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Acute Graft-versus-Host Disease and Steroid Treatment Impair CD11c$^+$ and CD123$^+$ Dendritic Cell Reconstitution after Allogeneic Peripheral Blood Stem Cell Transplantation

Mario Arpinati,[1] Gabriella Chirumbolo,[1] Benedetta Urbini,[1] Francesca Bonifazi,[1] Giuseppe Bandini,[1] Yogen Saunthararajah,[2] Alessandra Zagnoli,[1] Marta Stanzani,[1] Sadia Falcioni,[1] Giulia Perrone,[1] Sante Tura,[1] Michele Baccarani,[1] Damiano Rondelli[1,2]

[1]Research Center for Transplant Immunology, Institute of Hematology and Medical Oncology "Seràgnoli," University of Bologna, Bologna, Italy; [2]Stem Cell Transplant Program, Section of Hematology/Oncology, University of Illinois at Chicago, Chicago, Illinois

Correspondence and reprint requests: Mario Arpinati, MD, Institute of Hematology and Medical Oncology "Seràgnoli," University of Bologna, Ospedale S. Orsola, Via Massarenti, 9-40138 Bologna, Italy (e-mail: arpinati@med.unibo.it).

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ABSTRACT

Human dendritic cells (DC) comprise 2 subsets—plasmacytoid CD123$^+$ and myeloid CD11c$^+$ DC—that may have distinct roles in the regulation of immunity after allogeneic hematopoietic stem cell transplantation. In this study, we analyzed the kinetics of CD123$^+$ DC and CD11c$^+$ DC reconstitution in 31 patients who underwent transplantation with allogeneic granulocyte colony-stimulating factor–mobilized peripheral blood (PB) stem cells from HLA-identical sibling donors after myeloablative conditioning. Lineage marker–negative HLA-DR$^+$ CD11c$^+$ CD11c$^+$ DC and lineage marker–negative HLA-DR$^+$ CD123$^+$ CD123$^+$ DC, as well as monocytes and lymphoid subsets, were enumerated in donor grafts and in the PB of patients at various time points after transplantation. Reconstitution of both CD11c$^+$ DC and CD123$^+$ DC to normal levels occurred within 6 to 12 months and was not affected by the diagnosis, preparatory regimen, or graft composition. However, PB CD11c$^+$ DC and CD123$^+$ DC counts were significantly reduced in patients with acute GVHD grade II to IV (at 1 and 3 months) and grade I (at 1 month). Patients with chronic GVHD instead showed reduced CD123$^+$ DC counts only 6 months after transplantation. Moreover, treatment with steroids (>0.1 mg/kg) was significantly associated with reduced PB CD11c$^+$ DC and CD123$^+$ DC counts at all time points after transplantation. In multivariate analysis, only acute GVHD affected DC reconstitution early after transplantation. These results will prompt new studies addressing whether DC reconstitution correlates with immunity against infectious agents or with graft-versus-tumor reactions after PB stem cell allotransplantation.

KEY WORDS

Graft versus host disease • Dendritic cells • Plasmacytold DC • CD11c$^+$DC • Corticosteroids

INTRODUCTION

Dendritic cells (DC) are potent antigen-presenting cells (APC) that play an essential role in the initiation or regulation of immune responses [1]. In humans, 2 distinct subpopulations of circulating DC have been characterized within cells negative for lineage markers (lin$^-$) and positive for HLA class II molecules [2-6]. CD11c$^+$ DC (DC1) selectively express CD13, CD33, and CD45RO, as well as the granulocyte macrophage colony-stimulating factor receptor, and are therefore thought to originate from myeloid progenitors; CD123 (interleukin-3 receptor α chain)$^+$ DC (plasmacytoid DC; DC2) lack myeloid markers and, because of the selective expression of lymphoid-specific gene products (such as pre-Tα, λ-like and Spi-B) [7,8], have been suggested to originate from lymphoid progenitors [9]. CD11c$^+$ DC produce high levels of interleukin-12 and are potent

activators of T-helper 1 (Th1) differentiation, whereas $CD123^+$ DC produce high levels of interferon-α/β but are poor stimulators of T-cell proliferation [2,6,10-13]. Moreover, various stimuli, such as inflammatory cytokines TNF-α (tumor necrosis factor-α), T cell-derived molecules (CD40L), lipopolysaccharide, and other bacterial products—or viruses and their products—can drive $CD123^+$ DC to differentiate in vitro into potent APC that may be capable of selectively inducing Th2 (hence the name DC2) or T-regulatory type 1 cells, but also Th1 differentiation, depending on the experimental system [10-11,13-17]. It is therefore thought that $CD11c^+$ DC and $CD123^+$ DC might play distinct roles in alloimmune responses that occur in hematopoietic stem cell transplantation (HSCT) [18].

In recent years, several studies in murine models of allogeneic HSCT have suggested that DC may play a role both in initiating graft-versus-host disease (GVHD) and in promoting immune reconstitution after transplantation [19-21]. In humans, it has been proposed that graft $CD123^+$ DC may regulate GVHD after both bone marrow and peripheral blood stem cell (PBSC) transplantation [11,22]. Although it is conceivable that the reconstitution of the DC compartment after transplantation may be important for immune responses against both alloantigens and pathogens, very few data are available concerning the kinetics of myeloid and $CD123^+$ DC repopulation in peripheral blood (PB) after allogeneic transplantation. Moreover, conflicting data have been published regarding the relationship between various transplantation-related factors—including the type of preparatory regimen, GVHD prophylaxis, and GVHD—and the reconstitution of $CD11c^+$ DC and $CD123^+$ DC in PB after transplantation [23-27]. In this study, we show a correlation between the recovery of $CD11c^+$ myeloid DC and $CD123^+$ plasmacytoid DC after myeloablative PBSC allogeneic transplantation and the development of acute GVHD (aGVHD), as well as with treatment with corticosteroids (CS).

MATERIALS AND METHODS

Patients

Thirty-one adult patients who underwent an allogeneic PBSC transplantation from an HLA-matched related donor at the Institute "Seragnoli," University of Bologna, between June 1999 and October 2001 were consecutively enrolled in this study after written, informed consent was obtained. Patients' characteristics are shown in Table 1. All patients had aliquots of each PBSC harvest collected and processed for analysis of leukocyte subpopulations, as described below. In addition, patients' PB was drawn before and 1, 3, 6, and 12 months after transplantation to evaluate the immune reconstitution.

