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Pilot Analysis of Cytokines Levels in Stored G-CSF-Mobilized Peripheral Blood Stem Cell Concentrates

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Abstract

Background—The transfusion of peripheral blood stem cells (PBSC) concentrates are sometimes associated with febrile transfusion reactions. PBSC concentrates contain large numbers of leukocytes and during storage the levels of soluble cytokines which could cause transfusion reactions, may increase.

Methods—Aliquots of G-CSF-mobilized PBSC concentrates from 9 healthy subjects were stored in bags at 2 to 8°C for 48 hours. The levels of 19 growth factors and biologic response modifiers (BRMs) were measured in the plasma of PBSC concentrates at 0, 24, and 48 hours of storage using a nested ELISA. The same 19 factor levels were also measured in blood plasma from 6 healthy subjects.

Results—There were no significant differences in the PBSC and plasma levels of soluble IL-1 β , IL-6, and TNF- α which can cause febrile reactions. The levels of TGF- β 1, MMP-8, CCL5/RANTES, and PDGF-AB were significantly greater in PBSCs than in plasma and the level of CCL2/MCP-1 was significantly less in PBSCs. Duration of PBSC storage had no effect on the levels of these 5 factors. There was a trend for reduced levels of IL-1 β , IL-2, IL-7, IL-8, IL-12p70, IL-15, IFN- γ , CD40L and GM-CSF and increased levels of TNF- α and IL-10 levels in PBSC concentrates, but the differences were not significant.

Conclusions—There was no increase in stored PBSC concentrates of cytokines that have been associated with febrile transfusion reactions, however, the levels of other factors that were likely released by platelets and granulocytes during the collection process were elevated.

Keywords

peripheral blood stem cells; Granulocyte colony-stimulating factor; cytokines; growth factors; febrile transfusion reactions

Introduction

Hematopoietic stem cells for transplantation are often collected by apheresis from healthy subjects that are given a 5- to 6-day course of granulocyte colony-stimulating factor (G-CSF) to increase the concentration of circulating stem cells. Similar to other blood components the transfusion of these peripheral blood stem cell (PBSC) concentrates are sometimes associated with reactions. These reactions include fever, dyspnea, changes in blood pressure and hemolysis with febrile reactions being most common.

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Febrile transfusion reactions can be caused by the interaction of antibodies in the recipient with leukocytes in the transfused blood product or by cytokines in blood products. The levels of cytokines in blood products are determined by the balance between release from cells in the blood product and binding and/or degradation. In non-leukocyte-reduced platelet concentrates the levels of several cytokines rise during room temperature storage. After 3 to 5 days of storage levels of several factors become elevated including: IL-1 β , IL-6, IL-8, TNF α , transforming growth factor β (TGF- β), platelet factor 4, and CCL5/(reduced on activation normal T expressed and secreted (RANTES)).¹⁻⁹ Increased platelet concentrate levels of IL-1 β , IL-6 and TNF α have been associated with febrile transfusion reactions.^{1,2,10} High concentrations of the platelet-derived cytokine CCL5/RANTES in platelet concentrates have been associated with allergic transfusion reactions because CCL5 promotes chemotaxis of eosinophils, “memory” T cells, and basophils; induces histamine release; and stimulates activation of eosinophils^{11, 12}.

When PBSC concentrates are collected for transplants involving HLA-compatible siblings, they are generally collected, processed and transplanted within a few hours of collection. However, when PBSC concentrates are collected from unrelated donors they are most often collected at one site, transported, and transfused at another site. The transportation of the PBSC concentrates from the collection center to the transplant center typically takes 12 to 36 hours and the PBSC concentrates may be 48 hours old before they are transfused. Since PBSC concentrates contain large quantities of leukocytes, we hypothesized that when they are transported or stored, leukocytes in the concentrates might produce and release cytokines, some of which could cause febrile transfusion reactions. PBSC concentrates were collected from healthy subjects who were given 5 days of G-CSF and cytokine and growth factor levels were measured in aliquots that had been stored up to 48 hours at 2 to 8°C.

Materials and Methods

Study design

Nine healthy subjects were given 10 micrograms of G-CSF (Filgrastim, Amgen, Thousand Oaks, CA) for 5 days and a PBSC concentrate was collected for research studies with a blood cell separator (CS3000 Plus, Baxter Healthcare Corp., Fenwal Division, Deerfield, IL) on the fifth day. A 6 mL aliquot was removed from the concentrate and divided into three equal parts: one aliquot was tested immediately and two aliquots were stored in 6 mL Teflon bags (FEP bags 6 mL, American Fluoroseal, Gaithersburg, MD) at 2°C to 8°C. One sample was tested after 24 hours of storage and the other after 48 hours. These studies were approved by an NIH institutional review board on the use of human subjects in research.

