Kinetics of recovery of dendritic cell subsets after reduced-intensity conditioning allogeneic stem cell transplantation and clinical outcome

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ABSTRACT

Background and Objectives

Dendritic cells (DC) play a critical role in the regulation of alloimmune responses and might influence the outcome of allogeneic stem cell transplantation (allo-SCT). We studied the clinical relevance of early reconstitution of DC after reduced-intensity conditioning allo-SCT (allo-RIC).

Design and Methods

This study included 79 adult patients undergoing allo-RIC from HLA-identical siblings. Peripheral blood samples were drawn from patients at 1 month (+1m) and 3 months (+3m) after the transplant. DC were identified as positive for HLA-DR and negative for CD3, CD19, CD14 and CD56. The expression of CD33, CD123 and CD16 was used to identify myeloid DC, plasmacytoid DC and $CD16^+$ DC subpopulations, respectively.

Results

Patients whose DC count at +1m was lower than the median had a higher probability of treatment-related mortality (TRM) (60% *vs* 12%; p=0.02), poorer overall survival (OS) (15% *vs* 45%; p=0.002) and worse event-free survival (EFS) (20% *vs* 38%; p=0.03). A multivariate analysis confirmed that low DC counts had a detrimental effect on OS (RR 3.2; p=0.007), relapse (RR 4.1; p=0.01), and EFS (RR 6; p=0.001). Low $CD16^+$ DC counts were observed to have a detrimental effect on EFS, which was due to both a higher incidence of deaths caused by infections (50% *vs* 0%, p=0.05) and a higher incidence of relapse (57% *vs* 50%; p=0.03). Indeed, the number of $CD16^+$ DC at +3 m was the most important prognostic factor for EFS (RR 6; p=0.001).

Interpretations and Conclusions

This study shows the clinical importance of DC recovery, especially of the $CD16^+$ DC subset, in the outcome of patients treated with allo-RIC.

Key words: dendritic cells, $CD16^+$ DC, reduced-intensity conditioning allogeneic stem cell transplantation.

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Allogeneic stem cell transplantation (allo-SCT) has an important curative potential in many hematologic malignancies,[1] which is mainly due to the graft-versus-leukemia effect.[2] On the other hand, allo-SCT is associated with considerable treatment-related mortality as a result of graft-versus-host disease (GVHD) and severe infections.[3] The graft-versus-leukemia effect, GVHD and defence against infections are mediated through the innate and adaptive immune system. Not surprisingly, a rapid and robust reconstitution of neutrophils, monocytes, B, T and NK cells after the transplant is a very important factor for improved clinical outcome.[4-6] Dendritic cells (DC) are key players in the immune system. They have a particular capacity for antigen uptake and processing, and they are characterized by their capacity to prime naïve $CD4^+$ and $CD8^+$ T cells.[7-10] By activating antigen-specific T cells, DC constitute an essential link between the innate and the adaptive defence systems. The role of DC might be of special importance in allogeneic transplants with reduced conditioning regimen (allo-RIC), in which the cure of the patient is based on alloimmune reactions.

DC not only initiate but also direct alloimmune responses. Thus, depending on the subset and maturation state of DC, these cells can promote or inhibit antigen-specific T-cell activation.[10,11] DC were first identified by lack of lineage-specific markers (CD3, CD14, CD19, CD56) (Lin^-) and by the expression of high levels of major histocompatibility complex class II ($HLA\text{-}DR^{++}$). Typically two distinct lineages of DC have been described in human peripheral blood: myeloid DC ($CD33^+$ $CD123^{dim}$) and plasmacytoid DC ($CD33^-$ $CD123^{bright}$).[12,13] Myeloid DC and plasmacytoid DC are functionally distinguished by their patterns of cytokine production, capacity to polarize T-cell reactions, migration behavior, and handling of pathogens.[14-16] Recently, a third subset of DC has been identified by the M-DC8 monoclonal antibody: this new subset has high expression of FcγRIII (CD16), a marker absent in the other DC subsets[17-19] and shows reactivity for DC-SIGN, a C-type lectin exclusively expressed by DC.[20] DC $CD16^+$ share with myeloid DC high expression of CD11c and low expression of CD123 as well as antigens typical of myeloid cells.[21] This newly identified subset of DC appears to be one of the most potent allostimulatory Lin^- $HLA\text{-}DR^{++}$ cells types from peripheral blood[10] and is considered pro-inflammatory with a high potential to produce tumor necrosis factor-α (TNF-α). Almeida *et al.* demonstrated that myeloid DC, plasmacytoid DC and $CD16^+$ DC display different morphological, cytochemical, immunophenotypic, and functional characteristics compared to those of mature monocytes.[18]

