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Selective Depletion of αβ T Cells and B Cells for Human Leukocyte Antigen–Haploidentical Hematopoietic Stem Cell Transplantation. A Three-Year Follow-Up of Procedure Efficiency

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A B S T R A C T

HLA-haploidentical family donors represent a valuable option for children requiring allogeneic hematopoietic stem cell transplantation (HSCT). Because graft-versus-host diseases (GVHD) is a major complication of HLA-haploidentical HSCT because of alloreactive T cells in the graft, different methods have been used for ex vivo T cell depletion. Removal of donor αβ T cells, the subset responsible for GVHD, and of B cells, responsible for post-transplantation lymphoproliferative disorders, have been recently developed for HLA-haploidentical HSCT. This manipulation preserves, in addition to $CD34^+$ progenitors, natural killer, γδ T, and monocytes/dendritic cells, contributing to anti-leukemia activity and protection against infections. We analyzed depletion efficiency and cell yield in 200 procedures performed in the last 3 years at our center. Donors underwent $CD34^+$ hematopoietic stem cell (HSC) peripheral blood mobilization with granulocyte colony–stimulating factor (G-CSF). Poor $CD34^+$ cell mobilizers (48 of 189, 25%) received plerixafor in addition to G-CSF. Aphereses containing a median of 52.5×10^9 nucleated cells and 494×10^6 $CD34^+$ HSC were manipulated using the CliniMACS device. In comparison to the initial product, αβ T cell depletion produced a median 4.1-log reduction (range, 3.1 to 5.5) and B cell depletion led to a median 3.4-log reduction (range, 2.0 to 4.7). Graft products contained a median of 18.5×10^6 $CD34^+$ HSC/kg recipient body weight, with median values of residual αβ T cells and B cells of 29×10^3/kg and 33×10^3/kg, respectively. Depletion efficiency monitored at 6-month intervals demonstrated steady performance, while improved recovery of $CD34^+$ cells was observed after the first year ($P = .0005$). These data indicate that αβ T cell and B cell depletion of HSC grafts from HLA-haploidentical donors was efficient and reproducible.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) from an HLA-haploidentical relative has been successfully used for adult and pediatric recipients lacking either a related or unrelated HLA-matched donor [1-3]. Several manipulation strategies have been developed in order to remove T cells, responsible for graft-versus-host disease (GVHD), and B cells, from which a post-transplantation lymphoproliferative disease can arise. In particular, the approaches of graft manipulation developed over time included either positive selection of $CD34^+$ hematopoietic stem cells (HSC) or depletion of $CD3^+$ T cells and $CD19^+$ B cells, with pros and cons for each procedure, as recently reviewed [4,5]. Alloreactive precursors that mediate GVHD are confined to T cells expressing the αβ chains of the T cell receptor (TCR) [6-8]. Therefore, a procedure was developed for selective removal of this subset, in combination with removal of B cells [9-11]. This manipulation spares γδ TCR-expressing T cells, which provide an important contribution to control opportunistic infections and to exert an anti-leukemia effect [12-16], as well as natural killer (NK) cells and monocytes/dendritic cells (DC), which contribute to anti-leukemia effect and to antigen-presenting function, facilitating reconstitution of the T cell repertoire [8,17,18].

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In the present report, we describe our 3-year experience with the αβ T cell and B cell depletion procedure, which was performed using clinical grade reagents and an automated device. We manipulated 200 apheresis products to be administered for HLA-haploidentical HSCT to pediatric recipients affected by either malignant or nonmalignant hematological diseases. Performance of this procedure in terms of depletion efficiency, for cells to be removed, and of recovery efficiency, for cells to be spared, was also evaluated as follow-up during the whole study period.

MATERIALS AND METHODS

Donors

A total of 189 parental donors underwent 200 apheresis collections followed by the depletion procedure. Median donor age was 39 years (range, 18 to 56).

Leukapheresis Procedure

Donors received granulocyte colony–stimulating factor (G-CSF) for 4 days at 12 μg/kg body weight in 2 divided doses to induce peripheral mobilization of CD34⁺ HSC. Apheresis was performed on day 5. When on day 4 the CD34⁺ cell count was <40/μL and/or the predicted apheresis yields was $\leq 12.0 \times 10^6$ CD34⁺ HSC/kg recipient's body weight according to a previously reported formula [19], plerixafor (Mozobil [MZ]; Sanofi Genzyme, Modena, Italy) was given at .24 mg/kg with the aim of boosting mobilization of hematopoietic stem/progenitor cells. Plerixafor was usually given at midnight, 9 hours before HSC collection on day 5. Large volume apheresis was performed with the Spectra Optia Cell Separator (Terumo BCT, Leuven, Belgium). The bags containing the apheresis product (up to 60×10^9 nucleated blood cells) were filled to 600 mL with PBS-EDTA containing .5% human serum albumin (HSA) (CliniMACS washing buffer; Miltenyi Biotec, Bergish-Gladbach, Germany) and centrifuged at 200*g* for 15 minutes at room temperature with no brake to remove platelets. Cells were then resuspended in CliniMACS buffer containing 5% HSA at 150×10^6/mL to 200×10^6/mL for overnight storage at 4°C before processing. The procedures described here were approved by the institutional ethical committee (approval #938/LB) and a written informed consent was obtained from the adult donors.