Measurement of soluble factor levels and blood counts

White blood cell (WBC) and platelet counts were performed with an automated cell counter (Cell-Dyn 3500, Abbot Diagnostics, Abbott Park, IL). To measure cytokine levels the PBSC samples were centrifuged (2,500xg for 10 minutes) and the levels of 19 soluble factors were measured in the supernatant by nested ELISA (SearchLight, Aushon BioSystems, Inc., Billerica, MA). The factors measured were GM-CSF, IL-1 β , IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-15, IFN- γ , TNF- α , CD40L, MMP-8, TGF- β 1, CCL2/MCP-1, CCL3/MIP-1 α , CCL5/RANTES, and PDGF-AB. As a control, the plasma levels of the same 19 factors were measured in 6 healthy subjects who had not been given G-CSF.

Statistical Analysis

Values shown are mean $\pm$ one standard deviation. Groups were compared using two-tailed t-test and were calculated using Microsoft excel.

Results

The mean WBC and platelet counts measured in the 9 PBSC concentrates immediately after collection were $246 \pm 70 \times 10^9/\text{L}$ and $1,727 \pm 658 \times 10^9/\text{L}$ respectively. WBC and platelet counts after 24 and 48 hours of storage were available on 7 of the 9 PBSC concentrates and there was no change in either WBC or platelet counts with storage.

The levels of IL-4 and CCL3/MIP-1 α were below the levels of detection for all samples. The levels of the other 17 factors measured were compared between the PBSC concentrates and the plasma samples (Table 1). The levels of TGF- β 1, MMP-8, CCL5/RANTES, and PDGF-AB were significantly greater in PBSCs than in plasma and the level of CCL2/MCP-1 was significantly less in PBSCs. There was a trend for reduced levels of the following factors in PBSC concentrates: IL-1 β , IL-2, IL-7, IL-8, IL-12p70, IL-15, IFN- γ , CD40L and GM-CSF.

Discussion

We found that the levels of cytokines implicated in febrile reactions to platelet concentrates, IL-1 β , IL-6 and TNF α , were not increased in fresh or stored PBSC concentrates. The difference between these cytokines in PBSC and platelet concentrates may be due to differences in storage temperatures and storage duration. Platelet concentrates are stored while being agitated and at room temperature while the PBSC concentrates were stored without agitation and at 2 to 8°C since most centers transport PBSC concentrates at this temperature range. It may be that leukocytes in PBSC concentrates would produce IL-1 β , IL-6, and TNF- α if they were stored at room temperature. Cytokine levels increase in leukocyte containing RBC components store at 2 to 8°C, but at a much slow rate that in leukocyte containing platelet components.^{13,14} Since the levels of IL-1 β and IL-6 were not found to be elevated in platelet concentrates before 3 days of storage,^{1,4,6} it is possible that the levels of these cytokines might have increased in PBSC concentrates had they been stored longer. Although IL-1 β , IL-6 and TNF levels were not elevated in PBSCs stored for 48 hours, we cannot exclude the possibility that the levels of these cytokines maybe elevated in some PBSC concentrates and could contribute to transfusion reactions. A study which compared cytokine, chemokine and growth factor levels in specific products with reactions or lack of reactions in a large number of PBSC transfusion recipients is required to fully define the role of these factors in transfusion reactions.

We found that the levels of CCL5/RANTES, PDGF-AB, TGF- β 1, and MMP-8 were elevated in PBSC concentrates. CCL5/RANTES, PDGF-AB, TGF- β 1 are produced by platelets. A study by Foss et al. has found that the levels of PDGF-AB, along with β -thromboglobulin, and CXCL4/platelet factor 4 (PF4) were increased in G-CSF-mobilized PBSC concentrates, possibility due to the activation of platelets during the collection process.¹⁵ However, they did not measure CCL5/RANTES, MMP-8 or TGF- β 1 levels. Our results also suggest that these factors were released from platelets during collection rather than during storage since the levels of these factors were increased immediately after collection and did not change during storage.

The transfusion of these factors with PBSC concentrates may affect the outcome of the transplant. The volume of PBSC concentrates ranges from 200 to 400 mL and the levels of CCL5/RANTES, TGF- β 1, PDGF-AB, and MMP-8 may become transiently elevated following the transfusion of PBSC concentrates. CCL5/RANTES is a potent chemoattractant for monocytes, memory T helper cells and eosinophils.¹⁶ Platelet-derived biologic response modifiers including CCL5/RANTES, platelet factor 4 and TGF- β 1 have been studied to elucidate the factor(s) responsible for nonhemolytic febrile transfusion reactions in platelet transfusions.^{7,8} RANTES and platelet factor 4, have been shown to be released in stored platelet components and high levels of these factors may mediate allergic reactions by releasing histamine from basophils.^{7,9} TGF- β 1 has also been found in high concentration in