On this background, we hypothesized that DC reconstitution is important in the clinical evolution after allo-RIC and that the relative contributions of myeloid DC, plasmacytoid DC, and $CD16^+$ DC to the graft-versus-leukemia effect, GVHD and defense against infections after allo-RIC might be different. As a consequence, the influence of rapid reconstitution of total DC and DC subsets on survival parameters might also be different. We analyzed these issues in a homogeneous group of patients undergoing allo-RIC.

Design and Methods

Patients

This study included 79 adult patients undergoing an allogeneic transplantation for hematologic diseases between October 2002 and November 2004 in five Spanish hematopoietic cell transplant units. All patients were treated with reduced-intensity regimens and received peripheral blood stem cells from a sibling HLA-identical donor. The patient's characteristics are shown in Table 1. Peripheral blood samples were drawn from patients at 1 month (median 35 days, range, 22-52) and 3 months (median 96 days; range, 82-117) after the transplant to evaluate immune reconstitution. This study was approved by the local Ethics Committee and by the Spanish Health Department, and informed consent was obtained from all patients.

Conditioning regimens and GVHD prophylaxis

Two regimens for conditioning were used depending on the disease requiring transplantation; one was recommended for myeloid malignancies and the other for lymphoid malignancies.[22,23] The myeloid regimen consisted of fludarabine 30 mg/m^2 administered intravenously on days -9 to -5 together with ten doses of oral busulphan 1 mg/kg (days -6 to -4, total 10 mg/kg), with phenytoin given as anticonvulsant prophylaxis. The lymphoid regimen consisted of the same doses of fludarabine, followed by melphalan 70 mg/m^2 intravenously on days -3 and -2. Post-transplant GVHD prophylaxis was based on cyclosporine A plus methotrexate (78%), or cyclosporine A plus mycophenolate (22%) (Table 1). The diagnosis and grade of acute and chronic GVHD were determined according to the Seattle criteria.[24] Chronic GVHD was defined if GVHD was present at day 90 or onwards after transplantation.

Donors

Donors received granulocyte-colony stimulating factor (G-CSF; Filgrastim; Amgen) at a dose of 10 μg/kg/day subcutaneously for 5 days. Leukapheresis was started on day 5 and repeated the next day, if necessary, to achieve a target dose of $\geq 4\times10^6$ $CD34^+$ cells/kg of recipient weight.

Immunophenotypic studies

Samples were prepared as previously described.[25] Monoclonal antibodies used for analyzing DC were conjugated with different fluorochromes: fluorescein isothiocyanate (FITC) for monoclonal antibodies specific of

lineage (anti-Lin) such as CD3, CD14, CD19, and CD56; phycoerythrin (PE) for CD123 and CD16; peridin chlorophyll protein (PerCP) for anti-HLA-DR; and allophycocyanin (APC) for CD33. DC were identified as positive for HLA-DR and negative for CD3, CD19, CD14 and CD56 lineage markers. DC were classified as myeloid DC based on their expression of CD33, as plasmacytoid DC based on their expression of CD123 (interleukin-3 receptor α chain), and as $CD16^+$ DC if they expressed $CD16^+$ (Figure 1). All cell counts and immunophenotyping analyses were performed in a single center (Hospital Clinic, Barcelona, Spain).

