Brief report

G-CSF mobilizes slanDCs (6-sulfo LacNAc\(^+\) dendritic cells) with a high proinflammatory capacity

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Donor dendritic cells (DCs) play a pivotal role in the induction of immunity and tolerance after peripheral blood stem cell transplantation (PBSCT). Treatment of healthy donors with granulocyte-colony stimulating factor (G-CSF) increases the numbers of tolerogenic DCs and T cells among mobilized blood leukocytes in the graft. SlanDCs (6-sulfo LacNAc\(^+\) DCs), a major source of IL-12 and TNF-\(\alpha\) in blood, have not been studied in this respect. Here, we demonstrate that slanDCs (14.9 \(\times\) 10\(^6\)/L to 64.0 \(\times\) 10\(^6\)/L) are efficiently mobilized by G-CSF and retain their capacity to produce IL-12 and TNF-\(\alpha\) at high levels. Furthermore, G-CSF–mobilized slanDCs programmed the differentiation of Th1 cells and displayed a particularly strong capacity to stimulate the proliferation of naive allogeneic T cells. Thus, slanDCs transfused into recipients of allogeneic peripheral blood stem cell (PBSC) transplants are functionally fully capable and may be critical in supporting graft-versus-host disease as well as graft-versus-leukemia effects.

Introduction

Granulocyte-colony stimulating factor (G-CSF) is widely used to mobilize hematopoietic stem cells for allogeneic peripheral blood stem cell transplantation (PBSCT).\(^1,2\) Graft-versus-host disease (GVHD) leads to significant morbidity and mortality,\(^3,4\) and therefore remains a major complication of PBSCT. Several studies have indicated a tolerogenic effect of G-CSF. In T cells, G-CSF has been shown to directly inhibit interferon-\(\gamma\) (IFN-\(\gamma\)) production, to increase interleukin-4 (IL-4) production,\(^5,6\) and to induce regulatory T cells.\(^7\) G-CSF also mobilizes tolerogenic CD14\(^+\) cells expressing cell surface IL-10.\(^8,9\) So far, G-CSF has been shown to selectively mobilize plasmacytoid dendritic cells (pDCs) that induce T cells to produce high levels of IL-4 and IL-10, but low levels of IFN-\(\gamma\).\(^10,11\) The in vivo effects of G-CSF on the large population (1.2% of peripheral blood mononuclear cells [PBMCs]) of proinflammatory 6-sulfoLacNAc expressing DCs, now called slanDCs, have not been studied so far. SlanDCs are potent inducers of primary T-cell responses in vitro\(^12\) and have recently been described as the main producers of tumor-necrosis factor \(\alpha\) (TNF-\(\alpha\))\(^12\) and notably of IL-12 after stimulation with lipopolysaccharide (LPS), R848, or CD40 ligand.\(^13\) Given their high proinflammatory potential, these cells might play a crucial role in the immune balance after allogeneic PBSCT.

Materials and methods

This study was approved by the institutional review board of Technische Universität Dresden. Peripheral blood mononuclear cells (PBMCs) were prepared from paired blood samples obtained with the informed consent (in accordance with the Declaration of Helsinki) of healthy peripheral blood stem cell (PBSC) donors before and after receiving G-CSF (5 days, 7.5 \(\mu\)g/kg per day kenograsitm; Chugai, Tokyo, Japan). The percentage of DC subsets was determined by flow cytometry as described previously,\(^12\) and in parallel the white blood cell count was determined (Sysmex XE 2100; Sysmex, Hamburg, Germany). The absolute numbers of the DC subtypes were calculated from the absolute PBMC count, multiplied by the percentage of each subpopulation determined by flow cytometry.

Statistical analysis was performed using paired \(t\) tests.

Results and discussion

To determine the mobilizing effect of G-CSF on slanDCs, we studied PBMCs from healthy donors prior to and on day 5 of receiving G-CSF (\(n = 13\)). The flow cytometric analysis revealed a...
Notably, we observed that after G-CSF treatment of the donors, immature slanDCs have a slightly enhanced capacity to stimulate the proliferation of allogeneic naive CD4+CD45RA+ cord blood T cells (Figure 2C). Furthermore, we documented that G-CSF–mobilized slanDCs continued to program naive cord blood T cells for a strong Th1-dominated immune response in the presence of LPS as evidenced by high levels of IFN-γ and low levels of IL-4 in cell-free supernatants (Figure 2D).

The high frequency and unaltered proinflammatory capacity of slanDCs in PBSC grafts shed new light on the immune balance after allogeneic PBSCT. Myeloid DCs are regarded as key players in the initiation of GVHD as well as in maintaining the graft-versus-leukemia reaction (GVL). In contrast, pDCs have been shown to induce T-cell tolerance, and therefore increased numbers of pDCs in PBSC grafts were thought to account for the unexpectedly low incidence of acute GVHD (aGVHD) observed in initial studies. However, a recently performed meta-analysis revealed a significant increase of grades 3 to 4 aGVHD after PBSCT compared with bone marrow transplantation (BMT). In addition, chronic GVHD is known to be significantly increased in PBSCT compared with BMT.

According to current thinking, GVHD is triggered by the conditioning regimen. Mucosal damage induced by chemotherapy and irradiation allows microbial products such as LPS to enter the systemic circulation and to trigger the production of inflammatory cytokines such as TNF-α and IL-12, both of which are important mediators of GVHD. This early and high-level IL-12 production was shown to be crucial, as elevated IL-12p70 serum levels in the first month after PBSCT turned out to be a strong predictive factor for aGVHD development. However, elevated serum IL-12 levels may also enhance the GVL reaction, because they were shown to be associated with an improved relapse-free survival.

Our findings provide evidence that, in addition to the tolerogenic effects of G-CSF described by others, proinflammatory slanDCs in PBSC grafts are increased in numbers and are functionally competent. Thus, grafts containing slanDCs that are infused directly after conditioning therapy can be important for the immediate inflammatory response and for stimulating indirect T-cell immune responses against host tissues resulting in GVHD. This adverse function of slanDCs may be directly addressed by purging slanDCs from PBSC grafts. In this case GVHD may be reduced, however, slanDCs originating from donor stem cells after successful engraftment may well be able to contribute to the GVL immune responses as our previous studies demonstrated effective priming of tumor-specific cytotoxic T cells in vitro and enhancement of the tumorcidal activity of natural killer (NK) cells by high-level IL-12–producing slanDCs.

**Figure 1.** SlanDCs are mobilized by G-CSF and display an unaltered phenotype. Peripheral blood samples were collected from the same donors before and after G-CSF treatment. (A) Absolute cell counts of slanDCs (M-DC8+, CD1c+ DCs (lin−, HLA-DR+, CD11c+), and pDCs (lin−, HLA-DR+, CD11c+) were determined as described in “Materials and methods” (n = 13). Individual values and the median are shown. P values were determined using paired t tests. (B) The phenotypic analysis of slanDCs before and after G-CSF administration is compared. Results of one representative donor are shown (n = 5). Staining of PBMCs and gating were performed as described.

![Figure 1](image-url)

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**Authorship**

Contribution: S.H.C.B. performed the experiments and drafted the paper (this work is part of S.H.C.B.’s doctoral thesis); K.H. coordinated the blood sampling and the recruitment of donors; M.B. and E.P.R. contributed to the design and the writing of the paper; M.M. contributed to the writing of the paper; K.S. designed the study.

![Footnote Image](footnote-url)
the project, finalized the paper, and served as the thesis supervisor of S.H.C.B.

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References


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