Marrow Transplantation From HLA-Identical Siblings for Treatment of Aplastic Anemia: Is Exposure to Marrow Donor Blood Products 24 Hours Before High-Dose Cyclophosphamide Needed for Successful Engraftment?

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The present study in patients with aplastic anemia was undertaken to determine whether exposure of recipients to donor blood products 24 hr before preparation with cyclophosphamide (1) enhanced the rate of sustained engraftment of marrow from HLA-identical siblings as suggested by animal experiments, (2) increased the rejection rate, in particular in transfused patients who may already have been exposed to donor antigens by blood products, or (3) was of no relevance to the outcome of transplantation of marrow from HLA-identical siblings.

Transplantation of marrow from HLA-identical siblings is effective treatment for patients with aplastic anemia.1-7 Based on studies in rats,8 mice,9 dogs,10 rhesus monkeys,11 and patients with leukemia,12 preparation with cyclophosphamide (CY), 50 mg/kg intravenously on each of 4 successive days, has been widely used to suppress the patients' immune responses to non-HLA antigens of their marrow donors. Studies in mice showed that administration of donor antigen 24 hr before CY enhanced survival of hemopoietic cells from the same donor.9 Enhancement was thought to be due to sensitization and subsequent rapid replication of recipient lymphocytes, making the dividing cells particularly sensitive to destruction by the alkylating agent CY. Elimination of antigen-responsive cells would make the recipient "tolerant" to the sensitizing antigens. The data in mice were supported by those in dogs given marrow grafts from DLA-nondifferent unrelated donors.13 For this reason, patients were often given an infusion of 1–4 U of buffy coat cells or platelet-rich plasma from the marrow donor 24 hr before the first dose of CY.

One-hundred fifty-five patients were studied, of whom 78 received blood products from the marrow donor 24 hr before cyclophosphamide and 77 did not. A binary logistic regression analysis was applied to the data, simultaneously considering five previously known risk factors for rejection. Results showed that preceding transfusion of donor blood products had neither a significant beneficial nor detrimental effect on the incidence of sustained engraftment.

Most patients with aplastic anemia have been multiply transfused before marrow transplantation, and many are likely to be sensitized to HLA and non-HLA antigens in the transfused blood. Sensitizing antigens might include those for which the HLA-identical sibling marrow donor and the recipient differ, thus establishing a risk for marrow graft rejection.14 CY, like most immunosuppressive agents, is less effective in suppressing secondary than primary immune responses. It is conceivable, therefore, that in cases where immunity to donor antigens already exists, reexposure to the same antigens by infusion of donor blood products 24 hr before CY might heighten the risk of marrow graft rejection.

We have treated 175 patients with aplastic anemia with high-dose CY and marrow grafts from HLA-identical siblings. Approximately one-half of these were given blood products from their marrow donors immediately before CY and the remainder were not. This gave us an opportunity to examine whether exposure to donor antigen 24 hr before CY affected the graft rejection rate. To this purpose, a binary logistic regression analysis was applied to the data, simultaneously considering five previously known risk factors for graft rejection:14,15 (1) low numbers of marrow cells used for grafting; (2) a positive relative response in mixed leukocyte culture indicating transfusion-induced sensitization; (3) lack of viable donor buffy coat cells in addition to the marrow; (4) marrow from male donors; and (5) multiple preceding blood product transfusions.

MATERIALS AND METHODS

Between October 1970 and March 1981, 175 consecutive patients with severe aplastic anemia were treated with CY and marrow grafts from HLA-identical family members.1 All but 39 had multiple...
prior transfusions of red blood cells, platelets, or both. Details on the selection of patients and donors for transplantation, the CY regimen, the transplant procedure, and each patient's course before and after have been described. One-hundred sixty-five patients were treated with methotrexate for 100 days after grafting to obviate graft-versus-host disease, and 10 were given cyclosporine for 180 days. Sustained marrow engraftment and graft rejection were assessed by daily monitoring of peripheral blood counts, weekly determinations of marrow cellularity, and frequent monitoring of genetic markers, including peripheral blood and marrow cytogenetics, red blood cell antigen and enzyme phenotypes, and immunoglobulin allotypes.

Lymphocytes from each donor-recipient pair were examined in a mixed leukocyte culture. The response of patient cells to sibling cells was compared with their response to pooled, freshly drawn cells from two unrelated donors or to cryopreserved cells from two unrelated donors and then expressed as a percentage—the relative response. The average relative response in a group of 212 healthy HLA-identical siblings was 0.0% with 2 standard deviations, equating 2.6%. Hence, a positive relative response was defined as >2.6% and a negative one as <2.6%.

Fifty patients were given donor peripheral blood buffy coat cells on days 1–5 after marrow infusion to overcome transfusion-induced sensitization and reduce the incidence of graft rejection.

Seven of the 175 patients died between days 1 and 13 (day 0 is the day of marrow grafting), too early to evaluate success or failure of the graft, and were not considered in the analysis. In addition, the relative response could not be evaluated in 13 patients, most of whom were tested before a workshop-approved standardized mixed leukocyte culture technique was introduced. These 13 were also excluded from analysis.