Statistical analysis

The primary aim of the study was to analyze the association of the number of DC at 1 and 3 months post-transplant with survival parameters such as treatment-related mortality (TRM), overall survival (OS), and event-free survival (EFS). The secondary aim was to analyze the impact of DC counts on the graft-versus-leukemia effect, measured as relapse rate, and on GVHD. The analyses were performed first considering the total number of DC and then with respect to the numbers of myeloid DC, plasmacytoid DC, and $CD16^+$ DC. Patients who died of a transplant-related cause were censored at their last follow-up. OS was calculated from transplantation until death from any cause, and surviving patients were censored at their last follow-up. EFS was calculated from transplantation until disease progression or death, and those patients who did not have any response (complete or partial) after transplantation were considered to have experienced an event. OS and EFS were calculated using Kaplan-Meier product-limit estimates and compared with a log-rank test. Univariate Cox regression analysis was used to investigate the impact of time-dependent covariates on survival. The probability of TRM, relapse, and acute and chronic GVHD was determined by calculating the cumulative incidence and compared by Fine and Gray's method. The characteristics considered in this study were gender-pairing; patient's age, diagnosis; disease status at transplant; conditioning regimen; pre-transplant cytomegalovirus serology of the donor and recipient; numbers of DC, their subsets, and $CD34^+$ cells and $CD3^+$ cells infused with the inoculum; and reconstitution of DC and their subsets. All prognostic variables with a p value <0.2 in the univariate analysis were included in a stepwise proportional hazard Cox's regression model for OS and EFS, and using Fine and Gray's multivariate method, for TRM, relapse, and GVHD. Significance levels were set at $p \leq 0.05$. The proportional hazard assumption was checked separately for each covariate before performing the multivariate analysis. This check was done using a graphical and analytical

Table 1. Characteristics of the patients at the time of transplantation.

Characteristics	
Number	79
Age in years, median (range)	52 (23-69)
Female donor to male recipient	23 (29%)
Advanced disease status (*)	59 (75%)
Diagnosis	
Acute myeloid or lymphoid leukemia/myelodysplastic syndrome	34 (43%)
Multiple myeloma	19 (24%)
Non-Hodgkin's lymphoma	10 (12%)
Chronic lymphocytic leukemia	6 (8%)
Hodgkin's lymphoma	10 (12%)
Conditioning regimen	
Fludarabine + busulphan	33 (41%)
Fludarabine + melphalan	46 (58%)
GVHD prophylaxis	
CyA + methotrxate	62 (78%)
CyA + mycophenolate mofetil	17 (22%)
Graft characteristics	
$CD34^+ \times 10^6$/kg	5.9 (2.2-8.6)
$CD3^+ \times 10^8$/kg	2.3 (1.7-6.7)
Median follow-up, median (range)	266 days (37-660)

GVHD: graft- versus-host disease; CyA: cyclosporine A; (*) Advanced disease status was defined as the presence of acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) in second or later remission; AML or MDS refractory or in relapse; chronic myeloid leukemia in second chronic phase or accelerated phase or blastic-phase; lymphoid malignancy in second or later remission or in relapse.

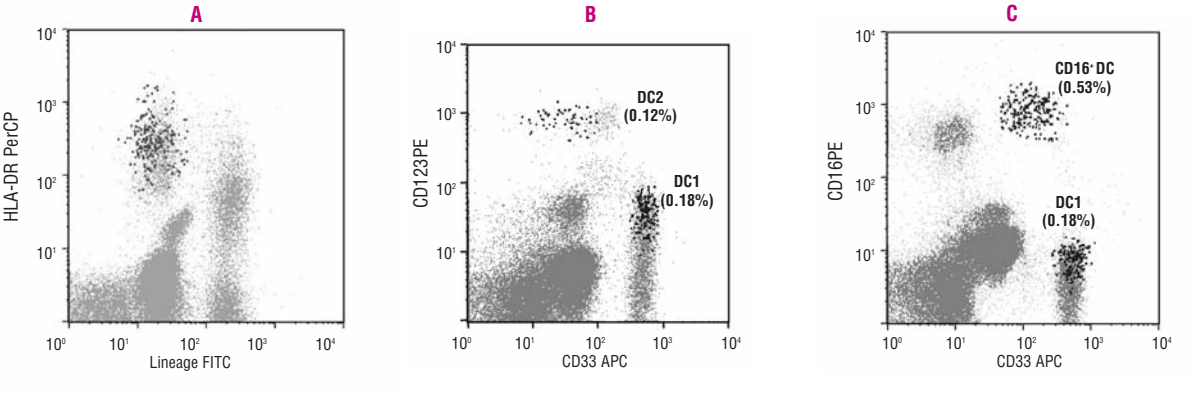

Figure 1. Four-color flow cytometry phenotypic analysis of DC subsets in peripheral blood from healthy donors before G-CSF treatment. A total of 150,000 events were collected from each tube. All nucleated cells present in peripheral blood are shown. (A) DC subsets were characterized by positivity for HLA-DR and negativity for lineage-specific markers. (B and C) The DC subsets were analyzed for the expression of CD33, CD123 and CD16, indicating myeloid DC, plasmacytoid DC and $CD16^+$ DC, respectively.

method for the Cox model and by Schoenfeld residuals for the Fine and Gray multivariate method. The statistical studies were performed using SPSS software (12.0 Chicago, IL, USA) and Cran R software (*cmpr* package).

