CONCISE REPORT

Recipient Immune-Competent T Lymphocytes Can Survive Intensive Conditioning for Bone Marrow Transplantation

By Anna Butturini, Robert C. Seeger, and Robert P. Gale

Bone marrow transplantation is usually preceded by intensive chemotherapy and radiation therapy designed to completely eliminate recipient immune-competent cells that might reject the donor bone marrow. We show that seven of 14 bone marrow transplant recipients who received intensive conditioning retained circulating T lymphocytes irreversibly. However, recent data in monkeys indicate that clonogenic T lymphocytes are present in peripheral blood following high-dose total-body irradiation, similar to that used in humans for pretransplant conditioning.

We studied the proliferative and functional properties of peripheral blood lymphocytes in transplant patients after intensive conditioning regimens. In half of the patients it was possible to recover considerable numbers of T lymphocytes able to proliferate in vitro in the presence of phytohemagglutinin (PHA) and recombinant interleukin 2 (IL 2). By expanding these cells in long-term culture, we showed that they exhibit many of the immunologic characteristics of normal T cells such as the ability to proliferate in mixed lymphocyte culture (MLC) and to develop into cytotoxic effector cells in an in vitro model of graft rejection.

MATERIALS AND METHODS

Fourteen consecutive patients were studied, six with acute lymphoblastic leukemia, five with acute myelogenous leukemia, two with chronic myelogenous leukemia, and one with advanced Hodgkin’s lymphoma. Clinical details are indicated in Table 1.

Ten to 20 mL of peripheral blood was obtained within 24 hours of the last dose of radiotherapy and prior to the infusion of donor bone marrow cells. Informed consent was obtained from all individuals.

Mononuclear cells were isolated on a Ficoll-Hypaque gradient, washed three times, and resuspended in RPMI 1640 supplemented with glutamine, antibiotics, 12% fetal calf serum, 20 mg/100 mL bovine albumin, 10 mg/100 mL human transferrin, and 0.1% of 0.1 mol/L FeCl3. Viability of mononuclear cells, tested by trypan blue dye exclusion, was >95%.

Cultures were established using 5 to 10 x 10⁴ cells per well in 24-well plates in 1.5 mL of medium in the presence of 1% PHA (Wellcome Reagents, Research Triangle Park, NC) and 100 U/mL of recombinant IL 2 (Biogen Research Corp, Cambridge, Mass). Culture medium was replaced on days 4, 9, and 15 with fresh medium supplemented with PHA and IL 2, and weekly thereafter with medium and IL 2. On days 4, 9, and 15, aliquots of cells were counted and analyzed for morphology and phenotyped using T3, T11, T4, and T8 monoclonal anti-T lymphocyte antibodies (Coulter Immunology, Hialeah, Fla) by immunoperoxidase staining. Between day 15 and 25 when cell numbers exceeded 2 x 10⁶, half of the cells were washed twice, incubated for 24 hours in medium alone, and assayed in MLC13 with irradiated allogeneic stimulator cells. Cells generated in MLC were tested for cytotoxicity against PHA-stimulated. ⁵¹Cr-labeled allogeneic lymphocytes. Natural killer (NK) activity was tested using K562 as target cells. Clonal growth of lymphocytes was assayed in semisolid matrix, and T colonies (>40 cells) were quantitated using an inverted microscope after ten days of culture.
Table 1. Patient Characteristics and Lymphoid Growth

<table>
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<tr>
<th>UPN</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Disease</th>
<th>Chemotherapy</th>
<th>TBI (Gy)</th>
<th>TLI</th>
<th>Growth</th>
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<td>7</td>
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<td>M</td>
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<td>ALL, rel</td>
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<td>ALL, rel</td>
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Abbreviations: UPN, unique patient number; rem, remission; rel, relapse; cp, chronic phase; acc, accelerated phase; TBI, total-body irradiation; TLI, total-lymphoid irradiation (2.25 Gy); CTX, Cytoxan (cyclophosphamide); Ara-C, cytosine arabinoside; VBL, vinblastine.

RESULTS

Lymphoid cell cultures were established from seven patients for periods of 29 to 52 days. Continuous stimulation with IL 2 was required for cell proliferation. Cells from the other patients showed little or no growth.

The initial proliferation was characterized by cells with abnormal morphology. These cells stained strongly with T11 and T3 but weakly with T8 and T4. After ten days, the cells were replaced by a population with the morphology of normal IL 2-activated lymphocytes. The lymphoid origin of these cells was further established by reactivity with Tl I, T8 and T4. After ten days, the cells proliferated when cocultured with allogeneic irradiated stimulator cells. Cells from four of these MLCs were cytotoxic for 51Cr-labeled target cells syngeneic to the stimulator cells (specific release: 12%, 16%, and 29%). None of seven lymphocyte cultures tested formed colonies in semisolid matrix; the plating efficiency was 10^{-2} to 10^{-3}.

T cells from four of five cultures proliferated when cocultured with allogeneic irradiated stimulator cells. Cells from three of four MLCs were cytotoxic for 51Cr-labeled target cells syngeneic to the stimulator cells (specific release: 12%, 16%, and 29%). None of seven lymphocyte cultures tested was directly cytotoxic for 51Cr-labeled K562 cells, indicating an absence of detectable NK activity.

DISCUSSION

This study indicates that viable and potentially functional T lymphocytes of both helper (T4) and suppressor (T8) phenotype survive intensive pretransplant conditioning regimens. These cells are able to proliferate after exposure to PHA and IL 2, react to disparate HLA antigens in MLC, and exhibit cell-mediated cytotoxicity. NK activity was absent. Considering the number of T lymphocytes in the peripheral blood at the time of the study (0.002 to 0.008 x 10^9/L) and the plating efficacy (10^{-2} to 10^{-3}), it is possible that a 70-kg recipient has at least 0.1 to 2.5 x 10^6 lymphocytes that can proliferate and react against the allogeneic donor bone marrow cells.

In most instances, the limited number of cells that survive the conditioning regimen are probably eliminated by donor immune-competent cells in the graft. When T cells are removed from the transplanted bone marrow, these residual host T cells may survive and react against the donor bone marrow. Our observations provide a possible explanation of graft failure/rejection occurring after bone marrow transplantation in humans. Furthermore, they suggest that graft rejection may be increased in T cell-depleted bone marrow transplantation or overcome by more intensive immune suppression. Preliminary data supporting both hypotheses are available.

The approach we describe may aid in developing more effective immunosuppressive conditioning regimens since the number and reactivity of residual T cells can be determined. Ultimately, studies of this nature may clarify the cellular mechanism underlying graft rejection.

REFERENCES

6. Smith BR, Guinan EC, Parkman R: Efficacy of a cyclophos-
phamide-procarbazine-antithymocyte serum regimen for prevention of graft rejection following bone marrow transplantation for transfused patients with aplastic anemia. Transplantation 39:671, 1985


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