platelets^{17,18} and has been shown to be released during platelet component storage.⁸ TGF- β 1 is a pleiotropic cytokine that plays a role in processes such as neoangiogenesis and Immunosuppression.¹⁹ It suppresses cell growth by inducing cell cycle arrest and it functions as an immune suppressant by inhibiting T cell proliferation, NK cell function, and antigen presentation.^{20,21} In addition, TGF- β 1 has been shown to play a critical role in the differentiation and induction of immunosuppressive CD4⁺Foxp3⁺ Treg cells.²² Furthermore, TGF- β together with IL-6 induces differentiation of IL-17-producing T-cell subsets called Th17 and Tc17 cells.²³

These studies revealed that the level of MMP-8 which is found in granulocytes and macrophages was also elevated in PBSC concentrates immediately after collection and there was a trend toward increased levels with storage. This is consistent with the release of MMP-8 by activated granulocytes during collection and further release from activated or apoptotic granulocytes during storage.

We found that the level of CCL2/MCP-1 was lower in PBSC concentrates than in plasma. PBSC concentrates typically contain very high concentrates of T cells and monocytes and the reduced levels of CCL2/MCP-1 may be due to binding to its receptor, CCR2, on monocytes and memory T cells. Since the leukocytes found in PBSC concentrates express a number of other cytokine receptors, it is not surprising that there was also a trend toward a reduction in the levels of IL-2, IL-6, IL-7 and IL-8 in PBSC concentrates.

CD40L is produced by platelets and the levels of soluble CD40L in platelet concentrates increase during storage.²⁴ Increased levels of CD40L in platelet concentrates have been associated with transfusion related acute lung injury.²⁴ Although PBSC concentrates contain large quantities of platelets, we did not detect an increase in CD40L in stored PBSC concentrates. It may be that platelets at 2 to 8°C do not release CD40L.

In conclusion, we found no increase in cytokines in stored PBSC concentrates that have been associated with febrile transfusion reactions, however, the levels of other factors were elevated. These factors were likely released by platelets and granulocytes or macrophages during the collection process, suggesting that methods used for preparation of PBSC concentrates may affect release and accumulation of some biologic response modifiers. The storage of PBSC concentrates at 2 to 8°C for 48 hours had little effect on soluble factor levels.

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Table 1

Soluble factor levels in PBSC concentrates and plasma

	Soluble Factor Levels (pg/mL)			
	PBSC Concentrate (n=9) (Storage time)			Blood Plasma (n=6)*
	0 hours	24 hours	48 hours	
IL-1β	2.7 ± 7.2	3.1 ± 7.9	3.4 ± 8.8	5.9 ± 12.4
IL-2	16.2 ±40.6	14.9 ± 39.6	18.9 ± 49.9	28.8 ± 38.8
IL-6	1.3 ± 2.6	1.3 ± 2.5	1.2 ± 2.5	4.5 ± 4.9
IL-7	0.7 ± 2.2	1.8 ± 4.6	0.9 ± 2.5	5.2 ± 10.0
IL-8	0.2 ± 0.4	0.5 ± 0.7	0.5 ± 1.0	1.3 ± 0.8
IL-10	2.7 ± 1.2	2.5 ± 1.0	2.5 ±1.0	1.4 ± 1.7
IL-12p70	0.6 ± 0.4	0.4 ± 0.3	0.5 ± 0.6	2.2 ± 5.2
IL-15	0.1 ± 0.2	0.1 ± 0.2	0.2 ± 0.3	0.6 ± 0.5
IFN-γ	0.3 ± 1.0	0.3 ± 0.8	1.2 ± 3.5	2.8 ± 4.5
TNF-α	5.0 ± 11.8	4.9 ± 10.6	7.3 ± 17.8	2.1 ± 2.9
TGF-β1	52,300 ± 56,000*	229,00 ± 540,00	94,100 ± 87,600*	2,230 ± 1,170
GM-CSF	107 ± 28.4	107 ± 281	105 ± 269	207 ± 360
CD40L	282 ± 422	338 ± 440	382 ± 429	481 ± 697
CCL2/MCP-1	75.9 ± 27.4*	83.6 ± 31.3*	79.4 ± 25.1*	391 ± 268
MMP-8	386,00 ± 21,400*	457,000 ± 332,000*	519,000 ± 503,000*	672 ± 543
CCL5/RANTES	46,100 ± 48,400*	128,000 ± 154,000	91,600 ± 87,700*	3,120 ± 1,370
PDGF-AB	6,740 ± 3,730*	25,000 ± 33,300	13,600 ± 7,260*	437 ± 296

* p<0.05 compared to plasma