Results

Dendritic cell counts

The median and the range of the counts of total DC and of the myeloid DC, plasmacytoid DC and $CD16^+$ DC subsets measured in peripheral blood of the recipients at 1 and 3 months after allo-RIC are shown in Table 2. The median value was used to group patients into those with low or high DC counts. There were no single associations between the patients' pre-transplant characteristics and DC counts at 1 or 3 months post-transplant (Table 3). Peripheral blood from 39 healthy donors was used to obtain normal values of DC.

Hematologic recovery

Patients reached levels of more than 0.5 granulocytes $\times 10^6$/mL at a median of 16 days (range, 0-27 days) post-transplantation and more than 20 platelets $\times 10^6$/mL at a median of 12 days (range, 0-32 days) post-transplantation. The time to granulocyte engraftment was 15 days (range, 0-24 days) *vs* 16 days (range, 1-27 days) in the group of patients with low *vs* high DC counts 1 month post-transplantation, respectively (*p=NS*). The time to platelet engraftment was 12 days (range, 0-22 days) *vs* 12 days (range, 0-32 days) in the group of patients with low *vs* high DC count at 1 month post-transplantation, respectively (*p=NS*).

DC count and survival parameters

There were 13 deaths associated with the transplantation procedure in the group of patients with a low DC count at 1 month post-transplantation as compared with five deaths in the high DC count group (TRM 45% *vs* 14%, χ^2 test p=0.01; 60% *vs* 12%, log rank p=0.02). A low DC count at 1 month after tranplantation was also associated with a significantly worse OS (15% *vs* 45%; p=0.002). Of note, a low DC count 1 month after transplantation was the most important independent prognostic factor for OS in the multivariate analysis (RR 3.2; 95% CI, 1.4–7.3; p=0.007); other prognostic factors for OS were acute GHVD grades II-IV (RR 2.7; 95% CI 1.25-5.83; p=0.01) and advanced disease (RR 2.3; 95% 1.01-

Table 2. DC recovery after transplantation.

	Time after transplantation		*Normal values*
	1 month	*3 months*	
Myeloid DC	4 (0-174)	4 (0-86.4)	15 (1.5-28)
Plasmacytoid DC	2.3 (0-124)	2 (0-42)	14 (1.1-45)
$CD16^+$ DC	23.5 (0-276)	15 (0-186)	40 (3.7-89)

Values are given as $\times 10^3$/mL and median (range).

Table 3. Characteristics of the groups with low or high DC counts after transplantation.

Characteristics	*1 month post-transplant*		*p*	*3 months post-transplant*		*p*
	Low	*High*		*Low*	*High*	
Age of patients, median (range)	52 years (20-69)	53 years (29-69)	NS	53.5 years (21-69)	48 years (17-69)	NS
Donor female to male	9/34	10/35	NS	9/26	7/26	NS
Advanced disease status	25/34	26/35	NS	23/26	19/26	NS
Diagnosis						
AML/ALL/MDS	14/34	15/35	NS	11/26	9/26	NS
MM/NHL/HD/CLL	18/34	18/35	NS	13/26	17/26	NS
Other	2/34	2/35	NS	2/26	0/26	NS
Conditioning regimen						
Fludarabine + busulphan	13/34	19/35	NS	10/26	10/26	NS
Fludarabine + melphalan	20/34	17/35	NS	15/26	16/26	NS
Graft characteristics						
$CD34^+ \times 10^6$/kg	5.55 (2.25-8.6)	6.04 (3.1-8)	NS	5.8 (2.7-8.6)	5.7 (3.9-8)	NS
$CD3^+ \times 10^8$/kg	2.26 (0.03-4.4)	2.33 (0.02-6.7)	NS	2.8 (0.42-6.7)	2.6 (0.02-5.6)	NS
Engraftment parameters						
Granulocytes 0.5×10^6/mL*	16 days (0-30)	16 days (1-27)	NS	16 days (0-22)	16 days (11-27)	NS
Platelet count 20×10^6/mL*	13 days (0-28)	12 days (0-32)	NS	12 days (0-28)	11 days (9-45)	NS
Complete donor chimerism**						
T cells	92% (28/30)	89% (29/32)	NS	100% (20/20)	94% (22/23)	NS
Granulocytes	100% (30/30)	92% (29/32)	NS	100% (20/20)	90% (21/23)	NS
Median follow-up (days)	199	307	0.01	211	350	0.003

