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Comparative analysis of naïve and memory CD4$^+$ and CD8$^+$ T-cell subsets in bone marrow and G-CSF–mobilized peripheral blood stem cell allografts: impact of donor characteristics

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Objective. **Donor T cells expressing lymph node homing receptors are the foremost initiators of acute graft-vs-host disease (aGVHD), and a high proportion of CD4$^+$CCR7$^+$ T cells in human leukocyte antigen–matched allografts has been shown to confer a high risk of aGVHD without interfering in other outcomes.**

Methods. **Naïve, central memory (T_{CM}), effector memory (T_{EM}), and terminally differentiated effector memory (T_{TD}) subsets, further subdivided by CD28 expression, were compared in 52 bone marrow and 37 granulocyte colony-stimulating factor–mobilized peripheral blood harvests.**

Results. **CCR7$^+$ cells (naïve and T_{CM}) predominated in the CD4$^+$ population, whereas CD8$^+$ memory cells were chiefly CCR7^{neg} in the grafts. Donor age, antecedent of chronic infections, and graft type were independent factors influencing graft composition. CD8$^+$ naïve cells negatively correlated and CD8$^+$ T_{EM} positively correlated with age. Cytomegalovirus seropositivity was associated with more CD8$^+$ T_{TD} and diminished CD28 expression. Toxoplasmosis seropositivity was associated with more CD4$^+$ T_{CM} ($p = 0.021$). Marrow grafts comprised more CD28$^+$ cells within CD8$^+$ T_{TD}, but the percentage of CD4$^+$CCR7$^+$ cells did not differ significantly between the two graft sources. Each of the four CD4$^+$ subsets and the percentage of CD4$^+$CCR7$^+$ cells ($p < 0.001$) were correlated between graft and venous blood analyzed in 42 donors before harvest procedures.**

Conclusion. **This study provides reference values for CD4$^+$ and CD8$^+$ naïve and memory subsets within allografts applicable to the healthy donor population and indicates that beforehand analysis of a whole-blood sample can help evaluating the risk of aGVHD conferred by each donor and, when possible, choosing the one conferring the lowest risk.**

Allogeneic stem cell transplantation provides the potential for T-cell therapy for various hematological malignancies not available in unmodified autologous transplants. Besides triggering a graft-vs-leukemia effect, donor-derived mature T cells are involved in engraftment and protection against infections, but also in graft-vs-host disease (GVHD) [1]. There are indications that acute GVHD can be partially separated from graft-vs-leukemia effects [2–4], but clinically feasible methods to prevent GVHD while preserving the beneficial effects conferred by donor-derived T cells remain challenging.

Compared with steady-state bone marrow stem cell (BMSC) grafts, granulocyte colony-stimulating factor (G-CSF) mobilized peripheral blood stem cell (PBSC) grafts contain many more mature T cells, but neither the incidence nor severity of acute GVHD appear to be higher [5,6]. Acute GVHD can indeed be induced by as low as 1×10^5 T cells/kg [7,8]. Thus, the occurrence of acute GVHD seems to depend more on the composition of the T-cell pool than on the overall quantity of mature T cells infused with the allograft [9].

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Peripheral T-cell pool includes a high frequency of cells capable of reacting with alloantigens [10], without history of prior exposure, which suggests that alloreactive T cells represent a substantial fraction of the naïve T-cell repertoire. However, memory cells accumulating after infections may cross-react with alloantigens [11–14]. It is now appreciated that acute GVHD necessitates the migration of donor T cells to secondary lymphoid organs, independently of allogeneic disparity, whereupon allospecific T cells can respond to host alloantigens and then traffic to the target organs [15–17]. Coexpression of L-selectin (CD62L) and CC-chemokine receptor 7 (CCR7) is crucial for lymphocyte migration to secondary lymphoid tissues. Irrespective of their antigenic specificity, naïve T cells uniformly express both CCR7 and CD62L, but the overall memory T-cell population is heterogeneous, with varying migratory behaviors. Central-memory cells (T_{CM}) maintain CCR7 and CD62L expression, but the other memory subsets are CCR7-negative (most, although not all, also CD62L-negative) and distributed among effector memory (T_{EM}, $CCR7^{neg}CD45RA^{neg}$) and terminally differentiated effector-memory cells (T_{TD}, $CCR7^{neg}CD45RA^{+}$) [18]. Transfer experiments in mice [11,15,19–22] and mixed lymphocyte reaction assays in humans [23] gave rise to the notion that $CD62L^{+}$ T cells respond to alloantigens and can induce GVHD in allogeneic hosts. Immunogenetic factors notwithstanding, a high proportion of $CD4^{+}CCR7^{+}$ T cells in BMSC and PBSC allografts has been shown to be an independent risk factor influencing the incidence, earliness of onset, and severity of acute GVHD in human leukocyte antigen–matched transplantations, without interfering in other clinical events, especially relapse [9].

