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Thrombopoietic cytokines in relation to platelet recovery after bone marrow transplantation

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Summary:

In order to evaluate the importance of different thrombopoietic stimulatory cytokines in accelerating platelet recovery after bone marrow transplantation (BMT), we assayed serial plasma concentrations of three cytokines, thrombopoietin (TPO), interleukin-6 (IL-6), and IL-11 through the course of platelet nadir and recovery after BMT. Both mean TPO and IL-6 levels showed a marked rise and later fall preceding or coincident with the platelet nadir and recovery, suggesting their potential role as circulating regulators or stimulators of thrombopoiesis. In contrast, IL-11 levels remained remarkably constant through the whole course suggesting that this cytokine, though capable of stimulating thrombopoiesis, does not serve as a circulating regulator of platelet production. Additionally, we assayed the levels of these three cytokines following initial platelet transfusion to assess the capacity of transfused platelets to adsorb these thrombopoietic cytokines from the plasma and reduce their circulating levels, thus potentially modifying their availability for stimulating megakaryocyte proliferation. No consistent falls in TPO, IL-6 or IL-11 levels were observed following the initial two platelet transfusions. These data support the importance of circulating TPO and IL-6 as hormones capable of stimulating platelet production. Their physiologic relevance as *in vivo* regulators of thrombopoiesis and clinical utility for therapy of thrombocytopenia need further investigation. *Bone Marrow Transplantation* (2000) **25,** 711–715.
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While the primary stimulators of erythropoiesis and granulopoiesis have been identified and incorporated into clinical practice, the clinical utility of cytokines possessing thrombopoietic stimulatory activity have not been as clearly delineated.[1–3] Mpl ligand (sometimes known as thrombopoietin (TPO)[4] or megakaryocyte growth and differentiation factor (MGDF)),[5–7] interleukin-6 (IL-6),[8–10] IL-11,[11–13] IL-3[14–16] and others have been identified as able to support megakaryocytopoiesis and platelet production. However, their *in vivo* interaction and contribution to platelet recovery after intensive myelosuppressive cancer therapy have not been fully explored. We sought to investigate three cytokines relevant to stimulating platelet recovery following bone marrow transplantation by evaluating plasma and serum levels of TPO, IL-6, and IL-11 in conjunction with platelet counts in patients following blood or marrow stem cell transplantation. In addition, serial cytokine levels were analyzed following platelet transfusion to evaluate the impact of transfused circulating platelets on these cytokine levels. Our data show that levels of TPO and IL-6, but not IL-11, inversely correlate with platelet count during the early post-transplant period, suggesting their possible role as circulating stimulators of thrombopoiesis.

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Patients and methods

Consenting, consecutive adult patients undergoing autologous or allogeneic blood stem cell or bone marrow transplantation at the University of Minnesota were enrolled. Their clinical characteristics are shown in Table 1. Patients were asked to participate in a protocol of serial blood sample collections to be performed in conjunction with their routine clinical blood drawing for diagnostic purposes. Ten ml of whole blood (collected to prepare serum and heparin anti-coagulated plasma) were obtained daily from preconditioning to day 30 and then three times weekly to day 42. Additionally, for seven previously untransfused patients, serial samples were collected over the initial 12 h following their first two platelet transfusions.

Specimens were centrifuged in the clinical specimen

Table 1 Clinical characteristics of patients assayed for cytokine levels during BMT

n		19
Sex	Male	10
Age (years)	Median 41 (3.6–64.8)	
Diagnosis		
Acute or chronic leukemia		11
Lymphoma; breast cancer		8
Type of transplant		
Allogeneic related donor		5
Allogeneic unrelated donor		3
Autologous (BM or blood)		11
Neutrophil engraftment[a]	Median 15 days (9–51)	
Platelet engraftment[b]	Median 29 days (9–126)	

[a]Time to absolute neutrophil count $>500/\mu$l.
[b]Time to platelet transfusion independence (no transfusions for 7 days).

receiving laboratory of the University of Minnesota Hospital, transferred to polyethylene cryovials and cryopreserved at −70°C until assayed.