*AML: acute myeloid leukemia; ALL: acute lymphoid leukemia; MDS: myelodysplastic syndrome; MM: multiple myeloma; NHL: non-Hodgkin's lymphoma; HD: Hodgkin's lymphoma; CLL: chronic lymphoid leukemia. NS, not significant. *median (range); **Chimerism analysis was performed in peripheral blood using a polymerase chain reaction-short tandem repeats method (as previously described by Fernandez-Avilés et al.[47]) in sorted T cells and granulocytes. Chimerism data were not available for seven and nine patients at 1 and 3 months post-transplantation, respectively.*

5.3; p=0.05). There was also a significant association between low DC count at 3 months post-transplantation with worse OS both in the univariate (0% *vs* 55%; p=0.009) and in the multivariate analysis (RR 8.2; 95% CI 1.9-34.5; p=0.004). Low DC count at 3 months post-transplantation was the most important predictive factor for poor EFS in both the univariate (26% *vs* 45%; p=0.002) and multivariate analyses (RR 6; 95% CI 2.1-17.25; p=0.001) (Figures 2 and 3; Tables 4 and 5). Table 6 lists the causes of death at 1 month and 3 months post-transplantation in both groups of patients.

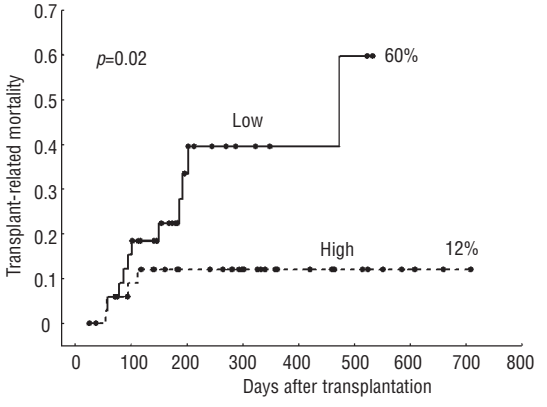

Figure 2. Transplant-related mortality according to median DC count 1 month after allo-RIC. Dashed lines, high DC recovery group; solid lines, low DC recovery group.

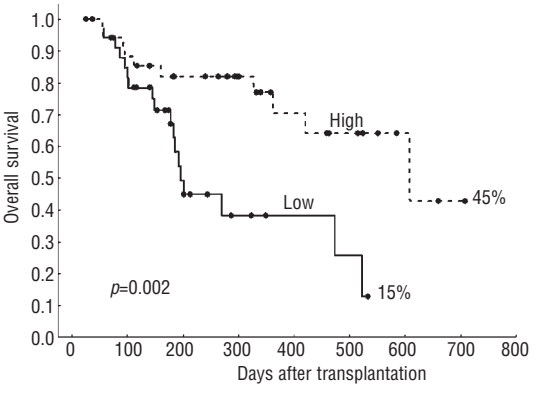

Figure 3. Overall survival according to median DC count recovery 1 month after allo-RIC. Dashed lines, high DC recovery group; solid lines, low DC recovery group.

Association of DC count with GVHD

Acute GVHD occurred in 39 patients (49%), and 30 (38%) developed acute GVHD grades II-IV at a median of 55 days post-transplantation (range, 12-100 days). Among 65 assessable patients, 17 (26%) developed extensive chronic GVHD at a median of 157 days after transplantation (range 100-300 days). There was no association between DC counts and acute GVHD II-IV or chronic GVHD. In the multivariate analysis, the only factor associated with acute GVHD II-IV was the quantity of $CD34^+$ cells infused with the graft (RR 3.5; 95% CI 1.15-10.7; p=0.03), and the only factor associated with the development of chronic GVHD was having had acute GVHD (RR 5.6; 95% CI 1.8-17.1; p=0.003).

Association of DC count with relapse

After a median follow-up of 202 days (range, 25-708), relapse was documented in 24 patients (30%) at a median of 128 days (range, 55-430). There was no association between DC counts at 1 month post-transplantation and the rate of relapse. In contrast, there was a significant association between poor DC recovery (lower than the median) at 3 months post-transplantation with a higher incidence of relapse (55% *vs* 49%; p=0.04) (Table 4). In the

Table 4. Univariate analysis of clinical outcome according to the median DC count recovery after transplantation.