Depletion Procedure

Manipulations were performed in a closed system. Clinical grade reagents, disposable kits and instrumentation were obtained from Miltenyi Biotec (Bergish-Gladbach, Germany). Procedures were performed with the fully automated CliniMACS device in a laminar-flow hood located in a clean room certified for sterile manipulations. The detailed instructions provided by the manufacturer were followed. Briefly, the apheresis bags stored overnight were filled with washing buffer and centrifuged at 300*g* for 15 minutes at room temperature with no brake. The cell pellet, resuspended in 95 mL washing buffer, was mixed with 3 mL of gamma-globulin solution at 50 mg/mL (Flebogamma, Grifols, Barcelona, Spain). After treatment for 10 minutes at room temperature to prevent nonspecific binding of selection antibodies to Fc receptors, the cells were incubated with the biotin-conjugated anti-αβ TCR antibody for 30 minutes at room temperature with gentle agitation. After 2 washing steps, the cells were treated with magnetic beads conjugated to antibiotin antibodies and with magnetic beads conjugated to the anti–B cell antibody (anti-CD19). After reagent inoculation, bag ports were carefully rinsed with buffer and cells thoroughly resuspended to make sure that all cells in suspension had been labeled. Following an additional 30-minute incubation and 1 washing step, cells were resuspended in washing buffer at 250×10^6/mL to 300×10^6/mL and loaded on the CliniMACS device equipped with the depletion tubing set. These cells were identified as precolumn sample. The procedure was run using the 3.1 Depletion Program. The eluted cells were identified as the graft. The retained cells were recovered from the column after removal of the magnetic field and defined as the nontarget (NT) fraction. Depletion efficiency was defined as -log10 of the ratio between cell numbers in the graft and cell numbers in the precolumn samples. Aliquots for quality controls were collected at different steps of the procedure (apheresis before labeling, precolumn sample, graft, and NT cells). These aliquots were used to enumerate CD34⁺ HSC, αβ T cells, B cells, γδ T cells, and NK cells. TCR αβ/CD19 cell–depleted grafts were reinfused into the patients within 2 hours from the end of processing. The patients received a median value of 18.5×10^6/kg body weight (range, 6.7×10^6/kg to 40×10^6/kg). When a number of CD34⁺ greater than 40×10^6 CD34⁺ cells/kg body weight was obtained, the exceeding cells were cryopreserved also to avoid the risk of infusing a residual αβ T cell number greater than 1×10^5/kg body weight. Microbiological testing for aerobic bacteria, nonaerobic bacteria, and fungi proved that all cellular products were sterile. Furthermore, no acute adverse reactions were recorded after reinfusion in any cases.

Phenotyping

Phenotypic analysis was performed with a FACScalibur BD cytometer (Becton Dickinson, San Jose, CA) using the CellQuest software on samples from apheresis products before and after labeling, from the graft and from the NT population. Cells were analyzed for expression of CD45, CD3, αβ TCR, CD4, CD8, γδ TCR, CD19, CD20, CD16-CD56, and CD34 and for viability using 7-AAD. All antibodies were purchased from BD Biosciences. The anti αβ TCR was obtained from Miltenyi Biotec (Berghish-Gladbach, Germany). Fluorochromes used for antibody labeling have been previously reported in detail [19]. CD34⁺ cell enumeration was performed using the BD Stem Cell Enumeration Kit (BD Becton Dickinson, San Jose, CA) according to the International Society of Hematotherapy and Graft Engineering guidelines (ISHAGE) [20]. Enumeration of residual αβ T cells and B cells (defined as CD20-positive cells) was performed as recommended in the Miltenyi protocol. Phenotypes were defined by enumeration of at least 5×10^4 events, while the frequency of residual αβ T cells and B cells was determined by acquiring 5×10^5 to 10×10^5 events, a value chosen because of their expected low frequency in the depleted graft.

Statistical Evaluation

Results are given as median values with upper and lower ranges or as mean values ± SD, whenever appropriate. The Mann-Whitney test was applied to define significant or nonsignificant differences between sets of data. The Pearson correlation coefficient was used to determine the strength of correlation between sets of paired data.

