incubated with 60 ng/mL of 4HC, washed, and cryopreserved in a liquid nitrogen freezer, as previously reported.15 Before ABMT, patients received oral busulfan (1.0 mg/kg/dose) every 6 hours for 16 doses and then intravenous cyclophosphamide (50 mg/kg/dose) daily for 4 days; during busulfan administration, patients received seizure prophylaxis with phenytoin.13 Forty-eight hours after the last dose of cyclophosphamide, the 4HC-treated autologous marrow suspensions were thawed immediately at the bedside and infused through a central venous catheter at a rate of 10 to 15 mL/min. Patients were nursed in single rooms equipped with high-efficiency particulate air filtration systems. All blood products were irradiated to at least 1,500 rad before administration to patients.

CSP administration. Patients received intravenous CSP as two 4-hour infusions daily for up to 28 days, beginning on the day of autologous marrow infusion. The starting daily dose of CSP was 1.0 mg/kg in the first group of patients and was increased in subsequent groups to 2.5 mg/kg/d and then to a maximum of 3.75 mg/kg/d, based on satisfactory engraftment and acceptable toxicity in the previous dosage group(s). CSP dosage was based on the lesser of the patient's ideal or actual body weight and was reduced by 25% if serum creatinine levels exceeded 2.2 mg/dL.

Evaluation for GVHD. Patients were examined daily for presence and extent of rashes, abdominal pain, hepatomegaly and/or hepatic tenderness, jaundice, and other potential clinical indicators of GVHD. Stool volume, blood chemistries (hepatic transaminases, alkaline phosphatase, bilirubin, creatinine, urea nitrogen), and complete peripheral blood cell counts were determined daily. In the absence of any cutaneous manifestations, 4-mm diameter punch biopsies of skin were obtained weekly beginning 7 days after ABMT and continuing through 49 days posttransplant; biopsies were also obtained at the time of appearance of any rash. Fixed, hematoxylin-stained sections of skin biopsies were independently examined under light microscopy by a dermatopathologist who was blinded as to the clinical status of the patients and were graded for severity.

### Table 1. Induction of Autologous GVHD by CSA in Patients With AML

| UPN  | Age/CR Status | CR Class | Duration of CSP Administration (d) | Daily Dose of CSP (mg/kg) | Duration of CSP Reduction of CSP (d) | Positive Skin Biopsy for GVHD | Interval Between ABMT and Positive Biopsy (d) | Positive ALC >0.2 x 10^9/L (d) | Time to Attain Positive ALC >0.2 x 10^9/L (d) | Outcome |
|------|---------------|----------|-----------------------------------|--------------------------|----------------------------------|---------------------------|-----------------------------|-----------------------------|--------------------------------|----------------|--------|
| 1163 | 41/F          | CR2      | 22/4.7*                           | 1.0                      | 28                               | Yes                       | No                          | —                           | 38/38                        | Died, 43 d (hepatic veno-occlusive disease) |
| 1175 | 19/M          | CR1      | 2.7*                              | 1.0                      | 28                               | Yes                       | Yes                        | 42                          | 41/175                      | CR1, 811 * d                    |
| 1204 | 23/M          | CR2      | 16/7.4*                           | 1.0                      | 18                               | No                        | Yes                        | 14                          | 27/39                       | CR2, 746 * d                    |
| 1256 | 33/F          | CR1      | 4.2*                              | 1.0                      | 28                               | No                        | Yes                        | 45                          | 34/ > 180                    | Died, 438 d (sepsis, marrow hypoplasia) |
| 1243 | 47/M          | CR3      | 12/8/2.7*                         | 1.0                      | 28                               | No                        | Yes                        | 46                          | 17/34                       | CR3, 648 * d                    |
| 1247 | 44/F          | CR1      | 2.3*                              | 1.0                      | 28                               | Yes                       | Yes                        | 26                          | 19/24                       | Relapse, 549 d                  |
| 1287 | 48/F          | CR1      | 2.2*                              | 1.0                      | 28                               | No                        | Yes                        | 14                          | 19/22                       | Relapse, 420 d                  |
| 1289 | 31/F          | CR2      | 10/2.4*                           | 2.5                      | 28                               | No                        | No                         | —                           | 38/69                       | Relapse, 178 d                  |
| 1298 | 21/F          | CR2      | 10/4.1*                           | 2.5                      | 28                               | Yes                       | Yes                        | 27                          | 41/66                       | Relapse, 459 d                  |
| 1300 | 50/F          | CR1      | 4.2*                              | 2.5                      | 28                               | No                        | Yes                        | 23                          | 14/15                       | CR1, 511 * d                    |
| 1308 | 20/M          | CR1      | 3.5*                              | 2.5                      | 28                               | Yes                       | Yes                        | 45                          | 39/187                      | CR1, 501 * d                    |
| 1319 | 34/F          | CR2      | 9/3.5*                            | 2.5                      | 28                               | Yes                       | Yes                        | 49                          | 22/24                       | Relapse, 276 d                  |
| 1327 | 20/F          | CR2      | 7/10*                             | 2.5                      | 14                               | No                        | No                         | —                           | 14/58                       | Died, 165 d (CMV pneumonitis)   |

