Imbalances Within the Peripheral Blood T-Helper (CD4⁺) and T-Suppressor (CD8⁺) Cell Populations in the Reconstitution Phase After Human Bone Marrow Transplantation

By Andrea Velardi, Adelmo Terenzi, Speranza Cucciaioni, Romano Millo, Carlo E. Grossi, Fausto Grignani, and Massimo F. Martelli

Peripheral blood T cell subsets were evaluated in 11 patients during the reconstitution phase after allogeneic bone marrow transplantation and compared with 11 age-matched controls. The proportion of cells coexpressing Leu7 and CD11b (C3bi receptor) markers was determined within the CD4⁺ (T-helper) and the CD8⁺ (T-suppressor) subsets by two-color immunofluorescence analysis. CD4⁺ and CD8⁺ T cells reached normal or near-normal values within the first year posttransplant. In contrast to normal controls, however, most of the cells in both subsets coexpressed the Leu7 and CD11b markers. T cells with such phenotype display the morphological features of granulocyte lymphocytes (GLs) and a functional inability to produce interleukin 2 (IL 2). These T cell imbalances were not related to graft vs host disease (GvHD) or to clinically detectable virus infections and may account for some defects of cellular and humoral immunity that occur after bone marrow transplantation.

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(McGraw Laboratories, Irvine, CA) at a final concentration of 0.66%, and the suspension was allowed to settle for 1 hour. The RBC-depleted, leukocyte-rich plasma fraction was collected and washed twice with phosphate-buffered saline (PBS). Soybean agglutinin (SBA) (1 mg/mL in PBS) was added, and agglutination was allowed to proceed for 5 minutes. The cell suspension was then layered on 5% (vol/vol) bovine serum albumin (BSA) in PBS and allowed to sediment at 1 g for 10 minutes. The top (nonagglutinated) cell fraction was washed with 0.2 mol/L d-galactose in PBS to remove SBA and was further depleted of residual T cells by rosetting with neuraminidase-treated sheep erythrocytes followed by Ficoll-Hypaque density-gradient centrifugation. As a result of the SBA agglutination and E rosetting procedure, residual T cells in the graft never exceeded 0.2%, as determined by immunofluorescence analysis. Cells were washed, suspended in sterile physiologic saline containing 1% human serum albumin, and infused over a period of 20 minutes.

Posttransplantation follow-up. The marrow graft recipients received no immunosuppressive therapy as GvHD prophylaxis in the posttransplant period. In each case, engraftment was documented by blood group or sex marker conversion, or Ph'-chromosome disappearance. Posttransplantation follow-up was 12 months in six patients, 10, 9, and 8 months in three patients, respectively, and 7 months in the remaining two patients. GvHD was minimal or absent in all patients, and clinically asymptomatic cytomegalovirus (CMV) infection was documented between 7 and 12 months postgrafting in 7 of the 11 patients by a sensitive enzyme-linked immunosorbent assay (ELISA) test (Behring, Marburg, FRG). In this assay, the antigen is prepared from human cell cultures infected with CMV. Antibody titers >1:40 were considered positive.

Immunofluorescence. Haptenized peripheral blood samples were obtained monthly from bone marrow recipients and normal controls after informed consent was obtained. Mononuclear cells were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density-gradient centrifugation, and partially depleted of monocytes by adherence to plastic dishes. The CD4⁺ and CD8⁺ cell subsets were enumerated and analyzed for coexpression of Leu7 and CD11b antigens by two-color immunofluorescence, using fluorescein-conjugated Leu3 or Leu2 mouse monoclonal antibodies, and rhodamine- or phycoerythrin-conjugated Leu7 or Leu15 antibodies (Becton Dickinson, Mountain View, CA). Alternatively, nonlabeled antibodies were used, followed by fluorescein-labeled or rhodamine-labeled anti-mouse immunoglobulin isotypes (technical details given in references 11-13). Coexpression of CD3 on CD4⁺ or CD8⁺ cells was determined using the same techniques and Leu4 antibodies (Becton Dickinson) with specificities for the CD3 complex. Immunofluorescence analyses were conducted on mononuclear cell fractions which, in the marrow graft recipients, were comprised of lymphocytes, residual monocytes and, occasionally, early myeloid cells. Absolute values for CD4⁺ and CD8⁺ lymphocytes (and their Leu11b⁺ or Leu11b⁻ subsets) were obtained based on the absolute count of the entire blood mononuclear cell population as determined by standard WBC counts and differentials. Wet preparations of viable cells were examined with a Leitz Orthoplan microscope equipped with epi-illumination and filters for fluorescein and rhodamine. Coexpression of two markers on the same cell was evaluated by scoring at least 200 cells positive for one marker and determining the percentage of cells also expressing the other marker.