Table 1. *Patient and Graft Characteristics*

Variable	Data
Median age, y (range)	42 (29-56)
Diagnosis	
Acute myeloid leukemia	6 (19%)
Acute lymphoid leukemia	1 (3%)
Myelodysplasia	2 (6%)
Chronic myeloid leukemia	7 (23%)
Multiple myeloma	10 (32%)
Non-Hodgkin lymphoma	4 (13%)
Hodgkin disease	1 (3%)
Conditioning regimen	
Bu-Cy	8 (26%)
Bu-Cy-ATG	8 (26%)
Cy-Mel-TBI	10 (32%)
Bu-Mel-ATG	5 (16%)
GVHD prophylaxis: CyA^+ MTX	31 (100%)
Median follow up, d (range)	392 (48-695)
Graft composition, median (range)*	
Total nucleated cells	1300 (592-2115)
Myeloid DC	2.1 (0.7-3.9)
Plasmacytoid DC	2.7 (1.1-6)
Monocytes	244 (86-577)
B cells	49 (18-158)
NK cells	48 (11-133)
T cells	259 (116-632)
$CD4^+$ T cells	139 (72-408)
$CD8^+$ T cells	107 (32-240)
$CD34^+$ cells	5.8 (3.3-26.2)

Bu indicates busulfan; Cy, cyclophosphamide; ATG, antithymocyte globulin; Mel, melphalan; TBI, total body irradiation; CyA, cyclosporin A; MTX, methotrexate; DC, dendritic cells.

*Values are expressed as 10^6 cells per kilogram of recipient's body weight.

Standard myeloablative regimens for conditioning were used, depending on the disease, as previously reported [28]. In particular, total body irradiation (TBI; 800 cGy single dose) was performed in multiple myeloma patients, who also received melphalan 120 mg/m^2 and cyclophosphamide 120 mg/kg. Busulfan 16 mg/kg and cyclophosphamide 200 mg/kg was used in all 7 acute leukemia and 1 of 2 myelodysplasia patients, whereas in 7 of 7 chronic myeloid leukemia patients and 1 myelodysplasia patient, antithymocyte globulin (ATG) was added to the standard busulfan/cyclophosphamide conditioning regimen. Five of 5 patients with either non-Hodgkin lymphoma (n = 4) or Hodgkin disease (n = 1) received busulfan 16 mg/kg, cyclophosphamide 120 mg/kg, and ATG in the conditioning regimen.

GVHD prophylaxis included cyclosporine and short methotrexate (days +1, +3, +6, and +11) in all patients. Acyclovir 10 mg/kg intravenously every 8 hours was given from day −5 to day +25 and then orally for 9 months. Preemptive treatment of cytomegalovirus disease was performed with intravenous or oral ganciclovir in case of cytomegalovirus-positive antigenemia, which was tested weekly until day +120 after transplantation. Acute GVHD was graded ac-

cording to Przepiorka et al. [29], whereas chronic GVHD (cGVHD) was classified according to the criteria described by Shulman et al. [30].

Donors

All donors were HLA-identical siblings. PBSC were mobilized with human recombinant granulocyte colony-stimulating factor (Lenograstin; Aventis, Milan, Italy), subcutaneously, twice daily, at 10 μg/kg/d. Leukaphereses were started on day 5 and repeated daily until the target $CD34^+$ cell dose (4×10^6 cells per kilogram of recipient's body weight) was reached. After written informed consent was obtained from the donors, aliquots of each apheresis product were processed for analysis of leukocyte subpopulations, as described below.

Flow Cytometry

Flow cytometry analysis of leukocyte subsets was performed on fresh samples of PB and apheresis products. The following monoclonal antibodies were used: fluorescein isothiocyanate (FITC)–conjugated anti-lin (a mixture of anti-CD3, -CD14, -CD16, -CD19, -CD20, and -CD56), anti-CD34 (clone 8G12), anti-CD19 (clone 4G7), and anti-CD4 (clone SK3); phycoerythrin (PE)–conjugated anti-CD11c (clone S-HCL-3), anti-CD123 (clone 9F5), anti-CD56 (clone My31), and anti-CD8 (clone SK1); Leukogate (a mixture of FITC-conjugated anti-CD45 and PE-conjugated anti-CD14); peridin chlorophyll protein (PerCP)–conjugated anti–HLA-DR (clone L243); and PerCP-carbocyanin 5–conjugated anti-CD3 (clone SK7), all from Becton Dickinson (Mountain View, CA). Appropriate isotype controls included FITC-, PE- and PerCP-conjugated immunoglobulin (Ig)G1 and PerCP-conjugated IgG2a (Becton Dickinson), as well as FITC- and PE-conjugated IgG2b (Pharmingen, San Diego, CA). $CD11c^+$ DC were identified as lin^- $CD34^-$ $CD11c^+$ $HLA\text{-}DR^+$, and $CD123^+$ DC were identified as lin^- $CD34^-$ $CD123^+$ and $HLA\text{-}DR^+$, as previously described [11,31]. Monocytes, B cells, natural killer (NK) cells, and $CD4^+$ and $CD8^+$ T cells were identified, respectively, as $CD45^+$ $CD14^+$, $CD19^+$, $CD56^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$. For staining, aliquots of whole blood or apheresis products containing up to 10^6 leukocytes were incubated with the appropriate amount of each monoclonal antibody for 15 minutes at room temperature. Red blood cell lysis was performed by using FACS Lysing Buffer (Becton Dickinson) according to the manufacturer's instructions. Stained cells were washed twice in phosphate-buffered saline, and then sample acquisition was performed within 2 hours on a FACSCalibur (Becton Dickinson). Analysis was performed by using Cell Quest (Becton Dickinson) software. Absolute numbers of leukocyte subpopulations in each sample were defined by multiplying the percentage of each cell subset, as determined by electronic gating during analysis, by the total white blood cell count, as determined by automated cell counting (Coulter Gen-S; Coulter Immunotech, Marseille, FR).

Statistical Analysis

Differences between groups were analyzed for their significance by using the Student *t* test, analysis of variance test, general linear model, nonparametric Kruskal-Wallis test, or χ^2 test, as indicated. In particular, the χ^2 test was used for categorical variables; for continuous variables, normality was tested by the Kolmogorov-Smirnov test. If the assumption of normality was not met, logarithmic transformation was applied. When the assumption of normality was met, differences between groups were tested by the Student *t* test or analysis of variance. Variables that showed nonnormal distribution were tested by using nonparametric analysis (Kruskal-Wallis test). Multivariate analysis was performed by the general linear model. Variables with a *P* value of at least .05 in univariate analysis were included. All statistical analyses were performed with SPSS software (SPSS Inc., Chicago, IL).