Abbreviation: ALC, absolute lymphocyte count.
histopathologic alterations of AGVHD according to published criteria.14

Statistical evaluation. The χ² test with Yates' correction factor and the Wilcoxon rank-sum test were used to compare the incidence and the time of onset of GVHD, respectively, among the three CSP dosage groups.15 Survival analysis was performed according to the product-limit estimates of Kaplan and Meier,16 using statistical software packages developed by the Biostatistics and Information Systems Division of The Johns Hopkins Oncology Center.

RESULTS

CSP dosage and duration of administration. In this dose-escalation trial, seven patients received 1.0 mg/kg/d of CSP, eight patients received 2.5 mg/kg/d, and four patients received 3.75 mg/kg/d (Table 1). CSP was administered at the prescribed dose for the entire 28-day course in eight patients (three at 1.0, three at 2.5, and two at 3.75 mg/kg/d); seven developed histopathologic changes of GVHD on skin biopsy. Eight patients (three at 1.0, three at 2.5, and two at 3.75 mg/kg/d) required reduction of CSP dosage by 25% because of elevated creatinine levels after ABMT; six had positive biopsies for GVH changes. In three patients (one at 1.0 and two at 2.5 mg/kg/d), CSP was discontinued after 14, 18, and 21 days, respectively, because of severe renal insufficiency associated with development of hepatic veno-occlusive disease; two patients had histopathologic GVH changes on skin biopsies. The difference between the proportion of patients with biopsy-documented GVH alterations was not significantly different in those receiving full-course, full-dose CSP (seven of eight patients) and those in whom CSP was decreased or discontinued early (8 of 11 patients) (χ² test with Yates' correction factor).

Appearance of GVHD. Fifteen patients (79%) developed positive skin biopsies for histopathologic grade 2 acute GVHD; six of seven patients administered 1.0 mg/kg of CSP, six of eight administered 2.5 mg/kg, and three of four patients administered 3.75 mg/kg (Table 1). Ten of the 15 patients had cutaneous manifestations at the time of positive biopsies, with macular and papular rashes involving less than 25% of the body surface; none had erythroderma or bullous lesions. In five patients, histopathologic grade 2 acute GVH changes were detected on routine biopsies without any evidence of skin rashes or other manifestations of GVHD. The median time to appearance of positive skin biopsies for GVH was 32 days after ABMT (range, 14 to 49) and was similar among the three CSP dosage groups (P = NS; Wilcoxon rank-sum test). Lymphocyte recovery was not essential for the development of positive skin biopsies; in 10 patients, peripheral blood lymphocyte counts had attained 0.2 × 10⁹/L at a median of 9 days (range, 1 to 29) before positive biopsies were observed, but in five patients positive skin biopsies were seen at a median of 13 days (range, 1 to 21) before peripheral blood lymphocytes exceeded 0.2 × 10⁹/L (Table 1). The development of autologous GVHD was apparently not dependent on neutrophil recovery; in 11 of the 15 patients, positive skin biopsies were observed at a median of 25 days (range, 3 to 180*) before the absolute neutrophil count (ANC) exceeded 0.5 × 10⁹/L. The cutaneous manifestations of AGVHD resolved spontaneously within 5 to 10 days after onset and did not require therapy such as corticosteroids. No patients had gastrointestinal or hepatic dysfunction that was attributable to GVHD.

