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Long-term persistent lymphopenia in hematopoietic stem cell donors after donation for donor lymphocyte infusion

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***Objectives*. To analyze the consequences of lymphocyte donations on lymphopoiesis in donors having previously undergone hematopoietic stem cell collection for allogeneic stem cell transplantation.**

***Methods*. Repeated analysis of leukocyte subsets in the peripheral blood of 76 hematopoietic stem cell donors undergoing lymphocyte donation(s) for DLI.**

***Results*. Grade I/II lymphopenia was present in 22 donors (29%) just before first apheresis for lymphocyte collection, demonstrating that former stem cell donation induced prolonged lymphopenia in a subset of donors. The monocytic lineage was not affected. Older age and history of PBSC harvest constituted 2 independent factors of lymphopenia, but had no influence on monocytopenia. The first apheresis induced lymphopenia in 36 donors (47%) and monocytopenia in 23 donors (39%). Lymphopenia before first apheresis and prior history of PBSC harvest were independent factors of apheresis-induced lymphopenia while those factors had no influence on monocytopenia. A time-dependent decrease in lymphocyte counts was observed in donors undergoing repeated aphereses, resulting in persistent and prolonged lymphopenia in 50% of donors. No persistent monocytopenia over time and aphereses was observed. Kaplan-Meier estimate of the risk to develop persistent lymphopenia after multiple aphereses was 21% ± 6% at 2 months, 38% ± 8% at 4 months, and 64% ± 10% at 12 months. After Cox regression analysis, previous PBSC harvest remained the unique factor associated with the risk for persistent lymphopenia.**

***Conclusions*. Monitoring the potential long-term effects of repeated aphereses in hematopoietic stem cell donors appears important. Selecting young bone marrow donors for subsequent DLI significantly reduces the risk for acute and prolonged lymphopenias.** **© 2004 International Society for Experimental Hematology. Published by Elsevier Inc.**

Introduction

In patients with recurrent hematological malignancies after allogeneic stem cell transplantation, adoptive immunotherapy with unprimed donor lymphocytes may restore durable, complete clinical and molecular remissions, and full complete chimerism. This has been successfully achieved in patients with recurrent chronic myelogenous leukemia [1], myeloma [2], lymphoma [3], and juvenile myelomonocytic leukemia [4]. Prophylactic donor lymphocyte infusions (DLI) are also performed after nonmyeloablative allogeneic stem cell transplantation [5], a promising therapeutic strategy for the treatment of hematological or nonhematological malignancies. In this context, injecting progressively increasing doses of infused donor $CD3^+$ cells strengthens the alloreactive effect of the procedure.

Donors can therefore be solicited at different time points, first for stem cell donation (either bone marrow [BM] or G-CSF-mobilized peripheral blood stem cells [PBSC]), and second for lymphocyte donation(s) for DLI. Whereas stem cell donation does not seem to induce any serious adverse event, very little is known of the precise impact of lymphocyte donation on donor lymphopoiesis, especially when repeated donations for DLI are performed. Indeed, repeated platelet aphereses or donations for transfusion were previously found to induce prolonged lymphopenia in a subset

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of donors [6–8]. As there is a dramatic increase in the use of DLI, it appears important to assess its possible side effects for the donor.

In the present study, we retrospectively analyzed available data about 127 lymphocyte donations via apheresis performed in our center between 1996 and 2003, in 76 consecutive healthy donors after previous stem cell harvest.

Patients and methods

Donor selection

Healthy donors undergoing unprimed apheresis for lymphocyte collection were HLA-matched related or unrelated donors who had previously given either BM (61 donations) or G-CSF-mobilized PBSC (15 donations) to the same recipient. In all cases, donor lymphocyte collection was performed (in the apheresis unit of the blood transfusion center) because of recipient relapse, persisting disease after transplantation, persisting mixed chimerism, or full chimerism loss, as analyzed by the variable number of tandem repeats (VNTRs) technique. After new physical examination, as well as serological (HIV-1, HIV-2, Hepatitis B, Hepatitis C, EBV, CMV, HTLV-1/2, syphilis) and blood cell count checks, cell preparations were harvested without previous growth factor administration, at steady state, in our apheresis unit. Written lymphocyte collection agreement was obtained from all donors after proper information, according to the legal guidelines of our institution. Collections were performed once or twice within a week.