RESULTS

Kinetics of $CD11c^+$ DC and $CD123^+$ DC Reconstitution in PB after Transplantation of Allogeneic PBSC

Thirty-one patients, whose characteristics are listed in Table 1 and who received an allogeneic granulocyte colony-stimulating factor–mobilized PBSC allotransplant, were enrolled in this study. After a median follow up of 392 days (range, 48-695 days), 23 patients (72%) were alive and in complete remission. Numbers of $CD11c^+$ DC, $CD123^+$ DC, and monocytes were measured in the PB of all patients at 1, 3, 6, and 12 months after transplantation and in the PB of 27 healthy donors, as normal controls. $CD11c^+$ DC and $CD123^+$ DC counts in the PB of control donors were (mean ± SD) $18.7 \pm 11.2 \times 10^6$/L and $11.6 \pm 9 \times 10^6$/L, respectively, as shown in Figure 1 (shaded area). As shown in Figure 1 (top), $CD123^+$ DC were very few or undetectable in the blood of most patients 1 month after transplantation, but their median count increased over the observation period up to 1 year after transplantation. Similarly, the median PB $CD11c^+$ DC count also increased until 1 year after transplantation, as shown in Figure 1 (center). However, monocyte counts were already within the normal range as early as 1 month after transplantation (Figure 1, bottom). These results suggest that a profound reduction of DC, but not monocytes, occurs after allogeneic PBSC transplants conditioned with my-

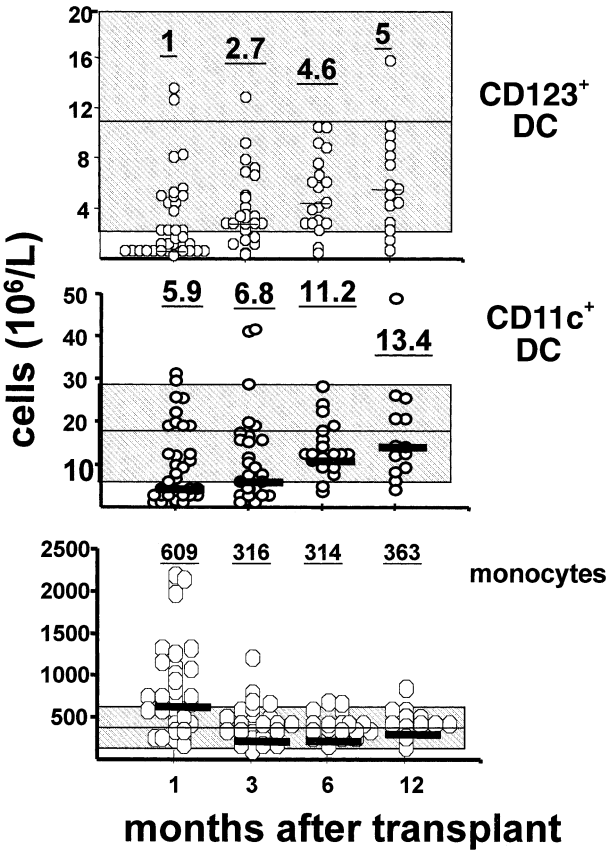

Figure 1. Kinetics of DC reconstitution in recipients' PB after allogeneic PBSC transplantation. $CD123^+$ DC (top), $CD11c^+$ DC (middle), and monocytes (bottom) circulating in the PB at 1 month (n = 31), 3 months (n = 27), 6 months (n = 20), and 12 months (n = 15) after transplantation were enumerated by flow cytometry, as described in Materials and Methods. Each circle represents an individual patient. The horizontal bars represent median values, which are also expressed as numbers on top of each dot plot. The shaded gray areas indicate the mean ± SD PB levels of $CD123^+$ DC, $CD11c^+$ DC, and monocytes in 27 age-matched healthy control donors.

eloablative regimens and that the full reconstitution of normal levels of $CD11c^+$ DC and $CD123^+$ DC may take as long as 6 months to 1 year after transplantation.

We then tested whether DC recovery correlated with patient-related factors (such as age or disease) or transplant-related factors (such as the type of preparatory regimen or the composition of the graft; Table 2). It is interesting to note that younger patients (age ≤41 years) had significantly greater median numbers of both circulating $CD11c^+$ DC (11.7×10^6/L; range, 2.3-30.5) and $CD123^+$ DC (4.2×10^6/L; range, 0.2-13.3) than the older ones (2.9×10^6/L; range, 0.3-17.9 and 0.6×10^6/L; range, 2.3-30.5, respectively, for $CD11c^+$ DC and $CD123^+$ DC; $P = .01$ and .05, respectively) at 1 month after transplantation, but the difference was no longer observed at 3 through 12 months. Furthermore, patients' disease and the presence of either ATG or TBI in the preparatory regimen did not affect DC recovery. Moreover, the recovery of $CD11c^+$ DC or $CD123^+$ DC was not different in patients who received a higher dose of graft $CD11c^+$ DC (>2.1×10^6/kg) or $CD123^+$ DC (>2.7×10^6/kg; Table 2).

Impaired DC Reconstitution in Patients Who Developed aGVHD

To test whether DC reconstitution was affected by aGVHD, patients were divided according to the grade of GVHD: 10 patients (32%) did not develop any signs or symptoms of aGVHD, 13 patients (42%) developed aGVHD grade I, and 8 patients (26%) developed grade II to IV aGVHD. The 3 groups were then compared for PB $CD11c^+$ DC and $CD123^+$ DC reconstitution at 1 and 3 months after transplantation. As shown in Figure 2 (top), patients with aGVHD grade I or II-IV had significantly lower PB $CD123^+$ DC median counts than patients with no GVHD at 1 month after transplantation ($P = .037$ and .027, respectively). At 3 months after transplantation, PB $CD123^+$ DC counts were similar in patients with no GVHD or aGVHD grade I, whereas they were still significantly lower in patients with aGVHD grade II to IV ($P = .03$). Acute GVHD had already developed at the time of the first flow cytometric analysis (ie, 30 days after transplantation) in 20 of 22 patients, whereas 2 patients developed GVHD after 39 and 45 days, respectively. It is interesting to note that in both these patients, the occurrence of aGVHD was followed by a profound reduction in PB $CD123^+$ DC counts at 3 months after transplantation (13.3×10^6 and 0.2×10^6, and 7.9×10^6 and 0.2×10^6 $CD123^+$ DC per liter, respectively, at 1 and 3 months). Median $CD11c^+$ DC counts were also reduced in patients with aGVHD grade I at 1 month after transplantation and in patients with aGVHD grade II to IV at 1 and 3 months after transplantation as compared with patients with no GVHD, but the differences were not statistically significant (Figure 2, center). The number of monocytes was similar in all 3 groups at each time point (Figure 2, bottom). Moreover, we did not detect any difference in PB B cell, NK cell, and $CD8^+$ T-cell counts among the 3 groups, either at 1 or at 3 months after transplantation, whereas PB $CD4^+$ T-cell counts were significantly reduced in patients with aGVHD grade II to IV at 3 months after transplantation (not shown).