RESULTS

Monitoring of CD34⁺ Cell Counts

Donor peripheral blood CD34⁺ cell counts were evaluated on day 4 to evaluate whether the donor could undergo the apheresis procedure the next day with the prediction of a sufficient cell yield or whether administration of MZ was advisable to enhance CD34 cell collection. Timing on day 4 (Figure 1A) shows a median CD34⁺ cell concentration of 31 cells/μL, with a range from 2 to 179. According to cell counts and considering the CD34⁺ cell numbers required as a function of recipient's body weight, the donors were split in 2 groups. Figure 1B shows CD34⁺ cell counts on day 4 and on day 5 before apheresis in 152 donors who did not require MZ. Starting from a median value of 37 CD34⁺ cells/μL on day 4, these donors reached a median value of 102 CD34⁺ cells/μL (range, 29 to 263) on day 5, with a 2.7-fold increase. Figure 1C shows the same information for 48 donors given MZ 9 hours before cell collection. In this group, median CD34⁺ cell count showed an 8.3-fold increase, with day 5 preapheresis median levels and ranges similar to the non–MZ-treated group. As an appropriate control, a comparison was also made with 56 donors picked up from Figure 1B (donors receiving G-CSF only) whose CD34⁺ cell counts on day 4 were lower than 40/μL (but with a predicted apheresis yield $>12.0 \times 10^6$ CD34⁺ HSC/kg recipient's body weight) and, thus, were comparable with the 48 donors who were given MZ (Figure 1C). As shown in Figure 1D, CD34⁺ cell counts showed a 4.1-fold increase, this result confirming the efficacy of MZ as a mobilization enhancer.

Effect of MZ on Cell Subsets in Apheresis Samples

Different cell subsets were analyzed in the apheresis products, comparing donors treated or not with MZ (Figure 2). Percentages of CD34⁺ HSC, CD3 T cells, αβ T cells, γδ T cells, and NK cells were not significantly different between the 2 groups (Figure 2A-E). By contrast, B cells exhibited significantly higher frequencies in samples from MZ-treated donors (Figure 2F). Similar results were observed when the total cell numbers collected in the aphereses were considered, with B cell counts being significantly higher in the MZ-treated group than in the untreated group (P .0005). A higher frequency of B cells, though, did not affect depletion efficiency

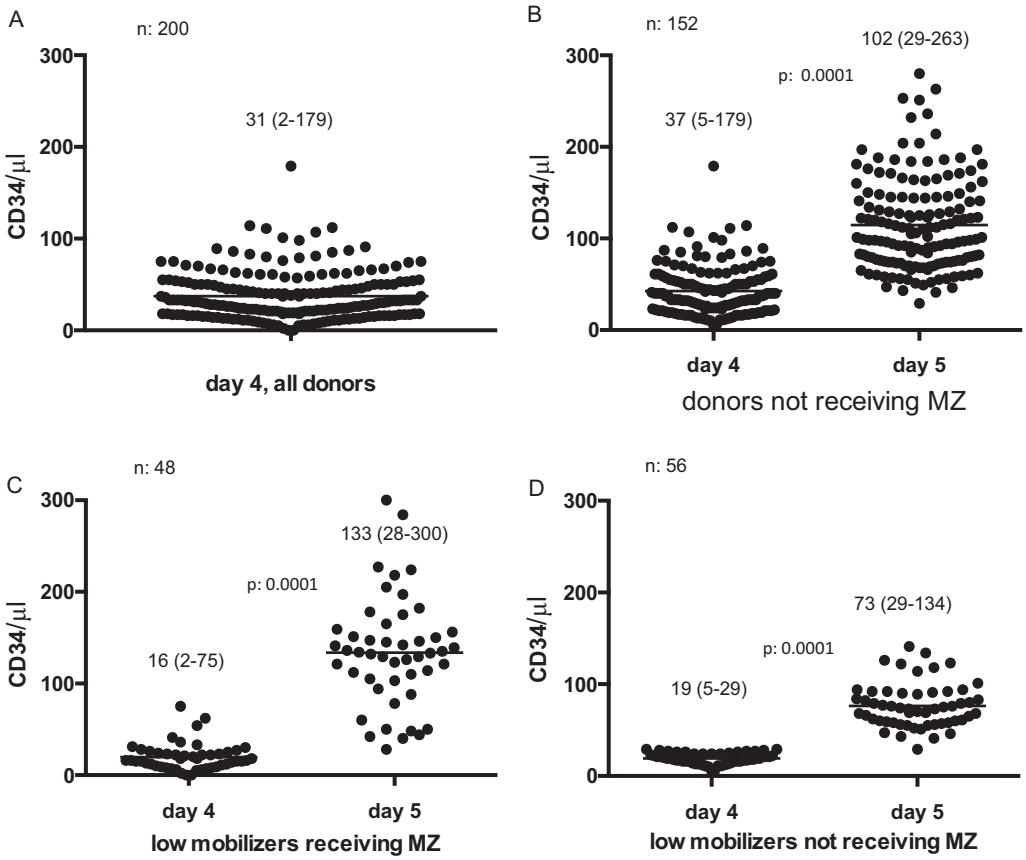


Figure 1. Preapheresis CD34⁺ HSC counts. (A) shows CD34⁺ HSC counts/µL on day 4 in donors mobilized with G-CSF, plus or minus MZ. (B) shows the increase of CD34⁺ HSC counts from day 4 to day 5 (2.7 fold, *P*, .0001) in 152 donors receiving G-CSF alone. (C) shows the increase of CD34⁺ HSC counts from day 4 to day 5 (8.3 fold, *P*: .0001) in 48 donors receiving MZ 9 hours before apheresis. For a better evaluation, (D) shows CD34⁺ HSC counts on days 4 and 5 in 56 donors not treated with MZ, who had CD34⁺ cell counts <40/µL (but with a predicted apheresis yield >12.0 × 10^6 CD34⁺ HSC/kg recipient's body weight) on day 4 and, thus, were comparable to the group of MZ-treated donors in Panel C.