Apheresis process and laboratory process

All initial stem cell donors underwent steady-state apheresis under identical technical conditions between 1996 and 2003 in our institution. The same process was used for all (i.e., single or multiple) donations. Enriched mononuclear cells were collected using a blood cell separator (Cobe Spectra device, Gambro BCT, Lakewood, CO, USA) with bilateral peripheral venous access. The mean amount of blood processed during leukapheresis was 15 liters total blood mass, once or twice a week when needed. Standard peripheral blood cell counts were performed immediately before and after each apheresis, with an automated Coulter cytometer (Beckman Coulter France SA, Roissy, France). The degrees of leukopenia, lymphopenia, and monocytopenia were classified according to the World Health Organization (WHO) criteria, as these criteria are compatible with normal ranges of the automated counter of the apheresis unit of the blood transfusion center. The blood cell counts performed for stem cell donors only (that did not undergo lymphocyte donation later on) were performed in another laboratory with another automated counter with other normal lymphocyte values ranging between 1 and 4×10^9/L. In this case, and in the case only, in this manuscript, lymphocyte data were exploited according to the normal values edicted by this laboratory, established on more than 500 healthy people.

Cell processing and laboratory tests

Bags of donor mononuclear cells were checked for mononuclear cell counts under good manufacturing practice conditions, and tested by flow cytometry (Becton-Dickinson [BD] FACScan) for lymphocyte subsets (T cells: $CD3^+$, B cells: $CD19^+$, NK cells: $CD3^-CD56^+$) with relevant antibodies (all from BD, Meylan, France] using direct immunofluorescence. Positive cells were analyzed within a propidium iodide (PI) (Sigma, Saint Quentin Fallavier, France) negative fraction. Harvests of donor lymphocytes were further fractionated (as far as possible) in order to cryopreserve doses of 1×10^6, 5×10^6, 10^7, 5×10^7 $CD3^+$ cells/kg in 10% dmiethylsulfoxide/human albumin (LFB, Les Ullys, France). In addition, an aliquot of cells was used to check bacterial and fungal sterility. In most cases, bags of donor lymphocytes were cryopreserved in liquid nitrogen and stored until use, whereas sometimes the first dose of donor lymphoid cells was infused fresh, while remaining cells were frozen.

Statistical and comparative analysis

SPSS statistical software version 11 was used for analysis. $p < 0.05$ was considered significant in all analyses. The chi-square test was used to detect differences between donor groups regarding age, delay between stem cell harvest and the first apheresis for DLI, and leukocyte, lymphocyte, and monocyte subset counts. The chi-square test and logistic procedure [9] were used to analyze the association of individual variables with the presence or absence of cytopenia before or after first apheresis. Logistic regression with backward selection [9] was used for the multivariate analysis of the occurrence of cytopenia before or after apheresis with covariables of age (years), delay between stem cell harvest and donation, sex, and type of stem cell harvest (bone marrow transplant [BMT] vs G-CSF-mobilized PBSC). Pearson correlation coefficient (r) was used to evaluate the correlation between paired values. In donors undergoing more than one apheresis, censored criteria (time without leukopenia or lymphopenia) were analyzed with the Kaplan-Meier estimate [10], the log-rank test [11], and Cox regression model [12]. In donors with multiple aphereses, risk factors for acquired cytopenia were assessed using the appropriate regression model, either logistic regression or Cox model. These factors were age at time of first apheresis, delay between stem cell harvest and donation, sex, type of stem cell harvest (BMT vs G-CSF-mobilized PBSC), and presence or absence of cytopenia before and/or after first donation. Degrees of (leuko- and lympho-) cytopenias are described according to the WHO classification. Although this classification is a grading system used to rate the clinical importance of cytopenias in *patients* and not in *healthy donors*, it seems the most suitable system to describe the deepness of cytopenias in this study.

Results

Donor characteristics

Between 1996 and 2003, 76 consecutive HLA-matched related or unrelated donors were harvested a total of 127 times, which represented a median value of 1.67 donations/donor. To control recipient disease and/or restore full donor chimerism, 26 of the 76 donors (34%) underwent 2 to 9 donations over a period of 0.25 to 49 months. At time of first donation, donor age ranged from 14 to 74 years (mean 40, median 38). Forty-two donors were male. Sixty-one had previously given their BM, while the remaining 15 had undergone G-CSF-mobilized PBSC collection. Lymphocyte donation was performed 1 to 174 months after stem cell harvest (mean 19 months, median 4 months), with no difference between BM