Seventy-eight of the 155 remaining patients were given 1–4 U of buffy coat cells or platelet-rich plasma, obtained by standard blood-banking techniques from the marrow donor 24 hr before CY administration, and 77 were not. Between 1976 and 1979, a prospective randomized trial was carried out in patients not refractory to the transplant procedure, and each patient's course before and after, while simultaneously considering the five other factors known to be associated with rejection. To take adequate account of possible changes in patients or patient care characteristics between 1970 and 1981, the year of transplant was also entered as a covariate. With a number of factors possibly predictive of marrow graft rejection, a regression analysis was used to separate factors that predicted graft rejection from those that could be explained by other patient or treatment characteristics. The binary logistic regression method permitted the probability, p(z), of graft rejection to depend simultaneously on a vector of numerically coded factors, z(1,z2, . . ., zn) via p(z) = exp(zb)[1 + exp(zb)], where "exp" denotes exponentiation. The data were then utilized to estimate the vector, b, of coefficients and their standard errors.

The marrow transplant protocols and consent forms were approved by the Human Subjects Review Committee of the Fred Hutchinson Cancer Research Center.

RESULTS

Table 1 shows graft rejection rates according to whether donor blood products in the form of buffy coat cells or platelets were administered 24 hr before CY. Rejection rates are given in eight categories defined by marrow cell dose, the result of the relative response, and donor sex. The upper portion of Table 1 shows the results on all 155 patients. The graft rejection rate was 13/78 (17%) among patients without and 23/77 (30%) among patients with prior donor antigen infusion. Within categories defined by the prognostic factors, however, there was little indication for a dependence of graft rejection on whether prior donor blood products were infused. The next two sections of Table 1 exclude

<table>
<thead>
<tr>
<th>Relative response: Donor sex:</th>
<th>Negative</th>
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<tr>
<td></td>
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untransfused patients and show graft rejection rates separately, according to whether the patients were given viable donor buffy coat cells in addition to the marrow after transplantation or not. Among 79 patients who did not receive postgrafting buffy coat cells, the graft rejection rates were 6/20 (30%) and 21/59 (36%) for those without and with prior donor antigen exposure, respectively. Among 42 patients with postgrafting buffy coat cells, the graft rejection rates were 4/28 (14%) and 2/14 (14%) for those without and with prior donor antigen exposure, respectively. Finally, among 35 untransfused patients, the graft rejection rates were 3/30 (10%) and 0/4 (0%) for those without and with prior donor blood transfusions, respectively. These tabulations suggested that pre-transplant exposure to donor blood products 24 hr before CY did not significantly affect graft rejection rates.

Binary logistic regression analyses were then carried out to examine more formally the relationship between prior exposure to donor antigen and graft rejection (Table 2). When graft rejection rates were permitted to depend simultaneously on marrow cell dose, relative response in mixed leukocyte culture, the infusion of postgrafting buffy coat cells, donor sex, and transfusion status (column 1 of Table 2), any suggestion of a relationship between pretransplant exposure to donor blood products and graft rejection disappeared. Column 2 of Table 2 restricted attention to the 79 transfused patients who did not receive postgrafting buffy coat cell infusions. The odds ratio estimate (1.73) is in the direction of higher rejection rates among patients with pretransplant exposure to donor blood products, but it is far from significant ($p = 0.42$).

**DISCUSSION**

The present study was undertaken to determine whether exposure of recipients to donor blood products 24 hr before CY (1) enhanced the rate of sustained marrow engraftment as suggested by mouse experiments, (2) increased the rejection rate, in particular in transfused patients who may already have been sensitized to donor antigens by blood products, or (3) was of no relevance to the outcome of transplantation. Results showed that there was neither a significant beneficial nor detrimental effect on the rate of sustained engraftment, although the direction among transfused patients was toward a minimal increase in the rejection rate among those who received donor blood products 24 hr before CY. This was perhaps related to recall antigen exposure after transfusion-induced sensitization to donor antigens, an immunologic response that is less easily suppressed by CY than a primary response. However, this trend was far from significant.

The current results, made both in univariate and in multivariate analyses simultaneously considering other known risk factors for rejection, can perhaps be explained by the fact that the CY dose used in man is very high, close to the range where fatal nonhemopoietic toxicity is expected to occur. In the previously untransfused mouse, the beneficial effect of preceding donor antigen exposure was made only at doses of CY that were relatively low for that species and the strain used (100–200 mg/kg), while no benefit was seen at doses of 250–400 mg/kg. No data have been reported in any animal model where recipients had multiple preceding transfusions.

We had previously shown in dogs that the marrow graft rejection rate decreased as the time interval between previous transfusions of marrow donor blood products 24 hr before CY.
and transplantation increased, perhaps related to a gradual decrease over time in the number of specifically sensitized immunologically active host cells. Presumably, reexposure of recipient dogs to donor blood immediately before transplantation would evoke immunologic memory responses requiring extraordinary drug and irradiation regimens to be suppressed. The animal studies, coupled with theoretical considerations and the slight trend seen in the present study, suggest that exposure to donor blood products immediately before CY should be avoided in multiply transfused patients to allay the possibility of reexposure to sensitizing antigens, thereby increasing the risk of graft rejection. An exception to this suggestion are patients refractory to random-donor platelets in whom transfusions of platelets from the HLA-identical marrow donor immediately before CY are essential to prevent serious hemorrhage. With regard to patients who have not been transfused, only four such patients received donor blood products before CY. Although no patient rejected the graft, the number is too small for a firm conclusion. However, based on the considerations outlined above, it might be advisable to administer donor blood products 24 hr before CY to previously untransfused patients. Perhaps the rare instances of graft rejection could be avoided by increasing the sensitivity of donor-reactive host lymphocytes to CY through priming by donor antigen. For practical purposes, untransfused patients with a low platelet count usually require donor platelets just before CY in order to avoid the danger of bleeding.

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REFERENCES

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