Characterization of Endogenous Cytokine Concentrations After High-Dose Chemotherapy With Autologous Bone Marrow Support

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Endogenous cytokines are thought to mediate numerous biologic processes and may account for some adverse effects experienced following the administration of recombinant proteins. This study describes the pattern of endogenous cytokine exposure following high-dose chemotherapy. Blood concentrations of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), macrophage colony-stimulating factor (M-CSF), and erythropoietin (EPO) were measured by enzyme-linked immunosorbent assay (ELISA) methods in 68 patients receiving the same ablative chemotherapy regimen (cyclophosphamide, cisplatin, carmustine). Patients were grouped according to cellular support (autologous bone marrow [BM] CSF–primed peripheral blood progenitor cells [PBPCs]) and prescribed growth factor (recombinant human granulocyte or granulocyte-macrophage colony-stimulating factor [rHuG-CSF or rHuGM-CSF]). Leukocyte reconstitution was most accelerated in the groups treated with PBPCs and rHuG-CSF. IL-6, M-CSF, and TNF-α concentrations were higher in the groups treated with rHuGM-CSF and without PBPCs. Maximal endogenous cytokine concentrations occurred approximately 12 days after BM reinfusion. High concentrations of EPO occurred in patients experiencing significant hypotension despite routine transfusions for hematocrit < 42%. High M-CSF and IL-6 levels were associated with increased platelet transfusion requirements. Concentrations of all four cytokines were significantly higher in patients experiencing renal or hepatic toxicity, with elevations occurring in a predictable sequence and M-CSF elevations occurring first. This report shows that endogenous cytokine concentrations may be influenced by either cellular or CSF support and are associated with differences in platelet reconstitution and organ toxicity.

ENDOGENOUSLY PRODUCED cytokines appear to influence multiple physiologic processes affecting regulation of the hematopoietic system. These proteins may also directly or indirectly contribute to treatment-associated toxicities, such as hypotension and liver or renal dysfunction after high-dose chemotherapy. Administration of cytokines such as recombinant human tumor necrosis factor-α (rHuTNF-α) produces some symptoms similar (although less in degree) to those found in patients developing major organ toxicity following bone marrow transplantation (BMT).[1,2] High concentrations of endogenous serum TNF have been reported to precede major transplant-related complications.[3,4] Elevated serum interleukin-6 (IL-6) and macrophage colony-stimulating factor (M-CSF) have also been found in patients developing major organ toxicity.[4-6] Previous studies in BMT patients have reported an association between endogenous serum erythropoietin (EPO) and anemia.[7,8] Cytostatic drugs may also stimulate EPO production for undetermined reasons.[9,10] Patients may experience significant exposure to endogenous cytokines such as granulocyte colony-stimulating factor (G-CSF) during the myeloablative phase following high-dose chemotherapy. The exact etiology of these elevations (accelerated production or reduced clearance) is unclear; however, some data suggest serum G-CSF concentrations may correlate to myeloid engraftment.[11]

Administration of recombinant human granulocyte colony-stimulating factor (rHuG-CSF) or recombinant human granulocyte-macrophage colony-stimulating factor (rHuGM-CSF) will accelerate hematopoietic recovery after high-dose chemotherapy with autologous BM support.[12-15] Addition of CSF-primed peripheral blood progenitor cells (PBPCs) to the treatment program will minimize leukopenia and further accelerate hematopoietic recovery.[16-18] However, toxicities related to colony-stimulating factor administration have also been reported.[19-22]

Recombinant proteins such as rHuGM-CSF can induce production of multiple cytokines by macrophages and neutrophils in vitro.[23-25] These secondarily released cytokines may play a role in the efficacy and/or toxicity of the administered product. Knowledge of endogenous cytokine release patterns may aid in the design of combination rHuCSF regimens or enable the development of toxicity monitoring and prevention strategies.

Previous work has, in general, evaluated one endogenous cytokine extensively or evaluated secretion of several cytokines in a limited number of patients. This report describes the endogenous concentrations of four different cytokines (EPO, M-CSF, IL-6, and TNF-α) in 68 patients, focusing on the interactions of these factors and their relationship to toxicity and hematopoietic recovery after high-dose chemotherapy with autologous BM support. Patients were grouped according to the CSF that was administered and whether they received autologous CSF-primed PBPCs in addition to BM. The relationship of endogenous cytokine concentrations to the clinical differences between the four patient groups also is described.