and PBSC donors. Leukocyte and monocyte counts were normal for all donors at the time of stem cell harvest. However, 14 donors (19.2%) had grade I (n = 10) or grade II (n = 4) lymphopenia (Table 1), with a mean lymphocyte count of 1.12×10^9/L at time of stem cell harvest, ranging from 0.62×10^9/L to 1.49×10^9/L (median 1.18×10^9/L). Whereas lymphocyte count before stem cell harvest was not influenced by donor sex, lymphopenic donors were significantly older than donors with normal lymphocyte counts: 39.2 vs 51.7 years ($p = 0.02$). In all cases, there was no obvious cause of acquired lymphopenia. To ensure that we did not select a population of donors with lymphopenia before stem cell harvest that further undergo lymphocyte donation for DLI, we concomitantly analyzed lymphocytic parameters of 44 donors before stem cell harvest, matched for age, sex, type of transplant, and year of harvest, but that did not undergo lymphocyte donation for DLI. According to the normal lymphocyte ranges ($1–4 \times 10^9$/L) of the laboratory that performed blood tests for donors who did not undergo lymphocyte donation later, only 2 donors among 44 (4.5%; 0/13 for PBSC and 2/31 for BM transplants) had a significant lymphopenia present at stem cell harvest, and this is not significantly different from donors who underwent further lymphocyte donation.

Frequent lymphopenia in lymphocyte donors as a consequence of stem cell harvest

Before first or unique apheresis, leukocyte, lymphocyte, and monocyte counts ranged from 3.8×10^9/L to 12.4×10^9/L (mean 6.53×10^9/L, median 7.3×10^9/L), from 0.55×10^9/L to 3.40×10^9/L (mean 1.71×10^9/L, median 1.72×10^9/L), and from 0.1×10^9/L to 0.71×10^9/L (mean 0.34×10^9/L, median 0.32×10^9/L), respectively. One donor had mild leukopenia (3.8×10^9/L), while 22 displayed grade I (n = 16) or grade II (n = 6) lymphopenia (Table 1). Only 6 of the 22 lymphopenic donors were found lymphopenic at the time of stem cell harvest, and the presence of lymphopenia before stem cell harvest was not associated with a significant risk of lymphopenia before first apheresis ($p = 0.13$, Fisher's exact test). There was no obvious cause of acquired lymphopenia such as infectious or systemic disease in the 22 lymphopenic donors. Together, these data suggest that lymphopenia in these donors could mainly be attributed to stem cell harvest. The mean lymphocyte count of the lymphopenic donors was 1.17×10^9/L, ranging from 0.59×10^9/L to 1.49×10^9/L (median 1.18×10^9/L). Donors with lymphopenia before first apheresis were significantly older (51.64 yrs vs 37.45 yrs, $p < 10^{-4}$) and had a significantly shorter delay between stem cell collection and first apheresis for DLI than donors with normal lymphocyte counts (25.3 months vs 7.32 months, $p = 0.045$). The proportion of lymphopenias was significantly higher in G-CSF-mobilized PBSC than in bone marrow donors: 10/15 (67%) vs 12/61 (20%) ($p = 0.008$). The lymphocyte count before apheresis was not influenced by donor sex. Both the nature of stem cell harvest (BM vs PBSC) and donor age were identified as independent risk factors for lymphopenia after logistic regression.

Effect of a single apheresis for DLI on donor lymphocytes and monocytes

We next investigated the effect of a single apheresis on donor leukocyte, lymphocyte, and monocyte counts. This was achieved by monitoring peripheral blood cell counts immediately before and after first apheresis, as detailed in the Materials and methods section. The mean absolute leukocyte, lymphocyte, and monocyte counts after first apheresis were respectively 5.7×10^9/L (median 5.7×10^9/L, range $3.0-9.7 \times 10^9$/L), 1.5×10^9/L (median 1.5×10^9/L, range $0.5–2.6 \times 10^9$/L), and 0.26×10^9/L (median 0.23×10^9/L, range $0.14–0.52 \times 10^9$/L). These values were significantly lower than those observed before apheresis ($p < 10^{-4}$ for all), paired-sample *t*-test). Overall, 7 donors (9%) became leukopenic after first lymphocyte donation whereas the remaining 69, including those with mild leukopenia prior to apheresis, had normal leukocyte count after first donation. All postapheresis leukopenic donors had grade I leukopenia and grade I monocytopenia, with an absolute leukocyte count ranging from 3×10^9/L to 3.8×10^9/L (mean 3.5×10^9/L, median 3.7×10^9/L). Thirty-six donors (47.4%), of whom 19 had been found lymphopenic before first apheresis, became or remained lymphopenic after the first blood collection for DLI (Table 1). There were respectively 25 grade I and 11