RESULTS

The absolute number of peripheral blood CD4⁺ cells/μL was evaluated monthly in bone marrow recipients and controls. As shown in Fig 1, this number reached the lower limit of the normal control range (~500 cells/μL) as early as 2 to 5 months after transplantation. In all patients, however, the

![Fig 1. A: Total numbers of CD4⁺ cells coexpressing NK-related (CD11b) antigens. Increase in the total CD4⁺ cell population is accounted for by a wave of CD4⁺ cells that coexpress CD11b. Shaded area represents 99% confidence limit for CD4⁺ cells coexpressing CD11b in control subjects. Similar data were obtained when coexpression of Leu 7 on CD4⁺ cells was determined (Table 1). As a consequence (B), in all cases except two, the absolute counts of CD4⁺ cells with a "normal" phenotype (ie, not coexpressing Leu7 and CD11b), stayed well below the 99% confidence limit of control values (shaded area indicates lower limit of control CD4⁺ cells not coexpressing NK-related antigens). Dotted lines refer to four patients without active CMV infection. CMV infection does not influence the expression of CD11b on CD4⁺ cells.](http://www.bloodjournal.org)
increase in the total CD4+ cell population was largely accounted for by cells that also expressed Leu7 and CD11b antigens. Peak values for the latter cells were generally reached in month 5 posttransplant and they decreased subsequently, at a rate that varied among patients. The absolute numbers of CD4+ cells coexpressing Leu7 and CD11b were largely above the normal values (Table 1). As a consequence, in most patients, the absolute count of CD4+ cells with “normal” phenotype (ie, not coexpressing Leu7 or CD11b) stayed well below the lower limits of control values (Fig 1).

We also evaluated the percentages of cells with cytotoxic/suppressor phenotype (CD8+) that coexpressed Leu7 and CD11b antigens. As with CD4+ cells, these CD8+ cells display GL morphology and have the ability to suppress T cell proliferation, pokeweed mitogen (PWM)-driven B cell differentiation, and IL 2 production. In all patients recovering from bone marrow transplantation, we found that CD8+ cells reached the control values within the third posttransplant month. However, cells coexpressing Leu7 and CD11b antigens were significantly above the normal control values (Table 1 and Fig 2). This imbalance within the CD8+ cell subset was apparent in all cases during the early recovery phase and was maintained consistently during the first posttransplant year.

CMV infection/reactivation, although clinically asymptomatic, was documented in 7 of the 11 patients between 7 and 12 months postgrafting. As shown in Figs 1 and 2, no correlation was found between the presence of anti-CMV antibodies and the expression of NK-related antigens on both CD4+ and CD8+ lymphocyte subsets.

**DISCUSSION**

Studies on the immunologic reconstitution of bone marrow transplant recipients have been conducted with the primary aim of understanding the mechanisms of the immunodeficiency and of predicting and preventing GvHD. Furthermore, the recovery phase after bone marrow transplantation has been viewed as a model in which the ontogeny of the immune system may be recapitulated. Phenotypic and functional abnormalities of peripheral blood lymphocytes of all lineages and imbalances among lymphocyte subsets have been described after bone marrow transplantation. In the absence of GvHD and/or severe infections, however, a return to normal lymphocyte phenotypes and functions is usually observed at the end of the first year posttransplant.

We describe a previously unrecognized imbalance within the T-helper population of blood lymphocytes that occurs after bone marrow transplantation. This imbalance consists of a predominance of CD3+CD4+ cells coexpressing Leu7 and CD11b antigens, a phenotype found in a minority population of T-helper cells from normal adult blood. These cells are already predominant in the early recovery phase, reach their peak value around the fifth month after bone marrow transplantation, and remain consistently elevated during and after the first posttransplant year. Phenotypically similar cells, when investigated in other anatomic sites and various pathologic conditions in which they are increased, display GL morphology. Functional studies at the population and the clonal levels have indicated that T-helper GLs differ from other nongranular T-helper cells in that they proliferate poorly in response to mitogens and do not produce IL 2. These functional properties are also a feature of GL with suppressor (CD8) phenotype that coexpresses Leu7 and CD11b antigens. After bone marrow transplantation, this subset of CD8+ GLs has also been shown to increase at the expense of nongranular CD8+ cells and to exert a potent suppressor function on IL 2 production by normal T cells.