	+1 month		*+3 months*	
	Low vs High	*p-value*	*Low vs High*	*p-value*
TRM	60% *vs* 12%	0.02	30% *vs* 0%	0.007
OS	15% *vs* 45%	0.002	0% *vs* 55%	0.009
EFS	20% *vs* 38%	0.03	26% *vs* 45%	0.002
Relapse	56% *vs* 59%	0.5	55% *vs* 49%	0.04
Acute GVHD II-IV	40% *vs* 31%	0.4	–	–
Chronic GVHD	34% *vs* 26%	0.4	28% *vs* 35%	0.67

TRM: transplant-related mortality; OS: overall survival; EFS: event-free survival; GVHD: graft-versus-host disease.

Table 5. Multivariate analysis for clinical outcome.

	TRM *RR; (95%CI); p*	*OS* *RR; (95%CI); p*	*EFS* *RR; (95%CI); p*	*Relapse* *RR; (95%CI); p*
DC recovery*				
DC at 1 month post-transplant	2.9; (0.92-9.26); 0.07	3.2; (1.4-7.3); 0.007	1.9; (0.9-4); 0.1	NS
at 3 months post-transplant	NS	8.2; (1.9-34.5); 0.004	6; (2.1-17.2); 0.001	4.1; (1.3-12.5); 0.01
Stage of disease	NS	2.3; (1.01-5.3); 0 .05	NS	NS
Acute GVHD II-IV	5; (1.6-16); 0.006	2.7; (1.2-5.8); 0.01	NS	NS
Chronic GVHD	NS	25.6; (2.7-247); 0.004	3.5; (1.36-9.27); 0.04	4.9; (1.4-17.1); 0.01

*TRM: transplant-related mortality; OS: overall survival; EFS: event-free survival; GVHD: graft-versus-host disease; NS: not significant. *Only one of these values was introduced in the model at any time.*

multivariate analysis, low DC count at 3 months post-transplantation and absence of chronic GVHD were the only independent factors predicting a higher incidence of this complication (RR 4.1; 95% CI 1.3-12.5; p=0.01 and RR 4.9; 95% CI 1.37-16.9; p=0.01, respectively) (Table 5).

Association of DC subsets with clinical parameters

The association of myeloid DC, plasmacytoid DC and CD16$^+$ DC counts at 1 and 3 months post-transplantation with TRM, OS and EFS are shown in Table 7. In the multivariate analysis of factors at 1 month post-transplantation, the most significant associations were found for plasmacytoid DC and CD16$^+$ DC counts with OS (RR 3.7; 95% CI 1.6-8.8; p=0.003 and RR 3; 95% CI 1.3-6.7; p=0.01, respectively). At 3 months post-transplantation there were significant associations of CD16$^+$ DC count with OS (RR 8.2; 95% CI 1.9-34.6; p=0.004) and EFS (RR 6; 95% CI 2.1-17.3; p=0.001) (Table 8). The strong association of low CD16$^+$ DC counts with poor EFS was due to a higher incidence of deaths caused by infections (50% *vs* 0%; p=0.05) and to a higher incidence of relapse (55% *vs* 50%; p=0.04). In the multivariate analysis there was a trend towards an association between low plasmacytoid DC count and a higher incidence of acute GVHD (RR 2.4; 95% CI 0.91-6.5; p=0.06) and between CD16$^+$ DC at 3 months post-transplantation with a higher incidence of chronic GVHD (RR 1.6; 95% CI 0.7-5.9; p=0.19).

Table 6. Causes of death in the groups with low or high DC counts 1 and 3 months after transplantation.

Causes of death	1 month post-transplant		3 months post-transplant	
	Low	High	Low	High
Relapse	4 (23%)	5 (50%)	2 (25%)	5 (100%)
GVHD	4 (23%)	1 (10%)	1 (12%)	0 (–)
Infection	8 (47%)	4 (40%)	4 (50%)	0 (–)
Other	1 (6%)	0 (–)	1 (12%)	0 (–)
Total	17	10	8	5