(data not shown). The final product (ie, grafts) from MZ-treated donors contained significantly higher mean numbers of residual B cells, compared with the group not treated with MZ (3.0 × 10^6 versus 1.8 × 10^6, *P* .001).

Depletion Efficiency

The depletion efficiency for each procedure is shown in Figure 3. This parameter was evaluated by comparing the final products (grafts) with the precolumn samples. The median log depletion value was 4.1 for αβ T cells and 3.4 for B cells. Depletion of CD3 T cells showed a median of 1.6 log, because non-αβ T cells, namely γδ T cells, which also express CD3 on their cell surface, are left in the graft. The table inserted below the graph demonstrates that MZ had no effect on depletion efficiency for the different subsets.

Evaluation of Cell Recovery

In addition to depletion efficiency, the other relevant parameter for this procedure is recovery of valuable cells. Table 1 reports the mean percentage of recovery for total nucleated cells and for the different subsets to be preserved. CD34⁺ cells showed a recovery close to 90% (92.6 for non-MZ and 88.8 for MZ groups) when the final graft product was compared with the apheresis samples (upper panel). On the contrary, CD34 cell recovery was close to 100% (98.1 for non-MZ and 103.0 for MZ groups), when the comparison was made with the pre-column samples (lower panel). These data suggest that an estimated 10% cell loss occurs during the labeling procedure (incubations and washings), while almost no loss occurs during the column separation procedure. The same consideration can be applied to recovery of γδ T cells and NK cells by comparing upper and lower panels. MZ administration showed no effect, with *P* values between the 2 groups higher than .05 in all cases (not shown in the table).

Effect of Polymorphonuclear Cell Frequency on Cell Depletion and Recovery

A possible influence of polymorphonuclear cells (PMNC) on depletion and recovery efficiency was analyzed. Figure 4A depicts the αβ T cell depletion efficiency plotted versus the percentage of PMNC. According to the Pearson correlation coefficient (ρ –.45), we observed a moderate inverse correlation, suggesting that PMNC have little effect on depletion efficiency. A weak inverse correlation (ρ –.23) was also observed between B cell log depletion and PMNC percentage. Similarly, PMNC percentage had no effect on CD34 cell recovery (data not shown).

Cell Composition of Graft Products

The absolute cell numbers collected after the depletion procedure are shown as median values in Figure 5A. CD34⁺ cells, in particular, were collected with a median value of 400 × 10^6, ranging from 85 × 10^6 to 1080 × 10^6. NK and γδ T cells were present with similar orders of magnitude.

