Correlation between the Numbers of Naive T Cells Infused with Blood Stem Cell Allografts and the Counts of Naive T Cells after Transplantation

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ABSTRACT
Naive T cells after allogeneic hematopoietic cell transplantation are thought to originate from the engrafted hematopoietic cells. In this report, we show that there is a correlation between the number of naive CD4 T cells infused with peripheral blood stem cell grafts and the absolute number of peripheral naive CD4 T cells on day 30 ($R = 0.65; P < .001$), day 80 ($R = 0.63; P < .001$), and day 180 ($R = 0.66; P < .001$) after transplantation. These results suggest that in the first 6 months after transplantation, most naive CD4 T cells are derived from the naive T cells infused with the graft.

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KEY WORDS
T cells ● Naive ● Blood stem cell allografts ● Hematopoietic cell transplantation

INTRODUCTION
Reconstitution of T cells after hematopoietic cell transplantation may occur either through de novo generation (from grafted hematopoietic cells) or peripheral expansion (of T cells infused with the graft; see reviews [1-3]). Generation of T cells de novo is minimal in the first 3 months after hematopoietic cell transplantation and leads to increasing numbers of naive T cells only thereafter [4-9]. However, phenotypically naive T cells are present in the first 3 months after transplantation [10,11]. These cells may be derived from the naive T cells infused with the graft; we present data in this report to support this hypothesis.

PATIENTS AND METHODS
We determined mononuclear cell (MNC) subsets in the grafts and in the peripheral blood of 122 allogeneic transplant recipients at least once during the first year after transplantation. We selected for this study 102 patients who did not die before 1 month after transplantation and who did not relapse by 1 year after transplantation. The reason for excluding patients with relapse was the fact that these patients were usually treated with chemotherapy, which could decrease T-cell counts, or could have received donor lymphocyte infusion, which could increase T-cell counts. Thirty-nine patients received filgrastim-mobilized peripheral blood stem cells (PBSC), and 63 patients received marrow [12]. Demographic and clinical characteristics of the patients are displayed in Table 1. All patients underwent transplantation for hematologic malignancies. For all patients, it was their first allogeneic transplantation. All conditioning regimens were myeloablative, typically intravenous cyclophosphamide (120 mg/kg) with oral busulfan (approximately 16 mg/kg) or intravenous cyclophosphamide (120 mg/kg) with fractionated total body irradiation (12.0-13.2 Gy). All patients received graft-versus-host disease prophylaxis with methotrexate (days 1, 3, 6, and 11) and cyclosporine (for 6 months, except for patients with clinical extensive chronic graft-versus-host disease). The study was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

Enumeration of MNC subsets was performed by 3-color flow cytometry as described previously [11,13]. Most patients participated in a randomized trial of PBSC versus marrow transplantation [12]; for
these patients, the numbers of MNC subsets in the grafts and in the blood after transplantation have been reported [11]. Naive CD4 T cells were defined as CD45RA^{high} CD4 T cells because this subset has been shown to contain thymic emigrants, and nearly all cord blood CD4 T cells are CD45RA^{high} [10,14,15]. Naive CD8 T cells were defined as CD11a^{low} CD8 T cells because virtually all cord blood CD8 T cells are CD11a^{low} and become CD11a^{high} after activation [16,17]. Moreover, after transplantation CD45RA^{high} CD4 T-cell counts correlate with T-cell receptor excision circle–containing CD4 T-cell counts, and CD11a^{low} CD8 T-cell counts correlate with T-cell receptor excision circle–containing CD8 T-cell counts [8].

Significance of correlation was tested by Spearman nonparametric tests. Correlations with \( P < .05 \) were considered significant.

**RESULTS AND DISCUSSION**

There was a significant correlation between the numbers of naive CD4 T cells in the PBSC graft and in the peripheral blood on days 30, 80, and 180 (Table 2, Figure 1). A similar correlation or trend toward a correlation was observed for the numbers of naive CD8 T cells in the PBSC graft and in the peripheral blood (Table 2, Figure 1). For naive CD4 or CD8 T cells in the marrow graft, the correlation was weak or nonexistent (Table 2), possibly because of a small sample size (the lower the number of cells, the greater the variability of the measurement; thus, a very large sample size would be needed to detect a correlation between the relatively low numbers of naive T cells in the marrow grafts and in the peripheral blood after transplantation). There was no significant correlation between the number of CD34 cells in the PBSC or marrow graft and the number of naive CD4 or CD8 T cells after transplantation (data not shown). The lack of correlation might be due to the fact that for the first several months after transplantation, most naive T cells do not originate from hematopoietic progenitors. Later, the number of de novo–generated T cells depends primarily on the thymic function rather than on the number of hematopoietic progenitors.

Concerning MNC subsets other than naive T cells, the numbers of MNC subsets in the grafts and in the blood after transplantation have been reported [11]. Naive CD4 T cells were defined as CD45RA^{high} CD4 T cells because this subset has been shown to contain thymic emigrants, and nearly all cord blood CD4 T cells are CD45RA^{high} [10,14,15]. Naive CD8 T cells were defined as CD11a^{low} CD8 T cells because virtually all cord blood CD8 T cells are CD11a^{low} and become CD11a^{high} after activation [16,17]. Moreover, after transplantation CD45RA^{high} CD4 T-cell counts correlate with T-cell receptor excision circle–containing CD4 T-cell counts, and CD11a^{low} CD8 T-cell counts correlate with T-cell receptor excision circle–containing CD8 T-cell counts [8].

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cells, there was no significant correlation between the number of memory/effectector (CD45RA<sup>low</sup> CD4 or CD11<sup>a</sup><sup>high</sup> CD8) T cells in the PBSC or marrow graft and in the blood on days 30, 80, 180, or 365 after transplantation (data not shown), except for CD45RA<sup>low</sup> CD4 T cells in the PBSC graft and in blood on day 180 (R = .44; P = .02). The reason for the lack of correlation may be that after transplantation, the grafted memory/effectector T-cell clones undergo expansion that depends on the encounter with their cognate antigen (eg, viral or alloantigen) rather than on the number of grafted T cells. There was no significant correlation between the number of monocytes or natural killer cells in the PBSC or marrow graft and in the blood on days 30, 80, 180, and 365 after transplantation (data not shown). For B cells, there was a correlation or a trend toward a correlation between the number of B cells in the PBSC (but not marrow) graft and in peripheral blood on day 30 (R = .38; P = .02) and day 80 (R = .30; P = .11) but not later after transplantation. Thus, early after PBSC transplantation, some B cells may originate from the B cells infused with the graft.

In conclusion, in the first 6 months after transplantation, most naive T cells seem to originate from the naive T cells infused with the graft.

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