To test whether DC reconstitution was also similarly affected by cGVHD, PB $CD11c^+$ DC and $CD123^+$ DC counts were analyzed in patients with cGVHD (n = 12; 10 extensive and 2 limited) and in those without cGVHD (n = 9). Twenty-one patients were evaluable, with a median follow up of 485 days (range, 131-695 days). Patients with cGVHD had lower PB $CD123^+$ DC counts at 6 months ($P = .04$),

Table 2. *Correlation Between Pretransplantation Characteristics and DC Recovery*

Variable	Myeloid DC			Plasmacytoid DC		
	1 mo	3 mo	6 mo	1 mo	3 mo	6 mo
Age	.01*	.38	.86	.05*	.85	.07
Disease						
Multiple myeloma	.53	.52	.84	.3	.2	.69
Acute leukemia	.29	.28	1	.26	.65	.67
Chronic myeloid leukemia	.17	.1	.6	.34	.29	.89
Preparatory regimen						
ATG containing	.63	.2	.62	.7	.18	.69
TBI containing	.68	1	.84	.37	.49	.69
Graft composition						
Plasmacytoid DC ≥2.7 × 10^6	ND	ND	ND	.2	.14	.54
Myeloid DC ≥2.1 × 10^6	.83	.08	.76	ND	ND	ND

Numbers of myeloid DC and plasmacytoid DC circulating in recipients' peripheral blood at the indicated time points after transplantation were compared according to the presence or absence of the indicated pretransplantation factors. The significance of each difference, determined as described in Materials and Methods, is expressed as *P* value.

ATG indicates antithymocyte globulin; TBI, total body irradiation; ND, not done.

P values ≤0.05 were considered significant.

but not at 1 year, after transplantation (Figure 3, top), whereas no difference was observed in PB $CD11c^+$ DC and monocytes (Figure 3) or in B-, T-, and NK-cell counts (not shown). To rule out the possibility that reduced PB $CD123^+$ DC counts in patients with cGVHD could depend on the earlier occurrence of aGVHD in those patients, PB $CD123^+$ DC counts at 6 months after transplantation were compared among aGVHD groups. Patients with previous aGVHD (n = 11; 9 grade I and 2 grade II to IV) had a median PB $CD123^+$ DC count of 5.4 × 10^6/L (range, 2.2-10.4 × 10^6/L) at 6 months after transplantation, and this was comparable to counts of patients who previously had not experienced any aGVHD (n = 7; 5.5 × 10^6/L; range, 2.4-7.3 × 10^6/L), thus excluding a delayed effect of aGVHD on PB $CD123^+$ DC reconstitution. Collectively, these data suggest that the development of aGVHD or cGVHD may selectively correlate with a slower $CD123^+$ DC and, possibly, $CD11c^+$ DC reconstitution for up to 6 months after allogeneic PBSC transplantation.

Effect of Steroids on DC Reconstitution

Because CS represent a first-line therapy for GVHD and may be responsible for many GVHD-related immunosuppressive effects, we hypothesized that they could play a role in the observed reduction of $CD123^+$ DC and $CD11c^+$ DC blood levels in patients with GVHD. To test whether treatment with steroids for at least 3 to 5 days correlated with the numbers of DC in PB, all collected samples, irrespective of the time of collection, were divided depending on the dose of steroids that the patient was taking at the time of the blood draw. As shown in Figure 4, patients treated with prednisone doses >0.1 mg/kg had significantly lower numbers of both $CD11c^+$ DC and $CD123^+$ DC. The effect seemed to be dose re-

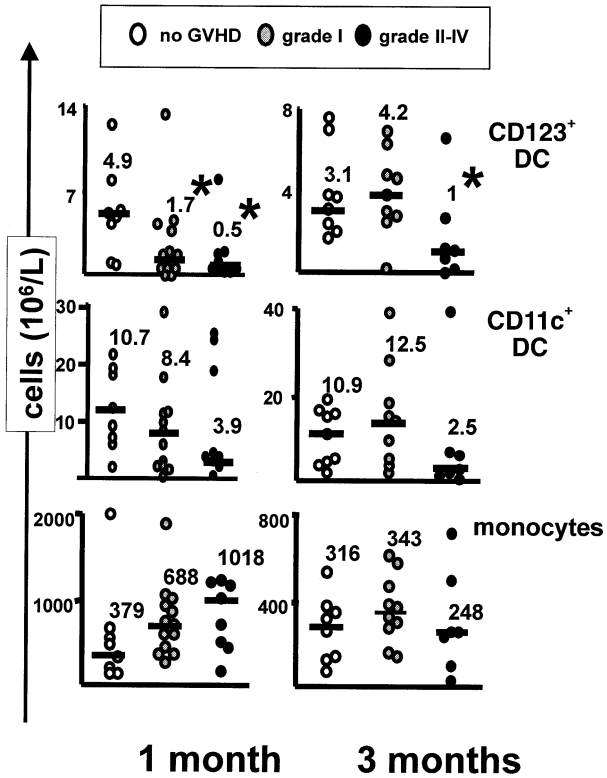

Figure 2. Acute GVHD correlates with reduced PB $CD123^+$ DC and $CD11c^+$ DC numbers at 1 and 3 months after transplantation. PB numbers of $CD123^+$ DC (top), $CD11c^+$ DC (middle), and monocytes (bottom) were compared among patients with no aGVHD (white circles), grade I aGVHD (gray circles), and grade II to IV aGVHD (black circles) at 1 month (n = 8, 13, and 9, respectively) and 3 months (n = 9, 10, and 7, respectively) after transplantation. Each circle represents an individual patient. Median values are indicated as described in the legend to Figure 1. *$P < .05$.