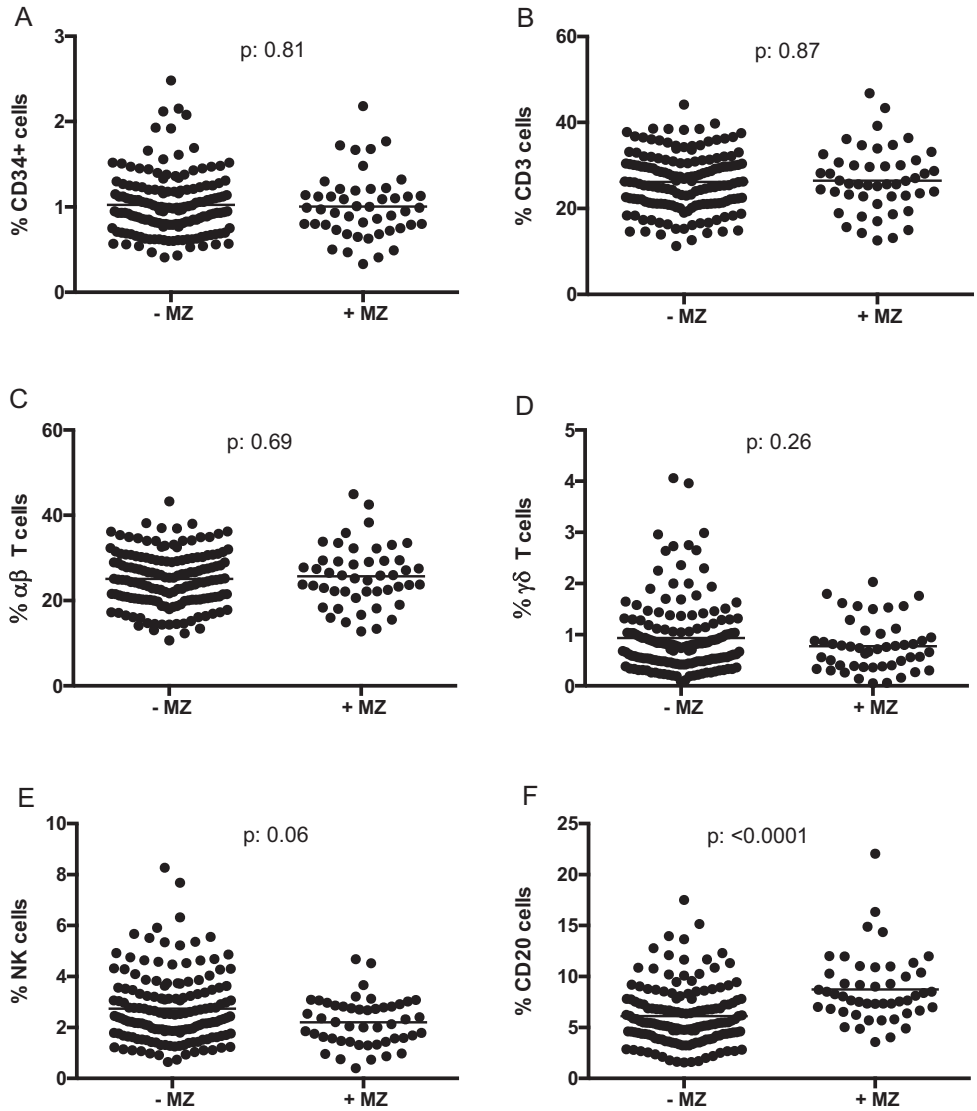


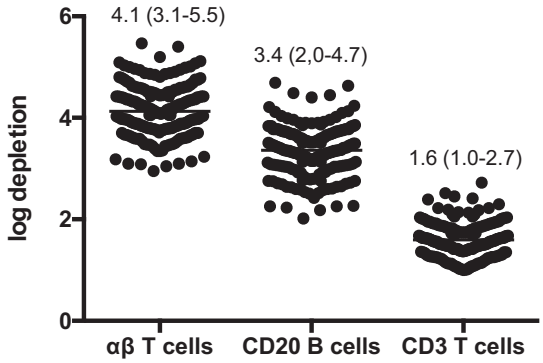
Figure 2. Effect of MZ on cell subsets in apheresis samples. The panels show the percentage of different subsets referred to total nucleated cells, in donors treated or not with MZ. (A) $CD34^+$ cells, (B) CD3 T cells, (C) αβ T cells, (D) γδ T cells, (E) NK cells, and (F) CD20 B cells. A highly significant effect of MZ (*P*: .0001) was observed on B cells only.

Figure 5B shows the number of different cell subsets reinfused per kg recipient's body weight. In particular, the number of $CD34^+$ HSC infused ranged between 6.7 $\times 10^6$/kg and 40×10^6/kg recipient body weight, with a median value of 18.5×10^6/kg recipient body weight. In some patients, we decided not to infuse the amount of $CD34^+$ HSC exceeding 40×10^6/kg recipient body weight. The residual αβ T cells and B cells in the infused grafts had a median value of .029 (range, .001 to .095) $\times 10^6$ and .033 (range, .002 to .9) $\times 10^6$/Kg recipient body weight, respectively.

It should be noted that even though MZ induced higher percentages of B cells in the apheresis samples (Figure 2F), recipients of MZ-mobilized grafts did not receive a significantly higher number of B cells/kg body weight in comparison to recipients of non–MZ-mobilized grafts ($.072 \times 10^6$ versus $.082 \times 10^6$, $P = .24$). This finding is explained by the significantly higher body weight of patients receiving MZ-mobilized grafts ($P < .0001$).

Monitoring of Procedure Performance

The data reported here have been collected over a 3-year period, namely from 2012 to 2015. The most relevant parameters (depletion efficiency for αβ T cells, B cells, and CD3 cells and recovery efficiency for $CD34^+$ cells, γδ T cells, and NK cells referred to precolumn values) are plotted on a time scale in Figure 6A,B. Mean depletion values with SD bars are shown for each semester. No statistically significant differences in cell depletion or cell recovery can be detected over time for any cell populations. Table 1 shows that overall CD34 cell recovery was around 90% if the final graft product is compared with the apheresis sample, whereas it was close to 100% if compared with the precolumn sample. This suggests that cell loss occurs during the manipulations that precede the magnetic column separation. To test whether cell loss changed over time, we plotted recovery of $CD34^+$ cells compared with the apheresis sample and with the precolumn sample at 6-month intervals, as shown in Figure 6C. Differences between



4.14 ± 0.50	3.38 ± 0.45	1.58 ± 0.31	- MZ
4.06 ± 0.46	3.30 ± 0.56	1.64 ± 0.35	+ MZ
0.35	0.53	0.56	p

Figure 3. Evaluation of depletion efficiency. Depletion efficiency of αβ T cells, CD20 B cells, and CD3 T cells, referred to the values observed in the precolumn samples. Depletion efficiency is given as median log10 depletion (with ranges in parentheses) as described in Methods. The inset table indicates the log depletion mean values in donors who received or did not receive MZ.