Cytokine assays of serum IL-6[9] and IL-11 were performed in the University of Minnesota Hospital Cytokine Reference Laboratory using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations. On each day samples were assayed and a standard curve was generated for each cytokine and compared with specifications for international NIBSC/WHO standards. Additionally, quality control samples were included in each assay. The normal value for IL-6 is <3.0 pg/ml (sensitivity 0.7 pg/ml) and for IL-11 is <31.2 pg/ml (sensitivity 4.0 pg/ml).[9] Plasma TPO levels were assayed at Amgen (Thousand Oaks, CA, USA) using a polyclonal antibody-based ELISA with a range in plasma of 80 ± 5 pg/ml from subjects with platelet counts in the normal range.[6–8,17] Serial platelet counts were performed in the clinical hospital laboratory using automated cell counting equipment.

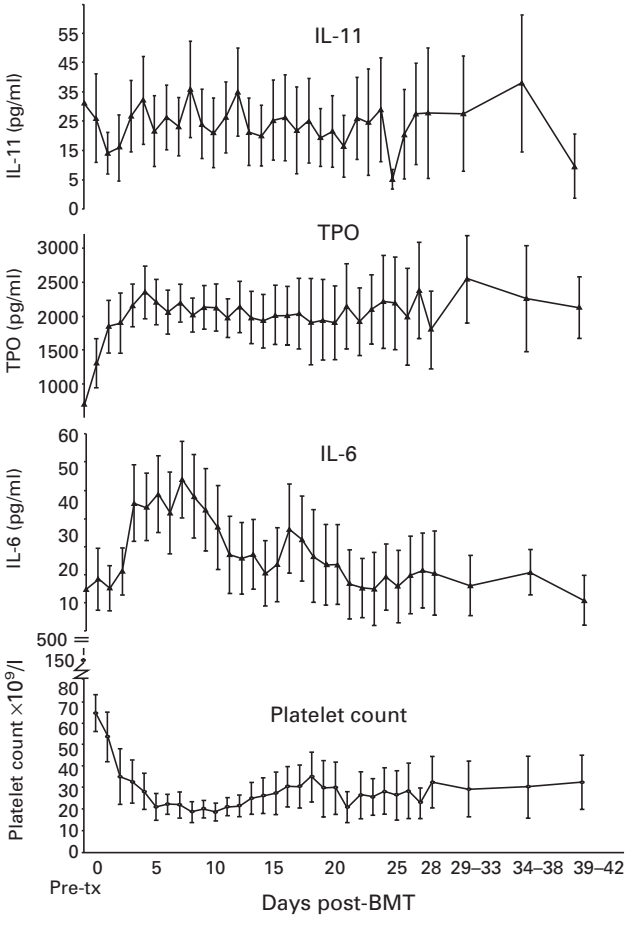

Figure 1 Circulating cytokine levels *vs* platelet counts over time after transplantation. Pre-transplant mean platelet count (± s.d.) shown as 150 000/μl. Correlations between TPO ($P < 0.01$) and IL-6 ($P = 0.02$) with platelet count were significant; IL-11 ($P > 0.8$) was not.

Statistical methods

Cytokine levels were assayed over time and results displayed with 95% confidence limits (Figure 1). Mean platelet counts $>150 \times 10^9$/l were displayed as 150×10^9/l. Spearman correlation coefficient estimates were determined for the mean daily cytokine levels in relation to the mean daily platelet counts and results expressed as correlation coefficients. Probability values shown are estimates of the significance of the relation between the plasma cytokine levels and platelet counts over time. Because not all patients had samples collected for platelet count daily and because not all specimens were sufficient for all three cytokine assays to be performed, the mean of all available platelet counts and cytokine assays is determined. From pretransplant to day +22 post-BMT, the median number of platelet counts assayed per day was 16 (range 10–19); from day 23 to 42 the median number assayed per day was 11 (6–12). Similarly, for the blood cytokines, to day 22 the median number of daily samples was 15 (11–19) while beyond day 22 a median of 11 (6–13) specimens per day was assayed for cytokines. The reduced sampling at later time points was due to patients' hospital discharge, generally accompanied by platelet recovery.