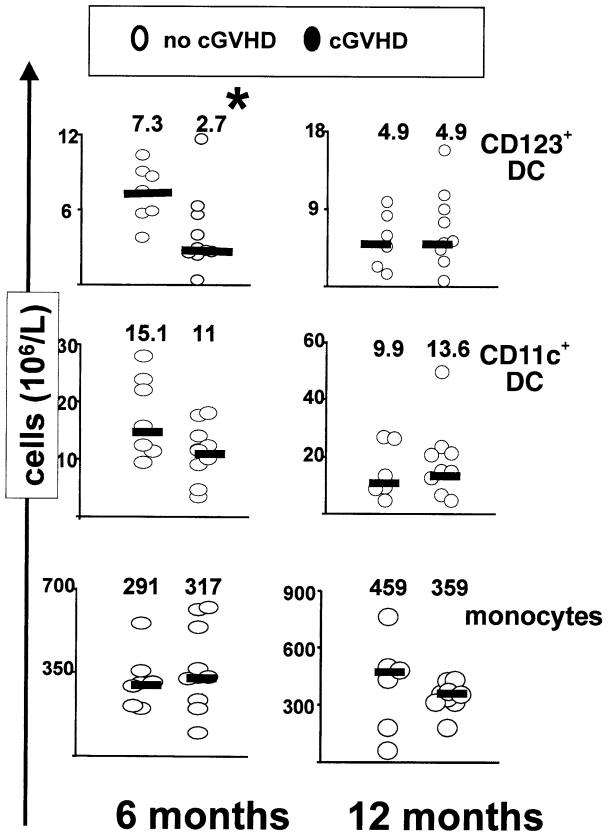

Figure 3. Chronic GVHD correlates with reduced PB CD123$^+$ DC numbers at 6 months after transplantation. PB numbers of CD123$^+$ DC (top), CD11c$^+$ DC (middle), and monocytes (bottom) were compared between patients with no cGVHD (white circles) and cGVHD (black circles) at 6 months (n = 7 and 10, respectively) and 12 months (n = 6 and 9, respectively) after transplantation. Each circle represents an individual patient. Median values are indicated as described in the legend to Figure 1. *$P < .05$.

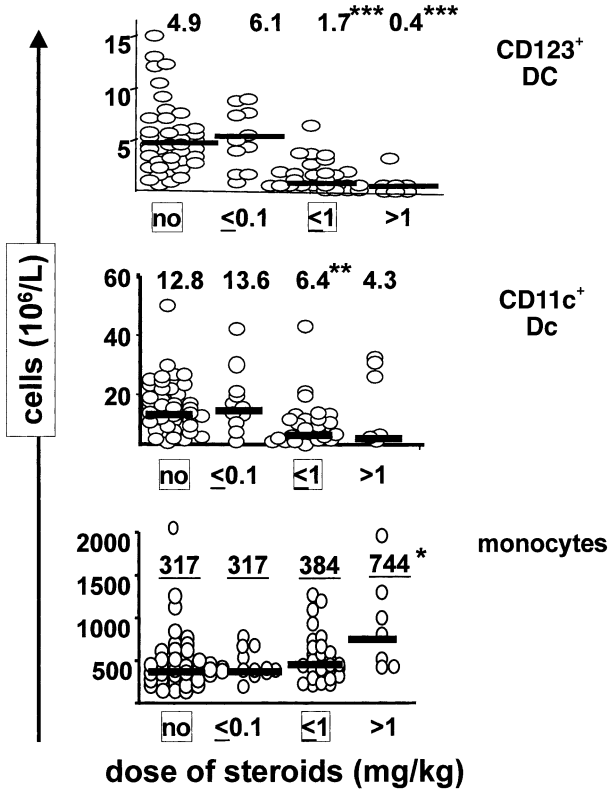

Figure 4. Dose-dependent correlation between steroid treatment and PB DC numbers after PBSC transplantation. Samples were divided according to whether the patient was receiving no steroids, up to 0.1 mg/kg, up to 1 mg/kg, or >1 mg/kg steroids at the time of blood draw, and then PB numbers of CD123$^+$ DC (top), CD11c$^+$ DC (middle), and monocytes (bottom) were compared among groups. Numbers of samples were 40, 11, 28, and 6 in the groups with no, ≤0.1, ≤1, and >1 mg/kg steroids, respectively. Each circle represents an individual sample. Median values are indicated as described in the legend to Figure 1. *$P < .05$; **$P < .01$; ***$P < .001$.

lated, because PB from patients receiving prednisone doses >1 mg/kg had even lower CD11c$^+$ DC and CD123$^+$ DC numbers. It is interesting to note that patients who were receiving lower doses of CS (ie, <0.1 mg/kg) had both CD11c$^+$ DC and CD123$^+$ DC numbers comparable to those of the patients who were not receiving steroids. However, PB numbers of other cell types, such as monocytes (Figure 4) and T and B lymphocytes (not shown), did not seem to vary depending on the CS dose. Therefore, these data suggest that treatment with steroids at a dose equivalent to >0.1 mg/kg prednisone may correlate with a selective reduction in the number of both circulating CD11c$^+$ DC and CD123$^+$ DC in HSCT patients.

To test whether the recovery of CD11c$^+$ DC and CD123$^+$ DC was affected by steroids at all time points after transplantation, patients were divided into 2 groups according to whether they were receiving >0.1 mg/kg CS at the time of each blood draw. As shown in Figure 5, patients receiving CS had significantly lower PB CD123$^+$ DC counts than other patients at all time points analyzed. PB CD11c$^+$ DC counts also showed a similar trend, although the difference was significant only at 6 months after transplantation ($P = .05$). Moreover, whereas in patients who were not receiving CS, both CD123$^+$ DC and CD11c$^+$ DC median counts were within the range observed in healthy donors throughout the entire observation period, in patients who were receiving CS, low numbers of DC were observed even as late as 1 year after transplantation (Figure 5). It is interesting to note that PB counts of other leukocytes, such as monocytes, B cells, and CD4$^+$ and CD8$^+$ T cells, did not differ in the 2 groups at any of the indicated time points, with the exception of NK cells, which were significantly reduced in patients who were receiving CS at 1 month after transplantation ($P = .02$; Figure 5 and data not shown).