Discussion

The present study shows that the absolute number of DC in peripheral blood was significantly associated with outcome after allo-RIC. Thus, patients whose DC count in peripheral blood was higher than the median 1 month after transplantation had a survival rate of 45%, whereas patients whose DC count was below the median at that time had a survival rate of 15% (p=0.002; Figure 3). Moreover, the impact of DC counts on clinical outcome was even higher than that of disease status at transplant or the development of acute GVHD grade II-IV. Accordingly, the DC count at 3 months post-transplantation was the most important factor for EFS in the multivariate analysis (RR 6; 95% CI 2.1-17.2; p=0.001) (Table 5). Similar to our findings, Reddy *et al.*[26] recently showed, in a series of patients treated with conventional myeloablative allo-SCT, that the reconstitution of absolute numbers of circulating DC was an independent predictor of post-transplantation survival and relapse. Interestingly, these and other authors[27,28] also found a correlation between DC counts and acute GVHD, unlike in our series in which high DC counts were associated with a lower relapse rate without an increased incidence of acute GVHD. This dissociated relation of DC numbers with the graft-versus-leukemia effect and GVHD was also found by Sato *et al.*,[29] who demonstrated that regulatory DC administered after allogeneic transplantation in mice protected against both relapse and acute GVHD. Prevention of acute GVHD might also be favored by a lack of host DC activity.[30] Most of the patients had predominantly full donor chimerism at 1 month and 3 months post transplantation (Table 3), precluding an analysis of the influence of mixed chimerism on outcome parameters. Although chimerism studies of selected DC of peripheral blood were not performed in our patients, recent studies showed that, in most of the patients, DC after allo-RIC were donor-derived.[31,32]

We focused our study on defining the clinical relevance of DC subsets after allo-RIC. Plasmacytoid DC recovery was strongly associated with TRM. Mohty *et al.* recently studied a series of patients undergoing allo-RIC and reported a relationship between plasmacytoid DC counts and TRM; like us, they did not find any correlation between high plasmacytoid DC counts and lower risk of disease progression or relapse.[33] The results from this series and those from Mohty *et al.* are in line with the findings of Waller *et al.*, who suggested that higher numbers of plasmacytoid DC were, in fact, associated with a higher incidence of relapse after myeloablative transplantation.[34] In this sense, it is known that plasmacytoid DC polarize T cells toward a Th2 phenotype, which reduces graft-versus-host reactions.[35,36] Very recently it has been demonstrated that plasmacytoid DC are able to prime interleukin-10-producing T regulatory cells by inducible co-stimulatory ligand and induce regulatory cytokines in effector T cells that can suppress bystander activity.[37,38] Indeed, we found a trend towards an association between high plasmacytoid DC counts and a lower incidence of acute GVHD in the multivariate analysis (RR 2.4; 95% CI 0.91-6.5; p=0.06). Altogether, these results suggest that plasmacytoid DC might have a limited role in anti-tumor immunity, but further functional studies are required to confirm this effect. Non-relapse mortality was significantly higher in the group with low plasmacytoid DC counts at 1 month after the transplant, with patients dying mainly from infections, further illustrating the importance of this DC subset in establishing immune competence by expanding specific cytotoxic T lymphocytes[39] and in handling of pathogens.[15,16]

We have previously shown that CD16$^+$ DC cells account for the majority of DC in blood and that G-CSF greatly increases the numbers of these cells, so that G-CSF-primed peripheral blood harvests contain much higher quantities of CD16$^+$ DC than do bone marrow harvests.[25] Up to now,

Table 7. Univariate analysis for clinical outcome according to the DC subsets.

	1 month post-transplant			*3 months post-transplant*		
	Myeloid DC Low vs High	*Plasmacytoid DC Low vs High*	*CD16⁺ DC Low vs High*	*Myeloid DC Low vs High*	*Plasmacytoid DC Low vs High*	*CD16⁺ DC Low vs High*
TRM	58% *vs* 20% *p*=0.21	60% *vs* 10% *p*=0.002	60% *vs* 12% *p*=0.02	NS	NS	30% *vs* 0% *p*=0.007
OS	10% *vs* 45% *p*=0.003	10% *vs* 44% *p*=0.001	10% *vs* 40% *p*=0.004	0% *vs* 55% *p*=0.05	28% *vs* 40% *p*=0.1	0% *vs* 50% *p*=0.009
EFS	10% *vs* 35% *p*=0.05	18% *vs* 32% *p*=0.1	20% *vs* 39% *p*=0.05	30% *vs* 41% *p*=0.08	30% *vs* 46% *p*=0.4	22% *vs* 45% *p*=0.002
Relapse	NS	NS	NS	55% *vs* 50% *p*=0.04	NS	57% *vs* 50% *p*=0.03
Acute GVHD	NS	45% *vs* 31% *p*=0.1	NS	–	–	–
Chronic GVHD	NS	NS	NS	NS	NS	60% *vs* 45% *p*=0.035

TRM: transplant-related mortality; OS: overall survival; EFS: event-free survival; GVHD: graft-versus-host disease; NS: not significant.