Table 1
Effect of MZ Administration on Recovery of Different Cell Subsets

A		Recovery versus Apheresis, %			
MZ	NC	CD34+	CD3	γδ	$CD16^+ 56^+$
–	57.4 ± 10.0	92.6 ± 9.4	2.8 ± 2.0	79.0 ± 28.8	79.2 ± 13.5
+	53.9 ± 10.1	88.8 ± 17.8	2.4 ± 1.5	87.0 ± 35.5	85.6 ± 24.6
B		Recovery versus Precolumn, %			
MZ	NC	CD34+	CD3	γδ	$CD16^+ 56^+$
–	62.7 ± 10.7	98.8 ± 9.4	3.3 ± 2.3	87.6 ± 27.5	90.7 ± 16.9
+	63.6 ± 16.4	103.0 ± 16.3	3.0 ± 2.0	100.3 ± 61.1	100.6 ± 31.2

Mean values (SD) of different cell subsets ($CD34^+$, γδ T cells, NK cells) expressed as percentage of recovery. Upper panel: recovery referred to apheresis samples. Lower panel: recovery referred to precolumn samples. No significant differences were observed for any cell subsets in the G-CSF and the $G\text{-}CSF^+$ MZ-treated groups.
NC indicates nucleated cells.

the 2 modes of recovery evaluation are highly significant during the first 2 semesters but lose significance later on. This suggests that cell manipulations preceding column loading were performed with increasing efficiency after the technical staff had acquired a better training and larger experience.

DISCUSSION

HLA-haploidentical HSCT, either T cell depleted or unmanipulated, has been increasingly used in the recent decades, even if several drawbacks must be kept in mind [1,2]. In particular, T cells in the graft, if not depleted or not strongly modulated in their alloreactive potential, are responsible for the occurrence of life-threatening or even lethal GVHD. Thus, in the past, ex vivo T cell–depleted HLA-haploidentical HSCT were performed either through a positive selection of $CD34^+$ cells or through a removal of $CD3^+$ T cells in combination with $CD19^+$ B cells [17,18]. Unfortunately, both these approaches result in loss of certain cell subsets that may play a positive role in HSCT recipients [21,22]. In fact, although T

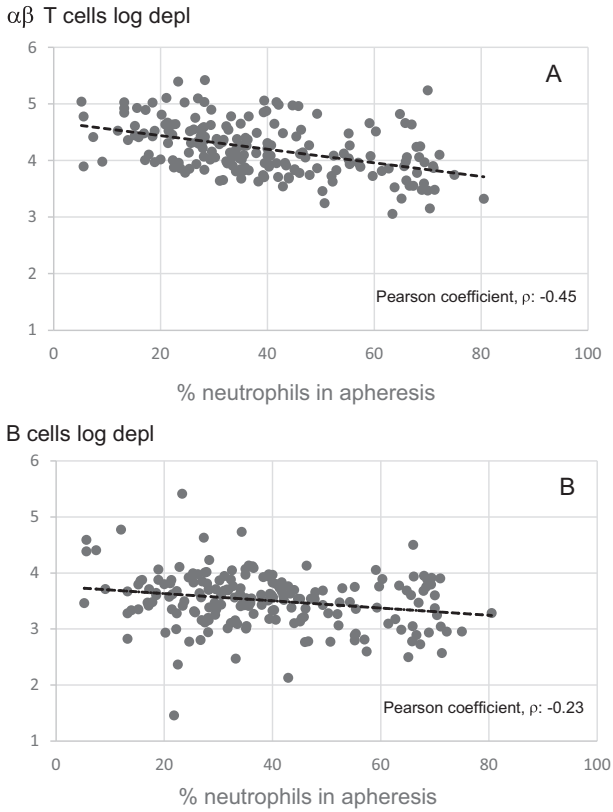


Figure 4. PMNC effect on cell depletion and recovery. Plotting PMNC percentages in apheresis samples versus αβ T cell log depletion (A) and versus B cell log depletion (B) shows a moderate and weak correlation respectively, according to the Pearson correlation coefficient.

cells displaying the αβ receptor are responsible for GVHD [15], T cells carrying the γδ receptor chains have no alloreactive capacity, but they contribute an important anti-infectious activity [15,16] in addition to a proposed anti-leukemia role [12,14,23]. Also NK cells, lost in procedure of positive selection of $CD34^+$ cells, exhibit an anti-leukemia effect [24-30], may act as graft-facilitating cells [31], and participate in control of opportunistic infections [32,33]. Finally, DC and monocytes can also play in vivo a role as graft-facilitating cells [34-38], both in humans and in animal models, and can act as professional antigen-presenting cells to help expansion of the reconstituted T cell repertoire. Keeping in mind these biological aspects, a more selective procedure for manipulation of HLA-haploidentical apheresis products has been introduced [4,5]. This strategy is based on selective removal of T cells that express the αβ receptor chains, a population that fully encompasses the alloreactive precursors and, thus, is responsible for GVHD [10]. During the same manipulation procedure, CD19 B cells are simultaneously removed to prevent Epstein Barr virus–related post-transplantation lymphoproliferative disease. This manipulation spares γδ T cells, NK cells, and DC monocytes, preserving the clinical benefits mentioned above.