Results

As shown (Figure 1), pretransplant (between day −8 and day −5) mean platelet counts ranged from 230 to 89×10^9/l (shown as 150×10^9/l) but by day 0 had fallen to a mean of 65×10^9/l ($56–73 \times 10^9$/l; 95% CI) and reached a nadir of 18×10^9/l ($14–23 \times 10^9$/l; 95% CI) on days 8 to 10. By day 28, platelet counts rose to 32×10^9/l ($20–44 \times 10^9$/l; 95% CI) and were sustained thereafter. For the 19 patients studied, the median day of recovery to a platelet count >20 000/μl was day 17 and to platelet transfusion independence (no transfusions for 7 days) was day 29 (range 9–126). Six patients were still platelet transfusion dependent beyond day +50.

Serum IL–6 levels (Figure 1) in pretransplant samples were a mean of 15 pg/ml (4.8–24.3; 95% CI) until day +3 when they rose to a mean of 45 pg/ml (32–58.8; 95% CI) and remained elevated to day +10 and returning towards baseline by day 21. IL-6 levels were sustained at baseline to the end of the study. Perhaps due to its known role as an acute phase reactant the rise in serum IL-6 preceded the thrombocytopenic nadir, but returned towards baseline as the platelet count gradually rose.

In contrast, mean serum IL-11 levels (Figure 1) remained remarkably stable throughout the entire pretransplant conditioning, early thrombocytopenic phase and post-transplant recovery. There was no measurable fluctuation accompanying the clinical events occurring during early neutropenia and thrombocytopenia or during later intervals as well.

TPO levels (Figure 1) ranged from 105 to 2288 pg/ml (mean 700 pg/ml; 95% CI; 362–1035) during the pretransplant week as platelet counts were falling and rose promptly and progressively to a sustained peak between 2000 and 2300 pg/ml between day +3 and day +28. TPO levels con-

tinued to be elevated to day +42 even as mean platelet counts rose above the transfusion-dependent range.

Statistical correlation of these mean plasma cytokine levels with platelet counts over time is shown in Table 2. Over the 33 time points assayed, there was a statistically significant inverse correlation of serial serum IL-6 levels compared to platelet counts ($P = 0.02$). Similarly plasma TPO levels correlated inversely and more strongly with the level of thrombocytopenia over time ($P < 0.01$). In contrast, the stable IL-11 levels measured serially through the transplant course showed no correlation ($P > 0.8$) with fluctuating platelet counts at any time from pretransplant through the acute course and into the post-BMT recovery from thrombocytopenia.

An established mechanism for regulation of growth factor concentration is end cell clearance. Because circulating platelets may adsorb or degrade cytokines, transfused circulating platelets might thereby reduce cytokine levels.[18,19] We assayed serial IL-6, IL-11, and TPO levels for the first two platelet transfusions in seven previously untransfused patients. Pre-transfusion platelet counts were $11\,000 \pm 2300/\mu$l (mean ± s.d.) and rose to $27\,000 \pm 24\,600/\mu$l at 1 h and were $27\,400 \pm 14\,500$ at 12 h. Pre transfusion serum IL 6 levels were 58 ± 32.4 pg/ml, rose to 69.1 ± 91.4 pg/ml at 1 h and 106.6 ± 144.4 at 12 h (P = NS). IL-11 at baseline was 40.7 ± 47.5 pg/ml, fell slightly to 39.8 ± 34.7 pg/ml at 1 h and 31.5 ± 30 pg/ml at 12 h (P = NS). TPO at baseline was 2629 ± 517 pg/ml, fell slightly to 2556 ± 284 pg/ml at 1 h and to 2263 ± 300 pg/ml by 12 h post-transfusion (P = NS). Overall, for each of the three cytokines assayed, no consistent fall in the circulating cytokine level was noted; thus no measurable cytokine clearance by the transfused platelets was apparent.