Table 8. Multivariate Cox regression analysis of clinical outcome.

	TRM RR; (95%CI); p	*OS RR; (95%CI); p*	*EFS RR; (95%CI); p*	*Relapse RR; (95%CI); p*
DC recovery*				
Plasmacytoid DC +1m	4.4; (1.2-15.6); 0.02	3.7; (1.6-8.8); 0.003	2.5; (1.1-5.5); 0.02	NS
CD16⁺ DC 1 m	2.9; (0.91-9.11); 0.07	3; (1.3-6.7); 0.01	NS	4.1; (1.3-12.5); 0.01
CD16⁺ DC 3 m	NS	8.2; (1.9-34.6); 0.004	6; (2-17.3); 0.001	NS
Stage of disease	NS	3.2; (1.4-7.3); 0.05	NS	NS
Acute GVHD II-IV	5.3; (1.7-17); 0.004	2.7; (1.2-5.8); 0.01	NS	NS
Chronic GVHD	NS	25.6; (2.7-247); 0.004	3.55; (1.36-9.27); 0.04	4.9; (1.4-17.1); 0.01

*TRM: transplant-related mortality; OS: overall survival; EFS: event-free survival; GVHD: graft-versus-host disease; NS: not significant.*Only one of these values were introduced in the model at any time.*

no single study had analyzed the clinical relevance of the kinetics of recovery of this particular DC subset after allo-SCT. We report for the first time a strong association between CD16⁺ DC recovery and TRM (mainly due to severe infections), relapse, and EFS. Indeed, the number of CD16⁺ DC 3 months post-transplantation was the most important independent prognostic factor for EFS in multivariate analysis (Table 8). The herein reported association of CD16⁺ DC in fighting against infections and in antitumoral immunity is in line with results of previous functional studies.[21,40-42] CD16⁺ DC constitute a novel pro-inflammatory subset of DC producing high quantities of TNF-α in response to the bacterial endotoxin lipopolysaccharide, thus having an important function in the induction and amplification of inflammatory reactions. CD16⁺ DC, but not myeloid DC or plasmacytoid DC, express molecules known to mediate recruitment to inflammatory sites such as C5aR, C3aR, and the Fcγ receptor III (CD16). Of interest, CD16⁺ DC tend to be localized in the subepithelial dome region of Peyer's patches, to where they are recruited in response to bacterial and other stimuli.[40] Of note, in patients with active Crohn's disease, abundant CD16⁺ DC are detected in inflamed ileal mucosa.[40] These findings suggest that this particular subset of DC might contribute to preventing micro-organisms from entering the body through the intestinal lumen, an important factor for TRM after allo-SCT,[43] but we cannot exclude that infection itself resulted in the reduced CD16⁺ DC counts in the blood rather than *vice versa*.

The observed association between high CD16⁺ DC counts and a lower probability of relapse might be explained by the potent capacity of these cells to prime T cells *in vitro* and their strong potential to produce TNF-α. In this sense, CD16⁺ DC have recently been identified as one of the most potent inducers of T-cell proliferation in allogeneic mixed leukocyte reactions,[11] and the role of TNF-α in eradicating residual disease and in preventing relapse has been increasingly recognized. This effect of TNF-α is directed against leukemic cells by enhancing the phenotypic and functional activation of DC, resulting in induction of a stronger antileukemic cytotoxic T-cell immune response,[44,45] or by activating the lytic function of NK cells against tumor cells.[46]

In conclusion, this study confirms the clinical importance of DC recovery after allo-RIC,[33] and shows that the recently identified subset of CD16⁺ DC might be key functional anti-infectious and anti-tumor cells in patients undergoing this treatment.

Authors' Contributions

CT and AUI interpreted the data, performed the statistical analysis and wrote the manuscript; CT performed the data analysis; RM and JAPS collected data and revised the manuscript; MB, CH, MG, AG, MT, FFA collected data; MA and PM contributed to the data analysis; JS and EM revised the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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