Our observations suggest that the defective IL 2 production in bone marrow recipients is due to the predominance of poor IL 2 producers in both the T-helper and the T-suppressor cell population. These data are supported by clonal analyses of peripheral blood T cells from the same patients, which show a very low frequency of IL 2-producing T cell clones in the recovery phase after bone marrow transplantation (A. Velardi et al, unpublished observations). In our study, the predominance of Leu7+CD11b+ GLs among the T-helper cells appeared unrelated to GvHD, which was absent or minimal, and to CMV infection. In contrast to that observed for GLs of other cells (eg, NK cells), GLs with T-helper phenotype have achieved adult proportions at birth. Therefore, their early emergence following bone marrow transplantation may recapitulate an ontogeny event. Alternatively, and in view of the localization of these cells into germinal centers, the elevated proportion of circulating T-helper GLs could result from lack of homing to the peripheral lymphoid environment. This is suggested by the observation that involution of lymphoid tissues occurs in the pretransplant induction phase and persists, with lack of germinal centers, in the posttransplant period. The postgrafting preferential expansion of CD11b+ granular T-cells may also be a consequence of T-depleted bone marrow transplantation. Following conventional unmanipulated marrow grafting, however, nongranular CD11b-T cells bearing the Tp44 surface molecule (CD28) are not transiently

**Table 1. Coexpression of Granular Lymphocyte Markers (Leu7 and CD11) on CD4+ or CD8+ Peripheral Blood Cells After Bone Marrow Transplantation**

<table>
<thead>
<tr>
<th>Cell Phenotypes</th>
<th>Patients (n = 11)</th>
<th>Controls (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ coexpressing Leu7 (%)</td>
<td>52.0 ± 15.0</td>
<td>2.0 ± 2.3</td>
</tr>
<tr>
<td>CD4+ coexpressing CD11b (%)</td>
<td>57.8 ± 17.0</td>
<td>4.0 ± 3.3</td>
</tr>
<tr>
<td>CD8+ coexpressing Leu7 (%)</td>
<td>78.0 ± 10.0</td>
<td>27.0 ± 12.0</td>
</tr>
<tr>
<td>CD8+ coexpressing CD11b (%)</td>
<td>81.0 ± 12.0</td>
<td>41.0 ± 20.0</td>
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</tbody>
</table>

Data refer to peak values determined during the follow-up (described in legend to Fig 2A).

*As determined by two-color immunofluorescence (described in the Materials and Methods section).

†Percentages of CD4+ or CD8+ cells also expressing Leu7 or CD11b. Values are means ± SD.

‡In all cases, differences between patients and controls were highly significant (P < .001 by Student's t test).
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Fig 2. Total numbers of CD8+ cells coexpressing NK-related (CD11b) antigens (A) and CD8+ cells not coexpressing NK-related antigens (B). Most of the recovering total CD8+ cell population is represented by cells that coexpress CD11b. As shown in A, in all cases except two, CD8+ cells coexpressing CD11b exceeded the control values (indicated by shaded area) as early as months 2 through 6 posttransplant and remained consistently elevated. On the other hand (B), in all cases except one, CD8+ cells not coexpressing CD11b were within or below the normal range (shaded area). Analysis of Leu7 coexpression on CD8+ cells yielded similar results (Table 1). Dotted lines refer to four patients without active CMV infection. CMV infection does not influence the expression of CD11b on CD8+ cells.

detected early postgrafting and subsequently decrease in number (P.J. Martin, personal communication), thus mirroring the progressive expansion of their reciprocal CD11b+ populations, as observed in our T-depleted marrow graft recipients. This suggests that the preferential expansion of granular T cells, or the selective loss of their reciprocal nongranular T cell populations, is not related to the T cell depletion of the marrow inoculum, but is a general feature of bone marrow transplantation per se.

ACKNOWLEDGMENT

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REFERENCES

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