MATERIALS AND METHODS

Patient set. All 68 patients in this study received high-dose chemotherapy with autologous BM support for the treatment of stages II to IV breast cancer or metastatic melanoma (Table 1). Patients were required to have normal laboratory indicators of renal function (serum creatine <1.5 mg/dL; creatinine clearance >60 mL/min) and liver function (serum total bilirubin <2 mg/dL; serum aspartate aminotransferase <2.5 times normal) before receiving this chemotherapy

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Table 1. Patient Demographics

Patient Group	Age*	Diagnosis		
		Breast (II)	Breast (IV)	Melanoma
G-CSF PBPC	37 (34-40)	10	4	0
GM-CSF PBPC	40 (36-44)	9	7	1
G-CSF	39 (34-44)	9	7	1
GM-CSF	39 (34-45)	3	14	3
		31	32	5

* Median (interquartile range).

regimen. All patients were given the same ablative treatment consisting of cyclophosphamide (5,625 mg/m^2), carmustine (600 mg/m^2), and cisplatin (165 mg/m^2) administered over the course of 4 days (day −6 to day −3), as previously described.[26] Chemotherapy was initiated between 8:00 AM and 12:00 (noon).

rHuCSF dosages expressed in this article are in unglycosylated equivalents. Patients on the PBPC regimen received daily infusions of either 6 μg/kg/d of subcutaneous unglycosylated rHuG-CSF (derived from *Escherichia coli;* Amgen, Inc, Thousand Oaks, CA) or 5.55 μg/kg/d of intravenous glycosylated rHuGM-CSF (derived from a Chinese hamster ovary system; 7.78 × 10^3 U/μg aglycoprotein; Sandoz/Schering-Plough, East Hanover, NJ) for 8 days as a means of peripheral blood progenitor cell priming. Peripheral blood progenitor cells were collected by leukapheresis 6, 8, and 9 days after initiation of growth factor. High-dose chemotherapy was begun approximately 1 week after the final day of leukapheresis. Peripheral blood progenitor cells were reinfused following chemotherapy over 3 days starting on either day −1 or day +1. The frequency of each reinfusion schedule was similar between the G-CSF and GM-CSF groups.

BM was reinfused 4 days after the end of chemotherapy (day +1) in all patients. Daily administration of rHuCSF was initiated 3 hours following the first autologous reinfusion (BM or PBPCs). Patients on the G-CSF PBPC regimen received 19.2 μg/kg/d of intravenous rHuG-CSF by daily 4-hour infusion for up to 21 days. Patients on the GM-CSF PBPC regimen received 11.10 μg/kg/d of intravenous rHuGM-CSF by daily 4-hour infusion for 7 days followed by 5.55 μg/kg/d for 14 days. Patients receiving G-CSF alone received 19.2 or 9.6 μg/kg/d by continuous infusion (for 14 days) or daily 4-hour infusion (for 21 days). Patients receiving GM-CSF alone received 11.10 or 5.55 μg/kg/d by continuous infusion (for 21 days) or daily 4-hour infusion (for 21 days). Patients not receiving PBPCs were evenly divided between the two dosages.

No drug intended to suppress TNF production, such as pentoxifylline, ciprofloxacin, or dexamethasone, was administered to any patient in this report. Patients were transfused with red blood cells to maintain a hematocrit >42% from day −6 until leukocyte engraftment. Platelet transfusions were prescribed when concentrations dropped below 25,000/μL. Serum creatinine >1.8 mg/dL or serum total bilirubin >3.6 mg/dL before day +22 was defined as clinically significant renal or hepatic toxicity for the purposes of this study.

Sample collection. Serum samples for the evaluation of endogenous cytokine concentrations in patients receiving growth factor and PBPCs were obtained between 4:00 AM and 6:00 AM on the following days of therapy: day −6 (before chemotherapy), day −1 (before PBPC reinfusion), day +1 (before BM reinfusion), and days +3, +6, +9, +12, +14, and +16 following marrow reinfusion. An aliquot of each sample was placed into one of four tubes and stored at −70°C to avoid multiple freeze-thaw cycles. Serum and EDTA-anticoagulated plasma samples for patients receiving growth factor without PBPCs were obtained between 4:00 AM and 6:00 AM on day −6 and approximately every third day following BM reinfusion. Samples were stored as described above. Plasma samples were used for IL-6 and TNF assays. Serum samples were used for M-CSF and EPO assays.

