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The healthy donor profile of immunoregulatory soluble mediators is altered by stem cell mobilization and apheresis

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Abstract

Background. Peripheral blood stem cells from healthy donors mobilized by granulocyte colony-stimulating factor (G-CSF) and thereafter harvested by leukapheresis are commonly used for allogeneic stem cell transplantation. *Methods.* Plasma levels of 38 soluble mediators (cytokines, soluble adhesion molecules, proteases, protease inhibitors) were analyzed in samples derived from healthy stem cell donors before G-CSF treatment and after 4 days, both immediately before and after leukapheresis. *Results.* Donors could be classified into two main subsets based on their plasma mediator profile before G-CSF treatment. Seventeen of 36 detectable mediators were significantly altered by G-CSF; generally an increase in mediator levels was seen, including pro-inflammatory cytokines, soluble adhesion molecules and proteases. Several leukocyte- and platelet-released mediators were increased during apheresis. Both plasma and graft mediator profiles were thus altered and showed correlations to graft concentrations of leukocytes and platelets; these concentrations were influenced by the apheresis device used. Finally, the mediator profile of the allotransplant recipients was altered by graft infusion, and based on their day +1 post-transplantation plasma profile our recipients could be divided into two major subsets that differed in overall survival. *Discussion.* G-CSF alters the short-term plasma mediator profile of healthy stem cell donors. These effects together with the leukocyte and platelet levels in the graft determine the mediator profile of the stem cell grafts. Graft infusion also alters the systemic mediator profile of the recipients, but further studies are required to clarify whether such graft-induced alterations have a prognostic impact.

Key Words: *allogeneic stem cell transplantation, apheresis, chemokine, granulocyte colony-stimulating factor, hematopoietic stem cell mobilization, interleukin, peripheral blood stem cell grafts, plasma profile, protease, soluble adhesion molecule*

Introduction

Allogeneic stem cell grafts from both bone marrow and peripheral blood allografts contain hematopoietic stem cells as well as large populations of immunocompetent cells and platelets [1]. Previous studies have demonstrated that T-cell graft depletion reduced the risk of severe graft-versus-host disease (GVHD) but increased the risk of leukemia relapse and graft failure [2–5]. Thus, the risk of immune-dependent post-transplantation complications was dependent on the number of graft immunocompetent cells and especially the number of T cells. One would therefore expect an increased frequency and/or severity of GVHD when using peripheral blood stem cells (PBSCs) mobilized by granulocyte colony-stimulating factor (G-CSF) because such grafts contain high T-cell numbers [6]. However, the incidence of acute GVHD after allogeneic peripheral blood stem cell transplantation (PBSCT) has been reported to be lower than expected [7–9], an observation indicating that T cells in blood grafts differ from bone marrow grafts. The plasma levels of soluble mediators may then reflect a G-CSF–induced immunomodulation that could involve the graft immunocompetent cells and thereby be important for the risk of post-transplantation immune-mediated complications. It is not known whether such G-CSF effects can influence donor

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health. A recent study showed that G-CSF therapy in healthy stem cell donors induced changes in the $CD34^+$ cell expression of more than 2000 genes and microRNAs involved in regulation of cell cycle progression, proliferation, angiogenesis and immune responses [10]. These changes increased during the first 30 days after G-CSF treatment, and lasted for at least 1 year. G-CSF also alters the short-term systemic metabolic regulation [11]. We previously described altered cytokine levels in autologous donors after both hematopoietic stem cell mobilization and apheresis [12,13] and an association between the pretransplantation serum cytokine profile of allotransplant recipients and risk of post-transplantation complications [14]. Taken together, these observations suggest that early cytokine-mediated effects are important for outcome after allotransplantation and donor immunoregulation seems to have an additional impact on patient outcome [15–19]. In this context we have examined the effects of G-CSF treatment and stem cell harvesting on plasma levels of immunoregulatory soluble mediators, the levels of these mediators in allogeneic stem cell grafts and the effects of stem cell infusion on the recipient cytokine network.

Materials and methods

Healthy allogeneic stem cell donors and allotransplant recipients

All studies were approved by the local ethics committee (REK III No.126.01, Regional Committee for Medical and Health Research Ethics of Western Norway: 2011/996, 2011/1237, 2011/1241 and 2013/634). The participants were included after signing a written informed consent. The present study includes 25 consecutive healthy HLA-matched related allogeneic stem cell donors, 16 males and 9 females with median age 54 years (range, 25–77 years), and 16 allogeneic stem cell transplant recipients, 7 males and 9 females with median age 47 years (range, 35–63 years).

Stem cell mobilization and harvesting in the healthy donors

The matched related donors received the stem cell mobilizing agent human non-glycosylated G-CSF 10 µg/kg/d for 4 days prior to stem cell harvesting. Stem cell quantification started on day 4 of G-CSF stimulation, and harvesting was performed when the stem cell count exceeded 15–20 x 10^3/mL. The first nine stem cell donors were harvested by large-volume leukapheresis with four times processing of the total blood volume, using the Mononuclear Cell Removal-protocol with the WBC kit for the Cobe Spectra cell separator version 7 (Cobe Laboratories). During the study the apheresis devices of our department were replaced due to timely equipment upgrade; hence the mononuclear cell (MNC) procedure with the Spectra Optia Collection Set on the Spectra Optia cell separator version 9 (Terumo BCT Inc.) was used for the 16 last donors.

Allogeneic stem cell transplantation

Eleven of the 16 allotransplant recipients were diagnosed with acute myeloid leukemia (AML), three with acute B cell lymphoblastic leukemia (B-ALL), one with myelofibrosis and one with myelodysplastic syndrome (MDS). At the time of transplantation, all leukemia patients were in complete hematologic remission; 14 patients received myeloablative conditioning with intravenous busulfan plus cyclophosphamide, and two patients received reduced intensity conditioning with intravenous fludarabine plus busulfan. All allotransplant recipients received G-CSF mobilized peripheral blood stem cell grafts derived from HLA-matched family donors and GVHD prophylaxis with cyclosporine A plus methotrexate. Neutrophil counts exceeding 0.5 x 10^9/L and stable platelet counts exceeding 50 x 10^9/L without platelet transfusions for at least 3 consecutive days were defined as neutrophil and platelet reconstitution, respectively.

Preparation of plasma and stem cell graft supernatant samples

Venous blood samples from the allogeneic stem cell donors were collected (i) at the time of the pretransplantation evaluation prior to G-CSF treatment, median 20.5 days before apheresis, (ii) during G-CSF therapy in the morning immediately before apheresis, (iii) immediately after apheresis and (iv) approximately 24 h after start of apheresis. From the allotransplant recipients venous blood samples were collected (i) between 0700 and 0900 AM the day of transplantation, (ii) between 0700 and 0900 AM the day after stem cell infusion and (iii) between 0700 and 0900 AM approximately 1 week after allogeneic stem cell transplantation (median, 6 days; variation range, 4–13 days). All venous blood samples were collected into Vacuette 9NC tubes with sodium citrate and acid-citrate-dextrose solution A (Greiner Bio-One GmbH). Plastic tubes without additives were used for samples from stem cell allografts. All blood and graft samples were centrifuged at 1310*g* for 10 min at room temperature within 30 min of sampling. The plasma supernatants were immediately transferred to plastic tubes, frozen and stored at -70°C until analyzed.

Plasma mediator levels

The concentrations of the following 38 mediators were determined using Luminex analyses (R&D Systems):