Table 1. Frequency of lymphopenia at different time points in donors for DLI

Lymphocyte count at	Overall (76 donors)	Type of stem cell harvest: PBSC (15 donors)	Type of stem cell harvest: Bone Marrow (61 donors)	p^1	Influence of Age (p)
Stem cell harvest	14 (18%)	4 (27%)	10 (16%)	ns	0.02
First apheresis, Before	22 (29%)	10 (67%)	12 (20%)	0.008	10^{-4}
First apheresis, After	36 (47%)	14 (93%)	22 (36%)	0.001	0.012
Subsequent apheresis (20 donors, before apheresis)	10/20 (50%)	5/5 (100%)	5/15 (33%)	$0.033^2-0.013^3$	ns

[1]Comparison of PBSC and bone marrow donors.

[2]Fisher's Exact Test.

[3]Log-rank test.

grade 2 lymphopenias, with an absolute lymphocyte count ranging from 0.53 to 1.48 × 10^9/L (mean 1.16 × 10^9/L, median 1.21 × 10^9/L), and 23 grade I monocytopenias with absolute monocyte counts ranging from 0.14 to 0.52 × 10^9/L (mean 0.26 × 10^9/L, median 0.23 × 10^9/L). After univariate analysis, main factors associated with the risk to develop lymphopenia after first apheresis were, in decreasing order: presence of lymphopenia just before first apheresis, donor age, and a history of PBSC harvest. In contrast, age and type of stem cell transplant harvested before had no influence on monocytopenia. Indeed, 14/15 (93%) donors having undergone PBSC harvest developed lymphopenia after first donation, while 19/22 (86%) donors with lymphopenia before first apheresis remained lymphopenic after donation, with a significant decrease of their lymphocyte count (1.09 vs 1.16, $p = 0.02$). In contrast, only 22/61 (36%) bone marrow donors and 16/54 (30%) non lymphopenic donors before first apheresis developed lymphopenia. The presence of lymphopenia before first apheresis and a prior history of PBSC harvest remained independent factors of apheresis-induced lymphopenia after multivariate analysis (Table 1). However, the presence of lymphopenia at time of stem cell harvest was not found to influence the lymphocyte count after first lymphocyte donation. Finally, we compared the PB leukocyte and PB lymphocyte counts between the group of donors that gave the highest numbers of total lymphocytes and the group of donors that gave the lowest numbers, and they were not statistically different before apheresis ($p = 0.05$ and 0.06 respectively, paired-sample t-test).

Repeated donations increase the frequency of lymphopenia but not monocytopenia

As DLI frequently requires multiple donations, we next investigated the effect of repeated aphereses on acute changes in donor leukocyte, lymphocyte, and monocyte counts before and after multiple aphereses. Fourteen, 6, 3, 2, and 1 donors respectively underwent 2, 3, 4, 5, and 9 donations. Absolute leukocyte, lymphocyte, and monocyte counts, measured after each apheresis, could be investigated over time in 20 out of the 26 donors (59 samples). Among them, 3 were found leukopenic after first apheresis: 1 remained leukopenic after the second donation performed 11.6 months later, whereas the remaining 2 recovered normal leukocyte count after their second donation performed 3 and 4 months later. Two of the remaining 17 donors without leukopenia after first apheresis acquired grade I leukopenia after subsequent donations. One underwent 2 aphereses at 1-month intervals and acquired grade I leukopenia after the second donation. The second one underwent 5 aphereses over a 14-month period and acquired grade I leukopenia after third, fourth, and fifth aphereses. Overall, repeated donations for DLI induced leukopenia in 5 of the 20 donors (25%). However, lymphopenias remained mild over time and did not worsen as a function of the number of aphereses.