Cytokine measurement. Immunoreactive TNF-α, IL-6, M-CSF, and EPO were measured using double-antibody sandwich techniques. Murine monoclonal antibody specific for the cytokine being measured was used as the capture antibody, and horseradish peroxidase-labeled antibody was used as the conjugate. EPO and IL-6 assay kits were kindly provided by Dr Larry Souza (Amgen, Inc, Thousand Oaks, CA) and M-CSF by Drs John Stoudemire and Edward Alderman (Genetics Institute, Boston, MA). TNF-α kits were purchased from R&D Systems, Inc (Minneapolis, MN). The lower limits of quantitation of these assays in our laboratory are TNF-α 7.5 pg/mL, IL-6 10 pg/mL, M-CSF 0.4 ng/mL, and EPO 2 mU/mL.

Statistical methods. Mean log concentration-time curves for IL-6, M-CSF, and white blood cells (WBCs) were obtained by regression analysis. Available data points were fit to the equation $\log y = A + Bx + Cx^2$, where y = concentration and x = day of therapy. Statistical comparisons were performed using the Mann Whitney U test for nonpaired group comparison, the Wilcoxon test for paired analysis, or the binomial proportions test. The criterion for statistical significance was defined as $P = .05$.

RESULTS

Clinical results. We evaluated endogenous cytokine concentrations in 68 patients receiving four different supportive regimens. Fourteen patients received G-CSF with PBPCs, 17 received GM-CSF with PBPCs, 17 received G-CSF without PBPCs, and 20 received GM-CSF without PBPCs. These different supportive regimens resulted in substantially different leukocyte recovery kinetics (Table 2). The use of PBPCs significantly enhanced leukocyte recovery for patients receiving either rHuG-CSF or rHuGM-CSF; however, patients receiving rHuG-CSF with PBPCs experienced more rapid leukocyte recovery compared with patients receiving rHuGM-CSF with PBPCs. The use of PBPCs also altered the shape of the WBC-time curve (Fig 1, A and D).

The different supportive regimens also affected the chances of a patient developing organ toxicity (serum creatinine >1.8 mg/dL or serum total bilirubin >3.6 mg/dL). The patients experiencing toxicity included 7.7% (1 of 13) of patients on G-CSF with PBPCs, 23.5% (4 of 17) of patients on GM-CSF with PBPCs, 35.3% (6 of 17) of patients on G-CSF, and 45.0% (9 of 20) of GM-CSF patients. A subset of five of the toxic patients died on the BM transplant unit, owing in part to renal and/or hepatic failure. This group consisted of one patient on G-CSF with PBPCs, one patient on GM-CSF with PBPCs, two patients on G-CSF, and one patient on GM-CSF.

Time dependency of cytokine concentrations. IL-6 concentrations rose over time for patients on the GM-CSF with PBPCs, G-CSF, and GM-CSF regimens (Fig 1, B and E). The largest and most rapid elevations occurred among patients treated with GM-CSF alone. IL-6 concentrations were not significantly elevated in patients on the G-CSF PBPC regimen. WBCs for many patients in the G-CSF PBPC group returned to >1,000 cells/mm^3 (ANC >500 cells/mm^3) between days +12 and +16, allowing them to leave the BM transplant unit during this time. Therefore, average cytokine concentrations after day +12 are not available in the G-CSF PBPC group.