Outcome. Three patients died in remission with ABMT-related complications. Two patients (unique patient numbers [UPNs] 1163 and 1327), neither of whom developed GVHD, died of hepatic veno-occlusive disease 43 days after ABMT and with cytomegalovirus (CMV) interstitial pneumonitis at 165 days after ABMT, respectively. One patient (UPN 1235), who had GVHD on skin biopsy, died with persistent marrow hypoplasia and presumed bacterial sepsis 438 days after ABMT. Seven patients (four receiving transplants in CR1 and three in CR2) had relapses of AML at a median of 411 days (range, 178 to 549) after ABMT; two had received 1.0 mg/kg of CSP and five had received 2.5 mg/kg. Six of the seven patients who relapsed had positive skin biopsies for GVHD. The actuarial relapse rate in this series is 57% (95% confidence interval, 26% to 88%) (Fig 1). Nine patients (eight in CR1, one in CR2, and one in CR3) are alive without leukemic relapse at a median of 501* days (range, 252* to 811*) after ABMT; eight had evidence of GVHD in the posttransplant period. The actuarial DFS in this group is 33% (95% confidence interval, 7% to 60%) (Fig 2).

DISCUSSION

Approaches to improving the DFS after ABMT for AML include better ex vivo treatment of the marrow to eliminate occult tumor cells and more aggressive in vivo cytoreductive preparative regimens to eradicate residual AML in the patient. A third strategy—post-ABMT immunologic manipulation to enhance antileukemic effects mediated by host cellular immunity—is supported by the observations of lower relapse rates in patients who develop clinically apparent GVHD after allogeneic BMT for AML.4,5 However, both acute and chronic GVHD remain major causes of morbidity and mortality in allogeneic BMT, and no proven techniques exist for minimization of GVHD without decreasing the graft-versus-leukemia effect in human marrow allograft recipients.  

![Fig 1. Probability of remaining in remission for 19 patients with AML who received post-ABMT CSP for induction of autologous GVHD.](https://example.com/image.png)
It has been suggested that immunologic graft-versus-tumor effects may be operative against neoplastic cells that bear Ia (class II) antigens on the cell surface.9,17,18 The autologous GVH reaction that follows administration of short-course CSP is associated with the development of anti-Ia cytotoxic T lymphocytes in rodents and, perhaps, in clinical ABMT settings, and can greatly decrease the number of Ia-positive tumor cells in a rat myeloma model.9 The development of autologous GVHD in patients with lymphoma or leukemia who receive CSP after ABMT is interesting and encouraging, but it is not known whether this disrupted immunoregulatory process is associated with an autograft-versus-tumor effect as well. Although eight of the nine patients in this series who are alive without recurrent AML had histopathologic evidence of cutaneous GVHD, six of the seven patients who relapsed after ABMT also had positive skin biopsies. Talbot et al could not demonstrate a consistent antileukemic effect in patients with AML who received oral or intravenous CSP after ABMT with unpurged marrow; in that series, two of the three patients who developed positive skin biopsies relapsed with AML shortly after ABMT.11