Of the 20 donors who underwent more than one apheresis and were monitored for lymphopenia over time, 7 were found to be lymphopenic after first apheresis, of whom 6 remained lymphopenic after subsequent donations. The remaining donor was found to be lymphopenic after first and second aphereses but not after third donation. Eight of the remaining 13 donors without cytopenia after first apheresis became lymphopenic after subsequent donations. These donors had undergone 3 to 9 aphereses (mean 5) and became lymphopenic at the time of donations 2 to 4 (mean 3.4). Overall, repeated donations for DLI induced lymphopenia in 15 of the 20 donors (75%), of whom 4 and 6 were lymphopenic before and after first apheresis, respectively. By contrast with the temporal fluctuation of the whole leukocyte count after apheresis, lymphocyte counts were found to decrease steadily as a function of the number of aphereses. This negative and linear correlation was statistically significant ($r = -0.332$; $p = 0.01$). However, this time-dependent decrease of lymphocyte counts was not associated with a significant increase of acute changes in lymphocyte counts before and after multiple aphereses, suggesting that both lymphocyte counts measured before and after multiple aphereses decreased over time.

Persistent and prolonged lymphopenia in lymphocyte donors undergoing repeated aphereses for DLI

Having observed that repeated aphereses increased the risk to develop leukopenia and lymphopenia without increasing acute changes in lymphocyte counts before and after multiple aphereses, we next investigated whether or not repeated aphereses could induce persistent cytopenias. To that end, we monitored the temporal fluctuation of leukocyte, lymphocyte, and monocyte counts measured before the 59 aphereses performed in the 20 donors followed over time. In addition to the donor with mild leukopenia at the time of first apheresis, a subject who underwent 5 aphereses over a 14-month period was found leukopenic only at the time of third donation. Both displayed normal leukocyte counts at subsequent aphereses. By contrast, a persistent lymphopenia was observed in 10 of the 20 donors followed over time (Table 1), including 3 who were found lymphopenic at time of stem cell harvest and 4 before first apheresis. It is of note that the median interval between the first apheresis for lymphocyte donation and the second is significantly longer (2.99 months ± 1.08) than between apheresis 2 and 3 (1.8 months ± 0.82; $p = 0.02$) and subsequent apheresis, and this may have influenced lymphocyte recovery after a third individual apheresis. The 10 donors, who underwent 2 to 9 aphereses (mean 3.8, median 3.5), developed lymphopenia at the time of first ($n = 4$) to fifth apheresis (mean 2.3, median 2). Figure 1 shows the lymphocyte count measured before first and last apheresis in the 20 donors followed over time. The lymphocyte count before apheresis was found to decrease with the number of aphereses. This negative and linear correlation

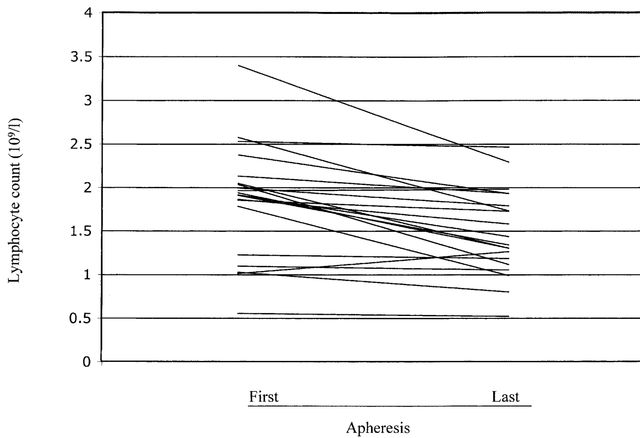

Figure 1. Lymphocyte counts before first and last apheresis in allogeneic donors having undergone more than one lymphocyte donation.

was statistically significant (Pearson's correlation coefficient, −0.348; $p = 0.007$). Lymphopenia occurred only at time of last apheresis in 2 donors harvested 2 and 4 times. In the remaining 8 donors, lymphopenia occurred before last apheresis and persisted at the time of all subsequent donations. In these 10 donors, lymphopenia was found to persist over 1.6 to 43 months (mean 9.6 months, median 3.7 months) from first apheresis, including 4 donors with grade II lymphopenia persisting 1.5 to 11 months. The Kaplan-Meier estimate of the risk to develop persistent lymphopenia after multiple aphereses was 21% ± 6% at 2 months, 38% ± 8% at 4 months, and 64% ± 10% at 12 months (Fig. 2). After Cox regression analysis, the type of stem cell harvest was the unique factor associated with the risk to develop persistent lymphopenia ($p = 0.033$). Figure 2 represents the Kaplan-Meier estimate of the risk to develop persistent lymphopenia after multiple aphereses according to the type of hematopoietic transplant. The median time to develop persistent lymphopenia in PBSC donors was 0 months vs 4 months for bone marrow donors. This difference was statistically significant ($p = 0.013$ by the two-sided log-rank test). Interestingly, as shown in Table 2, the monocytic lineage was not affected by repeated apheresis whatever kind of stem cell was harvested before, and remained normal along time.