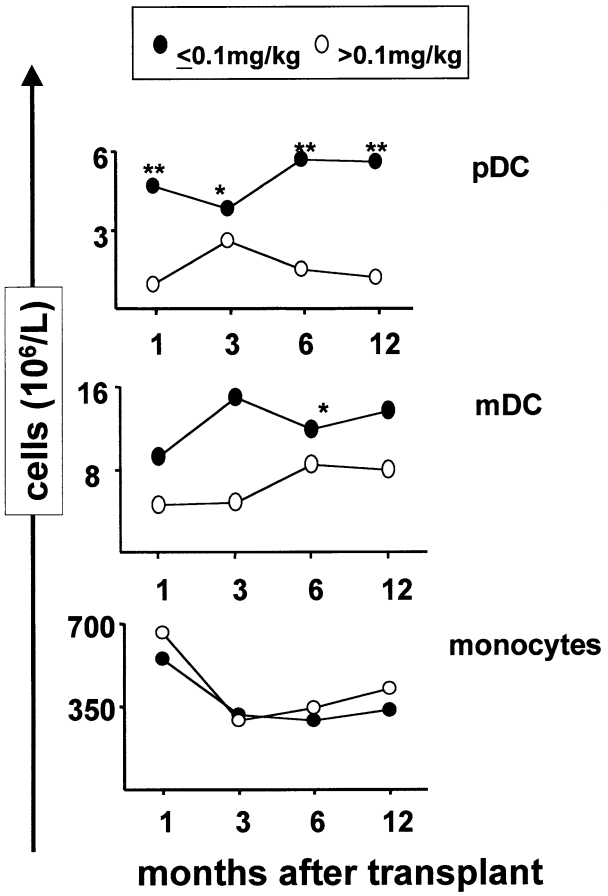

Figure 5. Treatment with steroids after transplantation correlates with reduced PB CD123$^+$ DC and CD11c$^+$ DC numbers. PB numbers of DC2 (top), DC1 (middle), and monocytes (bottom) were compared between patients who were being treated (white circles) or not (black circles) with steroids at the indicated time points. The numbers of patients who were receiving steroids, as compared with the others, were, respectively: 17 and 11 at 1 month; 10 and 13 at 3 months; 4 and 15 at 6 months; and 3 and 12 at 12 months. Circles represent median values. *$P < .05$; **$P < .01$.

Acute GVHD Is an Independent Factor That Impairs DC Recovery

Because DC recovery was apparently affected by age, aGVHD, and steroid treatment, we analyzed these factors in univariate and multivariate analysis. Samples obtained from patients at 1 and 3 months after transplantation were scored depending on the dose of CS that the patient was receiving, the presence of signs of active GVHD at the time of the blood draw, and age. At 1 month after transplantation, in univariate analysis, both aGVHD ($P = .0004$) and steroids ($P = .03$) affected the CD123$^+$ DC reconstitution negatively (Table 3). Therefore, these 2 factors were analyzed in a multivariate analysis that showed only aGVHD to be an independent cause of reduced CD123$^+$ DC after allo-PBSC transplantation ($P = .002$; Table 3). CD11c$^+$ DC reconstitution did not correlate significantly with any of the factors tested (Table 3).

CS, but Not cGVHD, Affect Late DC Reconstitution

It is interesting to note that among patients at risk for cGVHD, at 6 to 12 months after transplantation, only those receiving CS had significantly reduced PB CD123$^+$ DC and CD11c$^+$ DC median counts ($P = .003$ and $P = .015$, respectively). Chronic GVHD did not correlate with CD123$^+$ DC ($P = .693$) or with CD11c$^+$ DC ($P = .930$) PB counts. Therefore, these data show that although aGVHD independently correlates with reduced CD123$^+$ DC counts early (1 month) after transplantation, at later time points (3-12 months) CD123$^+$ DC and CD11c$^+$ DC counts are reduced only in patients receiving steroids.

DISCUSSION

In this study we have analyzed the kinetics of reconstitution of 2 distinct DC subpopulations (CD11c$^+$ DC and CD123$^+$ DC) in the PB of 31 patients receiving a fully myeloablative preparatory regimen followed by allogeneic PBSC transplantation from HLA-identical sibling donors. Reconstitution of both CD11c$^+$ DC and CD123$^+$ DC PB counts to normal levels takes 6 months to 1 year and is slower than that of monocytes. Moreover, factors that potentially affect DC reconstitution were identified, including the development of aGVHD after transplantation and the treatment with CS.

It is now well established that human circulating DC are composed of at least 2 subsets, CD11c$^+$ DC and CD123$^+$ DC, which possibly belong to distinct hematopoietic lineages, ie, myeloid and lymphoid, respectively [2-8]. Because CD11c$^+$ DC and CD123$^+$ DC may have different, even opposing, functions in the regulation of immunity, we have used a flow cytometry–based method that could identify each subset separately from the other. Although there is no general consensus yet as to the standard way to enumerate

Table 3. *Multivariate Analysis of the Significance of the Correlation between Glucocorticoid Dose, Acute GVHD, and Age and the Number of Circulating DC in Patients at 1 Month after Transplantation*

Independent Variable	Univariate Analysis		Multivariate Analysis	
	pDC	mDC	pDC	mDC
Cortisone dose	.03*	.40	.29	NA
Acute GVHD	.0004*	.16	.004*	NA
Age	.47	.12	NA	NA

NA indicates not applicable; pDC, plasmacytoid DC; mDC, myeloid DC.
*Significant.

$CD11c^+$ DC and $CD123^+$ DC in PB, our method has been previously validated in clinical HSCT studies by us and others [11,25,32]. Recently, new $CD11c^+$ DC– and $CD123^+$ DC–related antigens have been characterized (blood dendritic cell antigen [BDCA]-1 through -4) that may allow a more direct and simpler way to identify both subsets [33], and anti–BDCA-2 antibodies may be effectively used for the enumeration of $CD123^+$ DC in allogeneic HSCT patients [26,33].

Our data show that $CD123^+$ DC and $CD11c^+$ DC counts are variably reduced early after transplantation but, more importantly, that their reconstitution may be independently impaired by the development of aGVHD and the administration of CS. It is interesting to note that the recovery of both DC subsets after autologous transplantation of PBSC, either unmanipulated or $CD34^+$ selected, has been recently shown to be faster than after allogeneic transplantation; most patients show normal levels of both $CD11c^+$ DC and $CD123^+$ DC as early as 1 month after transplantation [34,35]. Therefore, it is conceivable that an early reconstitution of DC in the autologous transplant setting may be favored by the absence of GVHD or treatment with immunosuppression.