Discussion

It is well-established from extensive *in vitro* and more recent *in vivo* observations that TPO, IL-6, and IL-11, in addition to IL-3 and possibly other cytokines, all have the capacity to stimulate megakaryocytic proliferation, maturation and platelet production. However, the regulatory function of these cytokines in maintaining basal platelet counts and in the response to acquired thrombopoietic stress is uncertain. While some murine, lapine and limited human studies have suggested that TPO represents the prime circulating thrombopoietic hormone, its mechanism of regulation and its physiologic interplay with other cytokines is under active study.[5,14,18] Importantly, both plasma and bone marrow *in situ* interactions of cytokines and megakaryocytes may be important in regulating thrombopoiesis.

Constitutive TPO expression counterbalanced by its adsorption and degradation in platelets and megakaryocytes has yielded the hypothesis that free (unbound to platelets) TPO represents the available hormone for induction of differentiation and proliferation in the megakaryocytic lineage.[19–21] However, the regulated production of TPO in therapy-induced thrombocytopenia is still being explored, especially in extrahepatic tissue such as marrow stroma.[22] Shimazaki *et al*[23] reported assay of serial serum TPO levels through the course of autotransplantation in nine patients, as did Ishida *et al*[24] for three allotransplant recipients. Similar to the results reported herein, a powerful inverse correlation between platelet counts and circulating TPO levels was recognized. These independent observations suggest that TPO availability, or at least its circulation assayable in plasma, may represent an endocrine-like function; rising in response to the induction of thrombocytopenia. These observations, however, are consistent with the 'platelet as TPO sponge' theory which argues that circulating platelet and megakaryocyte mass determine the available free TPO to stimulate thrombopoiesis. Our data demonstrating no consistent fall in TPO levels with initial platelet transfusion may argue, at least in part, that the modest platelet mass increment represented by a single multipack platelet transfusion is not sufficient to measurably deplete plasma TPO.

In the current study, we observed IL-6 levels rising early after transplantation and inversely correlating with platelet count. However, IL-6 levels rose during the early fall in platelet counts, coincident with the acute toxicity of the early post-transplant period. IL-6 has a well recognized role as an acute phase reactant. Therefore, in addition to thrombocytopenia, peri-transplant toxicity may be related to the elevated IL-6 levels early after transplantation.[25–29] Steffen *et al*[30] described serial serum IL-6 determinations through the course of BMT. In this study, during aplasia, almost all patients demonstrated elevated IL-6 levels and a statistical association was recognized between elevations of IL-6, C-reactive protein and fever. While a weak statistical correlation between IL-6 and platelet count was identified, peak IL-6 levels were correlated with the time of platelet recovery, implying a relationship between IL-6 and thrombopoiesis early after transplantation. In the current study, a modest but non-significant rise in IL-6 levels after the first two platelet transfusions was recognized, though the heterogeneity in patients' responses allows no clear inference about a direct short-term interaction between IL-6 levels and platelet transfusions.

Interleukin-11 is recognized *in vitro* as having thrombopoietic activity, perhaps by enhancing the maturation of megakaryocytes, and was recently licensed in the US for its potential thrombopoietic function in cancer patients receiving chemotherapy.[29–33] Strikingly, IL-11 levels did not vary or correlate with platelet counts over the course of bone marrow transplantation. Circulating IL-11 levels were stable through the course of transplant and were unaffected by platelet transfusion. Our data concur with Ishida *et al*[24] but differ from that of Chang *et al*[12] who assayed

Table 2 Correlation of platelet counts and serum/plasma cytokine levels after BMT

	n	*Correlation coefficient*[a]	*P*
IL-6	33	−0.40	0.02
IL-11	33	−0.02	>0.8
TPO	33	−0.82	<0.01

[a]Spearman's correlation estimates of mean cytokine levels with platelet counts over time.

cytokine levels in a similar population but who experienced more modest mean platelet nadirs. They suggested IL-11 levels may be augmented by thrombocytopenia but also by undefined inflammatory mediators circulating over months following BMT, well after the severe thrombocytopenia had resolved. IL-11 elevations might also be blunted by chemoradiotherapy damage to its cell of origin. While our own data do not discount a potential interaction of IL-11 and thrombopoiesis locally within the marrow microenvironment, it suggests that IL-11 does not circulate as a plasma endocrine-like cytokine and may not be a major regulator of thrombopoiesis after marrow transplantation.

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