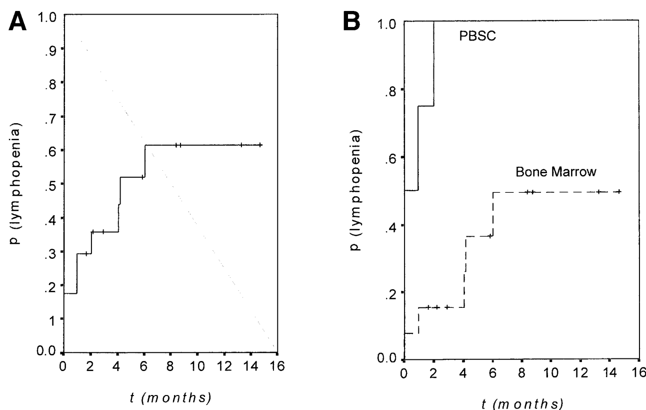

Figure 2. Kaplan-Meier estimate of the risk to develop lymphopenia in donors having undergone more than one lymphocyte donation. (**A**): Overall risk for apheresis-associated lymphopenia over time. (**B**): Risk for developing lymphopenia according to the type of initial hematopoietic stem cell donation.

Repeated aphereses have little effect on the yield of harvested lymphocyte subsets

We next investigated the consequences of repeated donations on the different lymphocyte subpopulations within harvested cells. The proportion of donor B, T, and NK cells was determined by analyzing aliquots of cells from apheresis bags over time. The number of $CD3^+$ cells per kg was found to decrease with the number of aphereses. This negative and linear correlation was statistically significant (r = −0.248; $p = 0.032$). Conversely, no significant fluctuation was observed over time for the mean number of total mononuclear cells (MNCs), total $CD3^+$ cells, total $CD19^+$ cells, and total $CD3^-CD56^+$ harvested.

Discussion

This is the first study addressing the consequences of donations for DLI on donor hematopoiesis. Our data show that this procedure significantly decreases the lymphocyte count, resulting in persistent lymphopenia in ~50% of repeatedly harvested donors. Univariate and multivariate analyses demonstrate that previous PBSC harvest is the main risk factor for apheresis-associated lymphopenia.

DLI is a direct demonstration of the graft-vs-leukemia (GVL) effect [5]. It induces durable complete remission at the molecular level in a majority (over 70%) of patients with chronic myelogenous leukemia, when treated at early relapse after stem cell transplantation. Results are less favorable for acute leukemias, although useful responses have been reported [13]. Data are scarce, though promising, for myelodysplastic syndromes [14] and multiple myeloma [2]. The engraftment of hematopoietic cells from donor origin can be achieved using a reduced-intensity conditioning procedure. Reducing transplant-related mortality by simplifying the stem cell transplant procedure with a reduced-intensity regimen, particularly nonmyeloablative conditioning, may have greater potential for the successful treatment of malignant and nonmalignant disorders. In this context, the use of prophylactic DLI contributes to reinforcing the GVL effect. The clear benefit of DLI explains the dramatic increase of its use for treating hematological patients.

In the present work, 14 donors (18%) had grade I (10) or grade II (4) lymphopenia at time of stem cell harvest. Lymphocyte count has been shown to decrease with aging [15]. The 14 lymphopenic donors were significantly older than donors with normal lymphocyte counts; all lymphopenic donors but 2 were over 50 years old (including 4 individuals over 60). Considering that, in either case, there was no known cause of acquired lymphopenia, such as infectious or systemic disease; the mild lymphopenia observed

Table 2. Frequency of monocytopenia at different points in donors for DLI

Monocyte count at		Overall (76 donors)	Type of stem cell harvest		p^1	Influence of Age (p)
			PBSC (15 donors)	Bone Marrow (61 donors)		
First apheresis	Before	9 (12%)	4 (26%)	5 (8%)	ns	ns
	After	24 (32%)	6 (40%)	18 (30%)	ns	ns
Subsequent apheresis (20 donors, before apheresis)		0/20 (0%)	0/15 (0%)	0/61 (0%)	ns	

[1]Comparison of PBSC and Bone Marrow donors.

in these donors could be mainly attributed to age and did not represent a contraindication for stem cell harvest. The presence of lymphopenia at time of stem cell harvest was not found to correlate with the risk to develop subsequent persistent lymphopenia.

