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CONCISE REPORT

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

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**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 that proliferate after incubation with interleukin 2 and phytohemagglutinin and function as effector cells in an in vitro model of graft rejection. These T cells may mediate graft rejection.**

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FAILURE of engraftment and graft rejection are important complications of bone marrow transplantation in humans. Graft failure occurs in 20% to 40% of individuals with aplastic anemia who receive HLA-identical transplants following pretransplantation conditioning with cyclophosphamide.[1] The incidence is even higher, 40% to 70%, in recipients of HLA-mismatched grafts.[2] Graft failure/rejection also occurs in recipients of bone marrow transplants for leukemia: graft rejection develops in approximately 1% of recipients of HLA-identical grafts and 15% of HLA-mismatched recipients.[3] These incidences increase substantially, to 15% and 30%, when T lymphocytes are removed from the transplanted bone marrow in attempts to prevent graft-*v*-host disease.[4]

The precise mechanism of graft failure/rejection is unknown, and there are no accurate in vitro tests to predict or define the mechanisms involved. Considerable indirect data suggest an immune basis of graft rejection in most instances. These include the observations that graft failure can be prevented or substantially reduced using more intensive pretransplant immune suppression[5,6] and that a lower incidence of graft rejection is reported in untransfused patients.[7] Extensive in vitro data and studies in animals including dogs and subhuman primates support the hypothesis that graft rejection is immune mediated.[8-11]

Despite these data that strongly suggest an immune basis of graft failure/rejection, there is no direct evidence in humans that immune-competent lymphocytes survive the pretransplant conditioning regimen. Most transplant patients receive high doses of drugs and radiation that are presumed to eradicate recipient lymphocytes completely and 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.[12]

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 exibit 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 $FeCl_3$. Viability of mononuclear cells, tested by trypan blue dye exclusion, was >95%.

Cultures were established using 5 to 10 × $10^4$ 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 × $10^6$, half of the cells were washed twice, incubated for 24 hours in medium alone, and assayed in MLC[13] with irradiated allogenic stimulator cells. Cells generated in MLC were tested for cytotoxicity against PHA-stimulated, $^{51}Cr$-labeled allogenic lymphocytes.[14] Natural killer (NK) activity was tested using K562 as target cells.[15] 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.[16]

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Table 1. Patient Characteristics and Lymphoid Growth

| UPN | Age (yr) | Sex | Disease | Chemotherapy | TBI (Gy) | TLI | Lymphoid Growth |
|---|---|---|---|---|---|---|---|
| 463 | 7 | F | AML, 2 rem | CTX + Ara-C | 10 | no | yes |
| 464 | 33 | F | AML, 1 rem | Ara-C | 13.50 | yes | no |
| 465 | 5 | M | ALL, rel | CTX + Ara-C | 10 | no | no |
| 466 | 18 | M | ALL, 1 rem | Ara-C | 13.50 | yes | yes |
| 467 | 25 | F | AML, 2 rem | Ara-C | 13.50 | yes | yes |
| 468 | 19 | M | ALL, rel | VBL + CTX | 11.25 | no | no |
| 469 | 16 | M | ALL, 2 rem | Ara-C | 13.50 | yes | yes |
| 470 | 31 | F | CML, cp | Ara-C | 13.50 | yes | yes |
| 471 | 20 | M | ALL, 2 rem | Ara-C | 13.50 | yes | yes |
| 472 | 22 | M | AML, 2 rem | Ara-C | 13.50 | yes | no |
| 473 | 30 | M | ALL, rel | VBL + CTX | 11.25 | no | no |
| 474 | 23 | M | CML, acc | CTX | 13.50 | yes | no |
| 475 | 34 | F | Hodgkin's | VBL + CTX | 11.25 | no | no |
| 476 | 39 | M | AML, 1 rem | Ara-C | 13.50 | yes | yes |

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 T11, T3, T4, and T8. The T8/T4 ratios in most cultures were 3 to 1.5:1. T cells from five patients (UPN 466, 467, 470, 471, and 476) formed colonies in semisolid matrix; the plating efficiency was $10^{-1}$ to $10^{-2}$.