There was substantial variation in the onset of autologous GVHD in these patients; in some, the syndrome occurred during CSP infusion, while in others it did not develop until 2 to 3 weeks after cessation of CSP. In rodents, there is also a range (14 to 28 days) over which autologous GVHD develops after CSP discontinuation, suggesting different rates of clonal expansion of autoreactive T lymphocytes.8,10 In the patients we studied, lymphocyte recovery in the peripheral blood did not consistently correlate with development of autologous GVHD; one-third of the patients who developed the syndrome were still profoundly lymphopenic (total peripheral blood lymphocytes <0.2 × 10^9/L) at the time of positive biopsies. Analyses of immunophenotypes of lymphocytes in the skin biopsies and in peripheral blood samples at selected times after ABMT were not performed in this study, but would be potentially instructive to elucidate the immunoregulatory imbalances induced by short-course CSP in patients with autologous GVHD. In this pilot study, we did not determine whether the lymphocytes in patients with CSP-induced autologous GVHD were capable of lysing allogeneic or pre-BMT autologous lymphocytes. Additionally, because these patients were referred in remission to our center, samples of their AML cells against which to measure cytotoxic responses of post-ABMT lymphocytes were not available.

Our observations suggest several practical points on CSP induction of autologous GVHD in recipients of ABMT with chemopurged marrow for AML. First, a daily dosage of as little as 1.0 mg/kg/d of CSP is sufficient to induce the syndrome. Higher doses of CSP did not induce autologous GVHD more rapidly or with any greater frequency or severity in these patients, somewhat different from observations in rodents, in which a correlation could be made between increasing CSP dosage and both frequency and intensity of autologous GVHD.7,8 The frequency with which autologous GVHD occurred in our patients treated with intravenous CSP was greater (15 of 19) than that reported in patients with AML administered unpurged autografts and either intravenous CSP (one of four patients) or oral CSP (two of five patients) at dosages of 1.0 mg/kg/d for 28 days.11 Second, as fewer than half of the patients received full courses of prescribed doses of CSP, it also appears that a duration of administration of at least 14 to 21 days is sufficient. Third, ex vivo chemopurging of the marrow cell suspensions apparently did not impede the development of autologous GVHD. Although incubation with 4HC has been shown to alter the lymphocyte content and subpopulations in autologous marrow samples,20 autologous GVHD can be induced by short-course CSP in irradiated rodents administered syngeneic BMT with 4HC-treated marrow.21 Furthermore, syngeneic GVHD can be induced in rats transplanted with lymphocyte-depleted marrow.22 The present studies corroborate these preclinical observations and indicate that sufficient lymphoid progenitors are present in the chemopurged autografts to allow the GVH effect to occur.

Most other preclinical and clinical studies of autologous GVHD have used pretransplant conditioning with regimens that contain total body irradiation, and it has been suggested that thymic irradiation plays an important role in the development of this syndrome.19 Therefore, it is of interest that almost 80% of the patients in this study (none of whom had received previous radiotherapy) developed autologous GVH-like reactions after a busulfan and cyclophosphamide conditioning regimen. Busulfan has negligible immunosuppressive activity and cannot be used as single-agent conditioning for allogeneic BMT;24,24 lymphoid as well as hematopoietic repopulation by donor cells occurs in murine recipients of high-dose busulfan and cyclophosphamide conditioning regimen. Busulfan has negligible immunosuppressive activity and cannot be used as single-agent conditioning for allogeneic BMT;24,24 lymphoid as well as hematopoietic repopulation by donor cells occurs in murine recipients of high-dose busulfan and cyclophosphamide conditioning regimen. Busulfan has negligible immunosuppressive activity and cannot be used as single-agent conditioning for allogeneic BMT;24,24 lymphoid as well as hematopoietic repopulation by donor cells occurs in murine recipients of high-dose busulfan and cyclophosphamide conditioning regimen. Busulfan has negligible immunosuppressive activity and cannot be used as single-agent conditioning for allogeneic BMT;24,24 lymphoid as well as hematopoietic repopulation by donor cells occurs in murine recipients of high-dose busulfan and cyclophosphamide conditioning regimen.
cated to determine whether CSP-induced autologous GVHD is associated with objective improvement in DFS and decreases in the relapse rate of patients undergoing ABMT with 4HC-treated marrow for AML in remission. Additionally, strategies to increase cellular expression of Ia antigen by measures such as administration of interferon may enhance the antitumor effects of autologous GVHD induced by CSP administration.

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