The αβ T cell and B cell procedure has been described in detail [9] and the application of the CliniMACS device for automated depletion has been reported [10]. It was also demonstrated that the clonogenic potential of $CD34^+$ HSC after depletion remained unaffected [10]. New developments are now in sight following the reports that a novel humanized

Figure 5. Median composition of the grafts. (A) shows the different cell subsets determined in the 200 grafts on a log scale as median numbers of cells present in the final products (grafts). Ranges are shown on top of the bars. (B) indicates the median cell composition and ranges of infused graft products per kg recipient body weight.

anti-αβ TCR antibody, endowed with an improved capacity to target $CD4^+$ T cells for GVHD prevention both in vitro and in vivo in a mouse-human model of HSCT [39,40], may become available for clinical use.

In the present study, we analyzed the efficiency of the separation procedure by using a sealed, unbreached system from start to end. In fact, all cell manipulations were performed using bags and sealing systems for tubing connections [41,42]. This method increases procedure safety and allows the use of a clean room instead of a Good Manufacturing Procedure (GMP) facility, as required for cell manipulation in open systems.

The depletion rates we attained were comparable to those reported in other studies [9,10,43,44], with median depletion logs of 4.1 for αβ TCR T cells and 3.4 for B cells. In addition, a recent report [11] describing data on immune recovery in 26 patients receiving αβ T cell– and B cell–depleted HLA-haploidentical HSCT showed depletion and recovery efficiencies similar to those we observed.

The present study showed that MZ had no effect on cell composition of the apheresis samples, with the only relevant exception of B cells, which were significantly increased. However, this did not translate in significantly higher numbers

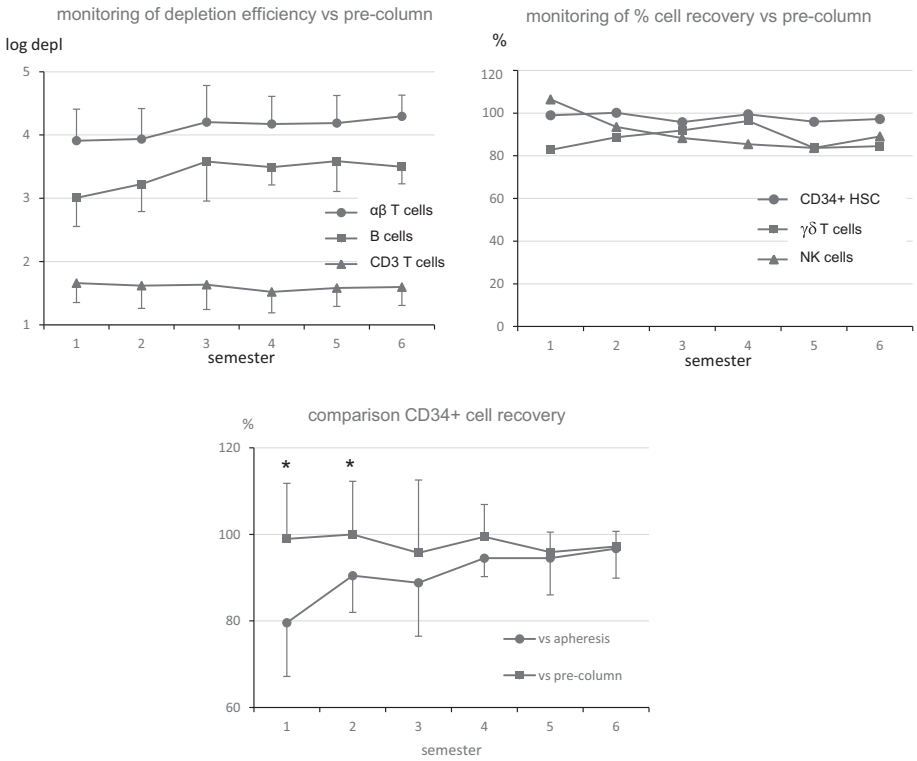


Figure 6. Monitoring of cell depletion and cell recovery. Data were analyzed at 6-month intervals. (A) Shows depletion efficiency for αβ T cells, B cells, and CD3 T cells over 6 semesters. The trend towards higher depletion efficiency for αβ T cells and B cells was not significant. (B) Shows percent cell recovery for CD34⁺ HSC, γδ T cells, and NK cells. Also in this case fluctuations were not significant. (C) Shows CD34⁺ HSC recovery in comparison with apheresis samples and with precolumn samples. The initial discrepancy, highly significant for the first 2 semesters (*P*, .0005 and .0008, respectively), vanished at later time points ($P > .05$).

of B cells reinfused into the patients. This observation complements previous data on a smaller cohort of HSC donors showing that mobilization with G-CSF alone or with MZ had no measurable effect on the relative frequency of naïve/memory B cell subsets [19].