T cells from four of five cultures proliferated when cocultured with allogeneic irradiated stimulator cells. Cells from three of four MLCs were cytotoxic for $^{51}$Cr-labeled target cells syngeneic to the stimulator cells (specific release: 12%, 16%, and 29%). None of seven lymphocyte cultures tested was directly cytotoxic for $^{51}$Cr-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 $0.008 \times 10^9$/L) and the plating efficacy ($10^{-1}$ to $10^{-2}$), it is possible that a 70-kg recipient has at least 0.1 to $2.5 \times 10^6$ 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.[4]

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

1. Storb R, Witherspoon RP, Sullivan KM, Stewart P, Sanders JE, Sale GE, Prentice RL, Hansen JA, Doney K, Deeg HJ, Clift RA, Buckner CD, Appelbaum FR, Thomas ED: Allogeneic marrow transplant for treatment of severe aplastic anemia, in Gale RP (ed): Recent Advances in Bone Marrow Transplantation. New York, Liss, 1983, p 3

2. Bortin MM, Gale RP, Rimm AA: Allogeneic bone marrow transplantation for 144 patients with severe aplastic anemia. JAMA 245:1132, 1981

3. Gale RP, Champlin RE: Bone marrow transplantation in leukemia: Critical analysis and controlled clinical trials, in Gale RP (ed): Recent Advances in Bone Marrow Transplantation. New York, Liss, 1983, p 71

4. Gale RP: T cells, bone marrow transplantation and immunotherapy, in Fahey JL (moderator): Immune Intervention Disease. Ann Intern Med 1986 (in press)

5. Gale RP, Ho W, Feig S, Champlin R, Tesler A, Arenson E, Ladish S, Young L, Winston D, Sparkes R, Fitchen J, Territo M, Sarna G, Wong L, Paik Y, Brison Y, Golde D, Fahey J, Cline M: Prevention of graft rejection following bone marrow transplantation. Blood 57:9, 1981

6. Smith BR, Guinan EC, Parkman R: Efficacy of a cyclophos-

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phamide-procarbazine-antithymocyte serum regimen for prevention of graft rejection following bone marrow transplantation for transfused patients with aplastic anemia. Transplantation 39:671, 1985

7. Storb R, Thomas ED, Buckner CD, Clift RA, Deeg HJ, Fefer A, Goodell BW, Sale GE, Sanders JE, Singer J, Stewart P, Weiden PL: Marrow transplantation in thirty "untransfused" patients with severe aplastic anemia. Ann Intern Med 92:30, 1980

8. Storb R, Weiden PL, Deeg HL, Graham TC, Atkinson K, Slicher SJ, Thomas ED: Rejection of marrow from DLA identical canine littermates given transfusion before grafting: Antigens involved are expressed on leukocytes and skin epithelial cells but not on platelets and red blood cells. Blood 54:477, 1979

9. Dallman MJ, Mason DW: Which T cells cause graft rejection? Transplantation 33:217, 1985

10. Steinmuller D: Which T cells cause allograft rejection? Transplantation 40:229, 1985

11. Wagenmaker G: Problems and prospects of histoincompatible bone marrow transplantation studied in Rhesus monkeys, in Hagenbeek A, Löwenberg B (eds): Minimal Residual Disease in Acute Leukemia. Dordrecht, Nijhoff, 1986, p 363 (abstr)

12. Reisner Y, BenBasat I, Douer D: A primate pre-clinical model for bone marrow transplantation: Definite proof for host radioresistant clonable T cells. Exp Hematol 13:321, 1985

13. Opelz G, Kuichi M, Takasuki M: Reactivity of lymphocyte subpopulation in human mixed lymphocyte culture. J Immunogenet 2:1, 1975

14. Schwartz A, Sutton SL, Gershan RK: Regulation of in vitro cytotoxic T lymphocyte generation 1: Evidence that killer cell precursors differentiate to effector cells in two steps. J Exp Med 155:783, 1982

15. Timonen T, Saksela E, Raucki A, Hayvey P: Fractionation, morphological and functional characterization of effector cells responsible for human natural killer activity against cell line targets. Cell Immunol 48:133, 1979

16. Goube de Laforest P, Thomas P, Pelletier D, Tanzer J: Studies on cell interactions using a micromethod for growing T-lymphocyte colonies in agar culture. Immunology 38:561, 1979

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