The balance of naïve and memory cells and the subset composition of the memory T-cell pool differ markedly between individuals. So, in a healthy population, individual subsets of memory cells accumulate at distinct rates in the peripheral blood [24]. Furthermore, memory T cells specific for common pathogens tend to accumulate within distinct memory subsets [25–30]. Finally, functional capacities (expression of effector cytokines and cytolytic molecules) and migratory properties (CCR7 and CD62L expression) overlap only partially in the memory compartment. The CCR7-negative subsets, but also some $CCR7^{+}$ T_{CM}, are now recognized as capable of rapid expression of effector cytokines upon short-term stimulation by their specific antigen. The expression (or lack thereof) of other surface molecules, such as the costimulatory receptors CD27 and CD28, might be more indicative of their functional properties. The loss of CD28 expression, in particular, has been correlated with a high expression of perforin and granzyme A and B and a superior ability to produce interferon-γ upon stimulation, both in the $CD4^{+}$ [29,31] and in the $CD8^{+}$ memory compartments [26,30,32–34]. By these phenotypic classifications, functional memory T cells may be considered as being predominantly distributed among five to six major subsets [25,32,33].

BMSC and PBSC grafts have not yet been analyzed in equivalent detail, particularly as regards the distribution within the $CD4^{+}$ pool, which contains the most alloreactivity [35] and provides the necessary help for subsequent long-term $CD8^{+}$ responses [36]. The objectives of this prospective study were 1) to compare the distribution of the naïve and the individual memory T-cell subsets, as defined by their phenotypic signatures, between PBSC and BMSC grafts; 2) to evaluate the influence of donor's characteristics on the composition of the grafts; 3) and to determine whether the balance of naïve and memory T-cell subsets differs between the graft and the peripheral blood.

Donors and methods

Donors, harvest procedures, and sampling

Between September 2003 and September 2006, ninety-nine consecutive allografts (52 BMSC and 37 PBSC) were analyzed. The study design has been approved by the ethics committee of the Lille University Hospital, and all donors have given their informed consent. Because in France use of G-CSF is not allowed in healthy minor donors, all of our PBSC donors were older than 18 years, and BMSC from minor donors were excluded from this comparative study. Donors' characteristics and results of serologic tests are summarized in Table 1.

Bone marrow allografts were procured by aspiration from the posterior iliac crests, and a minimum of 3×10^{8} nucleated cells/kg of recipient weight was collected. No specific procedure was performed on the harvest, other than red blood cell depletion in case of major ABO donor/recipient incompatibility. G-CSF was injected at the dose of 10 μg/kg of donor weight/day from day –5 before collection of a minimum of 3×10^{6} $CD34^{+}$ cells/kg of recipient weight. One milliliter final product of either BMSC or PBSC was processed in the laboratory within 6 hours.

Ethylenediamine tetraacetic acid–anticoagulated peripheral blood was obtained between 1 to 6 months (median, 1 month) before bone marrow harvesting in 30 donors or before the first administration of G-CSF in 12 of the PBSC donors, respectively.

Multiparameter flow-cytometric analysis

Whole-blood and graft samples were stained without further separation shortly after collection, and acquired by multiparameter flow cytometry (FC500, Coulter). Directly conjugated antibodies to CD3, CD4, CD8, CD45, CD45RA, CD28, and isotype controls were from Beckman Coulter (Galway, Ireland), and CCR7 from R&D Systems (Minneapolis, MN, USA). After staining (concentrations according to manufacturers' instructions), red cells lysis was carried out, either with ImmunoPrep reagent system (Beckman Coulter) using an automatic workstation (TQ-Prep, Coulter) for the PBSC and whole-blood samples, or