Besides the anesthetic risk associated with bone marrow harvest, the adverse events of peripheral stem cell mobilization and apheresis have been extensively analyzed [16–21]. They combine both growth factors and apheresis-associated secondary effects such as hyperleukocytosis, bone pain, asthenia, headache, nausea/vomiting, splenic rupture, thrombocytopenia, and minor electrolyte imbalances. Whereas the effects of cytokine treatment and apheresis on stem cell peripheralization and collection have been described, little is known of delayed effects of rhG-CSF treatment and apheresis on a normal hematopoietic system, and no long-term data allow to address safety issues. In the study of Körbling et al. [22], 10 patient-related donors having undergone 3- or 4-day rhuG-CSF treatment followed by single or tandem apheresis were monitored over 100 days after apheresis. Lymphocytes decreased, with a nadir at day 7 followed, for T cells, by a rebound at day 30 and a subnormal count at day 100. Stroncek et al. found no significant difference between blood counts before and one year after G-CSF-mobilized stem cell collection in 19 donors [23]. The effect of stem cell harvest combined with lymphocyte donation on donor lymphopoiesis has not been addressed to date. Twenty-two of the 76 donors (29%) of our study displayed lymphopenia before first apheresis. They had a significantly shorter delay between stem cell collection and first apheresis for DLI than donors with normal lymphocyte counts. Together with the absence of other causes of acquired lymphopenia, this strongly suggests that stem cell harvest induced a prolonged period of lymphopenia in these donors. Interestingly, advanced age and previous PBSC harvest constituted independent risk factors for lymphopenia.

Thirty-six donors (47%) were found lymphopenic after first stem cell harvest, including 25 grade I and 11 grade II lymphopenias. The mean 17% decrease in lymphocyte count after first donation is in the range of those previously published after whole-blood donation or cytapheresis [7,24–27]. However, 19 donors underwent a greater than 30% decrease (30.5–56%) in lymphocyte counts after first donation, meaning that this acute change in lymphocyte count might be variable between individuals. In fact, both the risk to develop and the degree of apheresis-associated lymphopenia significantly correlated with age, previous PBSC harvest, and the presence of lymphopenia before donation.

Repeated aphereses were found to significantly increase the frequency of lymphopenia; 75% of the donors followed over time developed at least one episode of lymphopenia after repeated donations. However, repeated donations did not significantly influence acute changes in lymphocyte count before and after apheresis. This was not associated with the recovery of normal lymphocyte counts between repeated donations. Lymphocyte counts both before and after apheresis significantly decreased over time, demonstrating that repeated aphereses were associated with persistent, prolonged, and worsened lymphopenia. Indeed, 50% of repeatedly harvested donors developed persistent lymphopenia. With a median follow-up of 4 months, it was possible to estimate the actuarial risk for persistent lymphopenia. Data from the present study suggest that this risk reaches a plateau of ~60% after 7-month exposure. Multivariate Cox regression analysis revealed that the type of initial stem cell donation constituted the main risk for persistent lymphopenia after repeated aphereses. Indeed, at 6 months, ~100% of PBSC donors became persistently lymphopenic vs 51% of bone marrow donors (Fig. 2). It is interesting to note that multiple-apheresis long-term lymphopenias seem to be a specific event affecting exclusively the lymphocytic lineage, as the monocytic compartment remains normal and stable over time.

Current information on the long-term immunological effects of the lymphocyte loss induced by repeated lymphocyte donations, particularly after stem cell donation, is limited. Various, and sometimes conflicting, results have been published on the frequency of donation-associated lymphopenia in whole-blood donors or long-term apheresis donors [6,7,26]. Both B cells and $CD4^+$ or $CD8^+$ T cells seem to be involved in donor cytopenia, but no lymphopenia-associated disease has been reported to date. It is well known that viral [28] or drug-associated [29] lymphopenia significantly increase the risk for infectious disease or cancer. Based on present results, it appears important to monitor potential long-term effects of repeated aphereses in stem-cell donors. Finally, using logistic regression and Cox regression analyses, our study shows that selecting young bone marrow donors

for subsequent DLI significantly reduces the risk for acute and prolonged lymphopenia.

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