Rabbit ATG has been shown to persist in the circulation for some time after transplantation and may bind to DC-related antigens, such as CD86 [36]. For this reason, it is rather surprising that in this study ATG did not affect $CD11c^+$ DC and $CD123^+$ DC reconstitution. Similarly, it has been recently observed that, although alemtuzumab (Campath-1G; Berlex Laboratories, Richmond, CA) may directly target PB DC in vivo, patients treated with alemtuzumab in their preparatory regimen did not show any impairment in DC recovery [25,37]. However, more recently, Morse et al. [38] have shown that alemtuzumab may selectively impair $CD123^+$, as opposed to $CD11c^+$, recovery. Moreover, DC recovery may be faster after reduced-intensity as compared with myeloablative preparatory regimens [27]. Also, graft $CD11c^+$ DC and $CD123^+$ DC, as well as graft $CD34^+$, cell doses did not correlate with DC reconstitution after transplantation [31]. It is interesting to note that in our series, neither TBI nor ATG correlated with the development of GVHD. However, these data should be interpreted with caution because of the low number of patients, which could limit the statistical power of the study.

Patients with aGVHD grade II to IV had fewer circulating $CD11c^+$ DC and $CD123^+$ DC, and the treatment with CS further reduced DC numbers, particularly $CD123^+$ DC, in the blood. However, blood levels of all the other cell types tested were unaffected by the development of aGVHD, with the exception of T cells, which seemed to be reduced in patients with severe aGVHD, as previously reported [28]. Although it is not clear whether the development of GVHD is followed by a decline in blood DC numbers or vice versa, because most patients had already developed aGVHD at the time of the first blood draw (ie, 1 month after transplantation), in the 2 patients who developed aGVHD >30 days after transplantation, $CD11c^+$ DC and $CD123^+$ DC levels strongly decreased after GVHD onset, suggesting that GVHD may indeed deplete circulating DC. Moreover, at present it is not clear why aGVHD may affect more $CD123^+$ DC than $CD11c^+$ DC levels. One possible hypothesis is that monocytic DC precursors, whose numbers are unaffected, may at least partially replenish the circulating $CD11c^+$ DC pool. Reduced numbers of circulating DC in patients with aGVHD have been previously reported also by Fearnley et al. [23], although they did not distinguish between $CD11c^+$ DC and $CD123^+$ DC. Nevertheless, our findings may also raise the question of whether early recovery of donor DC might prevent aGVHD. The observation that CS can reduce PB $CD123^+$ DC levels is in accordance with studies performed in clinical settings other than allogeneic HSCT, as well as in healthy donors [39]. However, although CS have been shown to affect the survival and function of $CD11c^+$ DC in vitro [40], their effect on $CD123^+$ DC has not yet been investigated.

In our study, cGVHD did not correlate with PB DC numbers. This is in contrast with a recent report by Clark et al. [26], which showed that cGVHD may be associated with increased numbers of circulating $CD123^+$ DC. This might be explained by differences in methods of $CD123^+$ DC identification or in drugs administered to the patients, eg, CS, that could possibly mask the increase in $CD123^+$ DC levels. Moreover, a recent report has suggested that the development of cGVHD may correlate with the presence of measurable mixed chimerism within the pool of circulating APC, particularly monocytic DC precursors [41]. However, in most studies PB $CD11c^+$ DC and $CD123^+$ DC have been shown to be consistently 100% of donor origin as early as 1 month after transplantation [24-26]. Further studies are needed to evaluate chimerism levels in circulating and tissue-resident DC subsets late after allogeneic transplantation in patients with or without GVHD.

In conclusion, our data suggest that the full reconstitution of blood $CD11c^+$ DC and $CD123^+$ DC levels may be significantly delayed after allogeneic HSCT because of aGVHD and its treatment with CS. Different experimental evidence, as well as some clinical studies in nontransplant settings, such as acquired immune deficiency syndrome [42], suggest that a lack of DC may enhance the risk of opportunistic infections. Therefore, future studies will address whether this is also the case in human HSCT. More impor-

tantly, because aGVHD is likely to be triggered by residual host DC interacting with donor T lymphocytes, it could be hypothesized that administering CS to HSCT patients shortly before starting the conditioning regimen might selectively eliminate tissue DC and thus reduce the risk of aGVHD. In addition, clinical trials are warranted to address whether depletion of $CD11c^+$ DC, $CD123^+$ DC, or both in the graft affects the incidence of GVHD and the dendritic and lymphoid immune reconstitution.

