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Depletion of T-cell receptor alpha/beta and CD19 positive cells from apheresis products with the CliniMACS device

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Abstract

Background aims. The CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany) was used for depletion of T-cell receptor alpha/beta positive (TCR$\alpha\beta^+$) and CD19 positive ($CD19^+$) cells from apheresis products. *Methods.* Investigators performed 102 separations. Apheresis products with a median 5.8 (minimum to maximum, 1.2–10.4) $\times 10^{10}$ mononuclear cells were used with a median 358 (92–1432) $\times 10^6$ $CD34^+$ cells. There were 24.8% (6.1–45.7%) median TCR$\alpha\beta^+$ cells and 4.4% (1.2–11.7%) median B cells in the apheresis product. *Results.* After depletion, a median 0.00097% (0.00025–0.0048%) of TCR$\alpha\beta^+$ cells could be detected, and B cells, as determined as $CD20^+$ cells, were reduced to 0.0072% (0.0008–0.072%). TCR$\alpha\beta^+$ cells were depleted by log 4.7 (3.8–5.5), and B cells were depleted by log 4.1 (3.0–4.7). Recovery of mononuclear cells was 55% (33–77%), and recovery of $CD34^+$ cells was 73% (43–98%). Recovery of $CD56^+/3^-$ natural killer cells was 80% (35–142%), recovery of TCR gamma/delta positive (TCR$\gamma\delta^+$) T cells was 83% (39–173%) and recovery of $CD14^+$ cells was 79% (22–141%). Viability of cells was 98% (93–99%) after separation (all values median). *Conclusions.* Profound depletion of TCR$\alpha\beta^+$ T cells can be achieved with the CliniMACS system. Recovery of $CD34^+$ stem cells is in the same range than after $CD34^+$ enrichment and CD3/CD19 depletion. Transplantation with $>4 \times 10^6$ $CD34^+$ cells/kg can be performed for every patient with 1–5 $\times 10^4$ TCR$\alpha\beta^+$ cells/kg and about 5–10 $\times 10^6$ TCR$\gamma\delta^+$ cells/kg with two rounds of apheresis.

Key Words: *allogeneic transplantation, B cell, CliniMACS, depletion, flow cytometry, T cell*

Introduction

Allogeneic hematopoietic cell transplantation from human leukocyte antigen-mismatched haploidentical donors is one possible strategy when a human leukocyte antigen-identical donor is unavailable (1). In most cases, *ex vivo* depletion of T cells and B cells has been used to avoid extensive graft-versus-host disease, which has been reported after transplantation of un-manipulated stem cell grafts (2). Depletion can be performed either by enrichment of CD34 cells or depletion of CD3 and CD19 positive T cells (3,4). However, using standard CD3 depletion techniques, T-cell receptor gamma/delta positive (TCR$\gamma\delta^+$) T cells are also eliminated despite not being involved in classic graft-versus-host disease. TCR$\gamma\delta^+$ T cells have been reported to exert anti-leukemic activity by several authors (5,6). Depletion of TCR alpha/beta positive ($\alpha\beta^+$) cells alone might be advantageous over the depletion of all T-cell subsets as illustrated in first reports of clinical transplantations with this strategy (7). Since 2009, a TCR$\alpha\beta$ depletion reagent has been available for the CliniMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany), which we used for validation of the procedure with mobilized apheresis products first and mobilized apheresis products not used for transplantation purposes thereafter. Clinical products were produced after manufacturing authorization was received from local authorities and used for transplantation within a compassionate use program in a first group of patients. We summarize our results using the CliniMACS device for combined TCR$\alpha\beta$ and CD19 depletion of apheresis products intended for transplantation from haploidentical donors.

Methods

All preparations were performed according to the manufacturing license under Good Manufacturing Practices conditions in appropriate clean room areas. Manipulation of open cell products was exclusively

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performed in a class A workbench with grade B background with continuous particle monitoring.