CD34⁺ cells present in the mobilized apheresis were recovered with high efficiency. Similar high efficiency was seen for recovery of γδ T cells and NK cells. It should be noted here that evaluation of depletion efficiency is based on enumeration of very few residual cells, whose frequency in the final graft product may be hard to estimate with accuracy and which, together with the wide range of the patient body weight in a pediatric population, can contribute to explain some outlier values. After an initial recommendation [10], a more recent paper [44] stressed this issue and presented an accurate discussion on flow cytometric quantification of low-frequency cells that persist as contaminants after αβ T cell and B cell depletion.

Labeling of all cells contained in the apheresis bag is essential for efficient depletion, especially in the perspective of reaching depletion of αβ T cells greater than 4 logs. In fact, some cells can escape labeling if clumped or hidden in recesses, such as bag corners or wrinkles and dead ends of tubing stumps. In these cases, antibodies may not reach all the cells, resulting in labeling efficiency lower than 100%. This risk can be reduced by thorough cell resuspension to disrupt clusters and to facilitate antibody access to all hidden cells. The impact inaccurate labeling may have on depletion efficiency can be quantitatively visualized by using a nomogram we have constructed (Figure 7). The nomogram demonstrates that, particularly for high-depletion efficiencies such

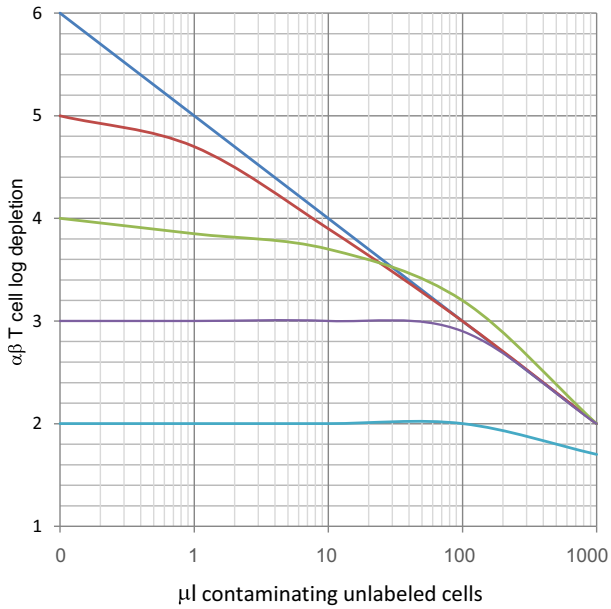


Figure 7. Nomogram to estimate reduction of depletion efficiency as a function of contamination with unlabeled cells. The nomogram was constructed to estimate the influence of unlabeled cells on cell depletion efficiency. Theoretical or expected log depletion is given on the Y axis. The X axis shows the µL of cells that escaped labeling and contaminated the processed product, with reference to a total manipulated volume of 100 mL. As an example, if a log depletion of 5 is expected with 0 µL contaminating cells, 10 µL of residual unlabeled cells that contaminate the final product decrease depletion efficiency by 1 log. This theoretical calculation is independent of the actual cell concentration in the bag. It can be noted that reduction of final log depletion correlates positively with the amount of contaminating cells and negatively with the expected depletion efficiency.

as those obtaining 4- or 5-logs removal of the original population, as often attained in standard procedures, a contamination of the final product with as little as 100-μL unlabeled cells may reduce depletion efficiency by 1 or 2 logs, respectively. In practice, a depletion efficiency of 4 logs may be the best possible achievement with 100% labeling efficiency for that procedure or could be a 5-log depletion in principle, reduced in practice by 1 log simply because 10 μL cells escaped labeling. The figure legend describes how this nomogram can be used as a theoretical predictor of graft contamination with residual unlabeled cells in relation to the expected and to the measured depletion efficiency.

$CD34^+$ cells present in the mobilized apheresis were recovered with high efficiency in the final product. In particular, the significant increase of recovery efficiency during the first year suggests, as already mentioned in the Results, that training and experience of the technical staff are important parameters for optimal performance. In contrast, no significant changes were observed over time for depletion efficiency of αβ T cells and B cells.

In summary, this study has shown that several issues should be considered to optimize the procedure in terms of cell depletion and recovery. Staff proficiency may help reduce cell loss during the initial manipulation phases, before the cells are loaded on the CliniMACS device. The technical staff must also be aware that even apparently negligible volumes of unlabeled cells may severely affect cell-depletion efficiency and, thus, they must ensure that cells are thoroughly dislodged from all bag recesses during the labeling step. The αβ T cell–depletion procedure performed in a single center with commercial reagents and supplies proved to be robust and reproducible, since our results are in agreement with the figures reported by other centers in studies based on smaller numbers of cases [10,11,40,43,44].

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