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REFERENCES

1. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annu Rev Immunol.* 2000;18:767-811.
2. Liu YJ, Kanzler H, Soumelis V, Gilliet M. Dendritic cell lineage, plasticity and cross-regulation. *Nat Immunol.* 2001;2: 585-589.
3. O'Doherty U, Peng M, Gezelter S, et al. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology.* 1994;82:487-493.
4. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu Y. The enigmatic plasmacytoid T cells develop into dendritic cells with IL-3 and CD40-ligand. *J Exp Med.* 1997;185: 1101-1111.
5. Robinson S, Patterson S, English N, Davies D, Knight S, Reid C. Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol.* 1999;29:2769-2778.
6. Kohrgruber N, Halanek N, Groger M, et al. Survival, maturation, and function of $CD11c^-$ and $CD11c^+$ peripheral blood dendritic cells are differentially regulated by cytokines. *J Immunol.* 1999;163:3250-3259.
7. Bruno L, Res P, Dessing M, Cella M, Spits H. Identification of a committed T cell precursor population in adult peripheral blood. *J Exp Med.* 1997;185:875-884.
8. Bendriss-Vermare N, Barthelemy C, Durand I, et al. Human thymus contains IFN-α–producing $CD11c^-$, myeloid $CD11c^+$, and mature interdigitating dendritic cells. *J Clin Invest.* 2001; 107:835-844.
9. Spits H, Couwenberg F, Bakker A, Weijer K, Uittenbogaart CH. Id2 and Id3 inhibit development of $CD34^+$ stem cells into predendritic cell (Pre-DC)2 but not into pre-DC1: evidence for a lymphoid origin of Pre-DC2. *J Exp Med.* 2000;192:1775-1783.
10. Rissoan M, Soumelis V, Kadowaki N, et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science.* 1999; 283:1183-1186.
11. Arpinati M, Green C, Heimfeld S, Heuser J, Anasetti C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood.* 2000;95:2484-2490.
12. Siegal FP, Kadowaki N, Shodell M, et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science.* 1999;284:1835-1837.
13. Cella M, Jarrossay D, Facchetti F, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med.* 1999;5:919-923.
14. Kuwana M, Kaburaki J, Wright T, Kawakami Y, Ikeda Y. Induction of antigen-specific human $CD4^+$ T cell anergy by peripheral blood DC2 precursors. *Eur J Immunol.* 2000;31: 2547-2557.
15. Kadowaki N, Antonenko S, Lau Y, Liu Y. Natural interferon α/β-producing cells link innate and adaptive immunity. *J Exp Med.* 2000;192:219-226.
16. Cella M, Facchetti F, Lanzavecchia A, Colonna M. Plasmacytoid dendritic cells activated by influenza virus and CD40 ligand drive a potent Th1 polarization. *Nat Immunol.* 2001;5: 305-310.
17. Gilliet M, Liu Y. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J Exp Med.* 2002;195:695-704.
18. Liu YJ, Blom B. Introduction: TH2-inducing DC2 for immunotherapy. *Blood.* 2000;95:2482-2483.
19. Shlomchick W, Couzens M, Tang Bi C, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science.* 1999;285:412-415.
20. Zhang Y, Louboutin JP, Zhu J, Rivera AJ, Emerson SG. Preterminal host dendritic cells in irradiated mice prime CD8+ T cell-mediated acute graft-versus-host disease. *J Clin Invest.* 2002;109:1335-1344.
21. Matsumoto M, Katoh Y, Nakamura Y, et al. Injection of CD4(+) and CD8(+) cells with donor or host accessory cells induces acute graft-vs-host disease in human skin in immunodeficient mice. *Exp Hematol.* 2001;29:720-727.
22. Waller EK, Rosenthal H, Jones TW, et al. Larger numbers of $CD4^{bright}$ dendritic cells in donor bone marrow are associated with increased relapse after allogeneic bone marrow transplantation. *Blood.* 2001;97:2948-2956.
23. Fearnley DB, Whyte LF, Carnoutsos SA, Cook AH, Hart DNJ. Monitoring human blood dendritic cell numbers in normal individuals and in stem cell transplantation. *Blood.* 1999;93:728-736.
24. Auffermann-Gretzinger S, Lossos IS, Vayntrub TA, et al. Rapid establishment of dendritic cell chimerism in allogeneic hematopoietic cell transplant recipients. *Blood.* 2002;99:1442-1448.
25. Klangsinsirikul P, Carter GI, Byrne JL, Hale G, Russell NH. Campath-1G causes rapid depletion of circulating host dendritic cells (DCs) before allogeneic transplantation but does not delay donor DC reconstitution. *Blood.* 2002;99:2586-2591.
26. Clark FJ, Freeman L, Dzionek A, et al. Origin and subset distribution of peripheral blood dendritic cells in patients with chronic graft-versus-host disease. *Transplantation.* 2003;27:221-225.
27. Mohty M, Gaugler B, Faucher C, et al. Recovery of lymphocyte and dendritic cell subsets following reduced intensity allogeneic bone marrow transplantation. *Hematology.* 2002;7:157-164.
28. Rondelli D, Raspadori D, Anasetti C, et al. Alloantigen presenting capacity, T cell alloreactivity and NK function of G-CSF-mobilized peripheral blood cells. *Bone Marrow Transplant.* 1998;22:631-637.

29. Przepiorka D, Weisdorf D, Martin P, et al. Consensus conference on acute GVHD grading. *Bone Marrow Transplant.* 1995; 15:825-828.
30. Shulman HM, Sullivan KM, Weidwn PL, et al. Chronic graft-versus-host syndrome in man. A long term clinico-pathologic study of 20 Seattle patients. *Am J Med.* 1980;69:204-217.
31. Urbini B, Arpinati M, Bonifazi F, et al. Allogeneic graft $CD34^+$ cell dose correlates with dendritic cell dose and clinical outcome but not with dendritic cell reconstitution after transplant. *Exp Hematol.* 2003;31:959-965.
32. Klangsinsirikul P, Russell N. Peripheral blood stem cell harvests from G-CSF-stimulated donors contain a skewed TH2 CD4 phenotype and a predominance of type 2 dendritic cells. *Exp Hematol.* 2002;30:495-501.
33. Arpinati M, Chirumbolo G, Urbini B, et al. Use of anti-BDCA-2 antibody for detection of dendritic cells type-2 (DC2) in allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2002;29:887-891.
34. Galy A, Rudraraju S, Baynes R, Klein J. Recovery of lymphocyte and dendritic cell subsets after autologous CD34+ cell transplantation. *Bone Marrow Transplant.* 2000;25:1249-1255.
35. Damiani D, Stocchi R, Masolini P, et al. Dendritic cell recovery after autologous stem cell transplantation. *Bone Marrow Transplant.* 2002;30:261-266.
36. Ayas M, Al-Mahr M, Al-Jefri A, Rifai S, Solh H. Does adding ATG to the GVHD prophylaxis regimen help reduce its incidence? *Bone Marrow Transplant.* 2003;31:311.
37. Ratzinger G, Reagan JL, Heller G, Busam KJ, Young JW. Differential CD52 expression by distinct myeloid dendritic cell subsets: implications for alemtuzumab activity at the level of antigen presentation in allogeneic graft-host interactions in transplantation. *Blood.* 2003;101:1422-1429.
38. Morse MA, Rizzieri D, Stenzel TT, et al. Dendritic cell recovery following nonmyeloablative allogeneic stem cell transplants. *J Hematother Stem Cell Res.* 2002;4:659-668.
39. Shodell M, Siegal FP. Corticosteroids depress IFN-α-producing plasmacytoid dendritic cells in human blood. *J Allergy Clin Immunol.* 2001;108:446-448.
40. Matyszak MK, Citterio S, Rescigno M, Ricciardi-Castagnoli P. Differential effects of corticosteroids during different stages of dendritic cell maturation. *Eur J Immunol.* 2000;30:1233-1242.
41. Chan GW, Gorgun G, Miller KB, Foss FM. Persistence of host dendritic cells after transplantation is associated with graft-versus-host disease. *Biol Blood Marrow Transplant.* 2003;9:170-176.
42. Soumelis V, Scott I, Gheyas F, et al. Depletion of circulating natural type 1 interferon-producing cells in HIV-infected AIDS patients. *Blood.* 2001;98:906-914.