Depletion was performed using the CliniMACS device. Apheresis products were from healthy donors after 5 days of mobilization with 2 × 5 mg/kg body weight of granulocyte colony-stimulating factor administered subcutaneously. Products obtained from validation runs before manufacturing license were not used for transplantation. Apheresis cells were stored overnight and processed the next day. Cells were filled into two to four sterile conical centrifuge tubes (175 mL), filled up with CliniMACS buffer and centrifuged at 400*g* for 15 min, 10°C, brake 8 out of 10. Supernatant was discarded, and cells were re-suspended using plastic pipettes. The volume was adjusted to 45 mL in each tube, and 3.75 ml of CliniMACS TCR$\alpha\beta$ Biotin Reagent (Miltenyi Biotec) was added. Cells were incubated at room temperature on a rotating shaker at 2 rpm for 30 min. Tubes were filled with MACS buffer, centrifuged and re-suspended to 45 mL each. CliniMACS Anti-Biotin Reagent, 7.5 mL, and CD19 reagent, 3.75 mL or in some cases 7.5 mL, were added, and cells were incubated again as previously. Cells were washed and re-suspended again, transferred to a transfer bag and adjusted to a defined volume of 100 mL, 150 mL, 200 mL or 250 mL depending on the overall cell count. The bag was connected to the CliniMACS Depletion Tubing Set (Miltenyi Biotec) and CliniMACS device in a class A environment. Program Depletion 3.1 was used. After depletion, cells were transferred to tubes again, washed and re-suspended in 0.9% NaCl solution for infusion. Samples were taken from apheresis cells (apheresis), cells after magnetic labeling (labeled) and cells in the bag after separation (negative fraction).

Samples for quality control analysis were taken to obtain cell number and volume in all samples, flow cytometry in apheresis cells and negative fraction, sterility in apheresis cells and cells ready for infusion. CD34 enrichment and CD3 depletion used for comparison of techniques were performed as described previously (8,9).

For flow cytometry, viable TCR$\alpha\beta^+$ cells were defined by their cell scatter characteristics of lymphocytes, negativity for propidium iodide staining and positivity for CD3 and TCR$\alpha\beta$ without positivity for TCR$\gamma\delta$ (Table I). A sample of depleted cells was spiked with about 3% of cells from the positive fraction, and the gate for TCR$\alpha\beta^+$ cells was set using the dot plot of this sample. A minimum of 1×10^6 events was acquired for analysis, and the gate for detection of TCR$\alpha\beta^+$ cells was set first on the TCR$\alpha\beta^+$ population in the TCR$\alpha\beta^+$/CD19$^+$ positive cell sample (Figure 1).

Table I. Antibody panel for multi-gating analysis after depletion.

	FITC	PE	PerCP	APC	No. events
1	msIgG1	msIgG1	PI	msIgG1	50,000
2	20	19	PI	45	1,000,000
3	45	34	PI	3	50,000
4	45	34	PI		50,000
5	14	56	PI	3	50,000
6	TCR$\gamma\delta$	3	PI	TCR$\alpha\beta$	1,000,000
7	TCR$\gamma\delta$	3	PI	TCR$\alpha\beta$	1,000,000

APC, allophycocyanine; FITC, fluorescein isothiocyanate; msIgG1, mouse immunoglobuline G1; PE, phycoerythrin; PerCP, peridinin chlorophyll protein complex.

Results

We performed 102 separations. Apheresis products with a median 5.8 (minimum to maximum, 1.2−10.4) × 10^{10} mononuclear cells and 358 (92−1432) × 10^6 CD34$^+$ cells were used. There were 24.8% (6.1−45.7%) median TCR$\alpha\beta^+$ cells and 4.4% (1.2−11.7%) median B cells in the apheresis product. After depletion, a median of 0.00097% (0.00025−0.0048%) of TCR$\alpha\beta^+$ cells could be detected, and B cells, as determined as CD20$^+$ cells, were reduced to 0.0072% (0.0008−0.072%). TCR$\alpha\beta^+$ cells were depleted by log 4.7 (3.8−5.5), and B cells were depleted by log 4.1 (3.0−4.7) (Table II). Recovery of mononuclear cells was 55% (33−77%), and recovery of CD34$^+$ cells was 73% (43−98%). Recovery of CD56$^+$/3$^-$ natural killer cells was 80% (35−142%), recovery of TCR$\gamma\delta^+$ cells was 83% (39−173%) and recovery of CD14$^+$ cells was 79% (22−141%) (Table III). Viability of cells was 98% (93−99%) after separation (all values median).

A median reduction of platelets of 88% was achieved with <5% of standard deviation by the staining and washing procedure (Table IV). Cell numbers were reduced by the staining and washing procedure to 93% of the starting number (Table V).

Comparison with other techniques of depletion (i.e., CD34 enrichment and CD3/CD19 depletion) revealed comparable performance of TCR$\alpha\beta$ depletion with log 4.7 with CD34 enrichment (log 4.6) and significantly better performance than CD3/CD19 depletion (log 4.0, $P < 0.0001$). Recovery of CD34$^+$ stem cells (74.3%) was comparable to CD34 enrichment (72.8%) and CD3/CD19 depletion (69.3%). In addition, results were more constant with TCR$\alpha\beta$ depletion; co-efficients of variation were lower than in both other techniques (Figures 2 and 3).

Discussion

Depletion of peripheral blood hematopoietic cells with the CliniMACS CD3 reagent and system is an