Table 2. Clinical Data Segregated Based on Treatment Group

	Patient Group	Median	Interquartile Range	Compared With	P
WBC ($\times 10^6$/mL)	G-CSF PBPC	6.7	4.1-12.9	G-CSF	<.0001
Day +12	GM-CSF PBPC	1.3	0.8-1.9	G-CSF PBPC	<.0001
	G-CSF	1.0	0.6-2.0	GM-CSF	.0119
	GM-CSF	0.5	0.4-0.8	GM-CSF PBPC	.0015
ANC ($\times 10^6$/mL)	G-CSF PBPC	4.4	2.9-12.3	G-CSF	<.0001
Day +12	GM-CSF PBPC	0.4	0.1-0.9	G-CSF PBPC	<.0001
	G-CSF	0.4	0.2-0.6	GM-CSF	.0002
	GM-CSF	0.2	0.1-0.2	GM-CSF PBPC	.0342
Hematocrit (%)	G-CSF PBPC	45	42-46	G-CSF	.8670
Day +12	GM-CSF PBPC	43	42-45	G-CSF PBPC	.3687
	G-CSF	45	42-46	GM-CSF	.1394
	GM-CSF	42	40-45	GM-CSF PBPC	.1661
RBC transfusions	G-CSF PBPC	12	11-15	G-CSF	.0029
(Total units)	GM-CSF PBPC	23	18-24	G-CSF PBPC	.0002
Day < +26	G-CSF	18	14-24	GM-CSF	.6696
	GM-CSF	21	18-23	GM-CSF PBPC	.2407
Platelet transfusions	G-CSF PBPC	8	7-10	G-CSF	.0005
(Total)	GM-CSF PBPC	21	12-30	G-CSF PBPC	.0014
Day < +26	G-CSF	19	12-30	GM-CSF	.4739
	GM-CSF	26	21-27	GM-CSF PBPC	.2595
Cr (mg/dL)	G-CSF PBPC	0.9	0.8-1.1	G-CSF	.0139
Maximum	GM-CSF PBPC	1.2	1.0-1.4	G-CSF PBPC	.0290
Day < +22	G-CSF	1.3	1.0-1.8	GM-CSF	.1564
	GM-CSF	1.4	1.2-1.9	GM-CSF PBPC	.0182
T Bil (mg/dL)	G-CSF PBPC	0.9	0.8-1.5	G-CSF	.0995
Maximum	GM-CSF PBPC	1.2	0.8-4.8	G-CSF PBPC	.2497
Day < +22	G-CSF	1.6	0.9-3.4	GM-CSF	.5123
	GM-CSF	2.6	1.0-4.7	GM-CSF PBPC	.2931

Abbreviations: ANC, absolute neutrophil count; Cr, serum creatinine; T Bil, total serum bilirubin.

M-CSF concentrations followed a pattern similar to IL-6 concentrations, rising most rapidly in the GM-CSF group (Fig 1, C and F). Concentrations in the G-CSF PBPC group decreased after transplantation, whereas M-CSF for the GM-CSF with PBPCs and G-CSF groups increased early (days +2 to +9) following transplantation and then declined as WBC recovery occurred.

We were unable to detect significant amounts of TNF-α in most samples (85.7%; 336 of 392). Therefore, no average log concentration-time curve for TNF-α could be generated. The median day on which TNF-α first rose to a detectable concentration was day +10.5. The day in which TNF-α increase was first detectable did not vary significantly between treatment groups.

EPO concentrations were not substantially elevated from baseline values in most patients. A few patients demonstrated very high levels late (days +10 to +17) in the post-transplant period. EPO concentrations increased above 100 mU/mL in six patients. Hematocrit was maintained at greater than 40% on most days, never falling below 36%. Hypotensive episodes (not associated with carmustine infusion or BM reinfusion) occurred concurrent with EPO elevation. The minimum mean arterial pressure (MAP) was below 60 mm Hg in each of these six patients near the time of the peak EPO concentration.

Comparison of rHuCSF regimens. Day +12 was chosen as the optimal day for statistical comparison of cytokine concentrations between the four patient groups and between toxic and nontoxic patients. This day was chosen because it was the last day with universal sampling for the patients on the G-CSF with PBPCs regimen, as many of them left the BM transplant unit shortly after this time, and because the kinetic curves displayed near maximal concentrations at this time point. Cytokine concentrations are summarized in Table 3 (segregated based on treatment group) and Table 4 (segregated based on patient toxicity).

Day +12 TNF-α concentrations were greatest in the GM-CSF group (median 8.54 pg/mL). Median concentrations for the other three treatment groups were not detectable; however, the percentage of patients with detectable TNF-α varied by group. Patients experiencing major organ toxicity were more likely to have detectable TNF-α on day +12 than patients not experiencing toxicity ($P < .0001$).

IL-6 concentrations were greatest in the GM-CSF group. The median day +12 IL-6 concentration was over six times greater in the group treated with GM-CSF alone than with G-CSF alone (212 pg/mL *v* 34 pg/mL; $P = .0003$). Similarly, patients in the GM-CSF with PBPCs group tended to have more IL-6 than patients in the G-CSF with PBPCs group ($P < .0001$). Patients with detectable TNF-α on day +12 had

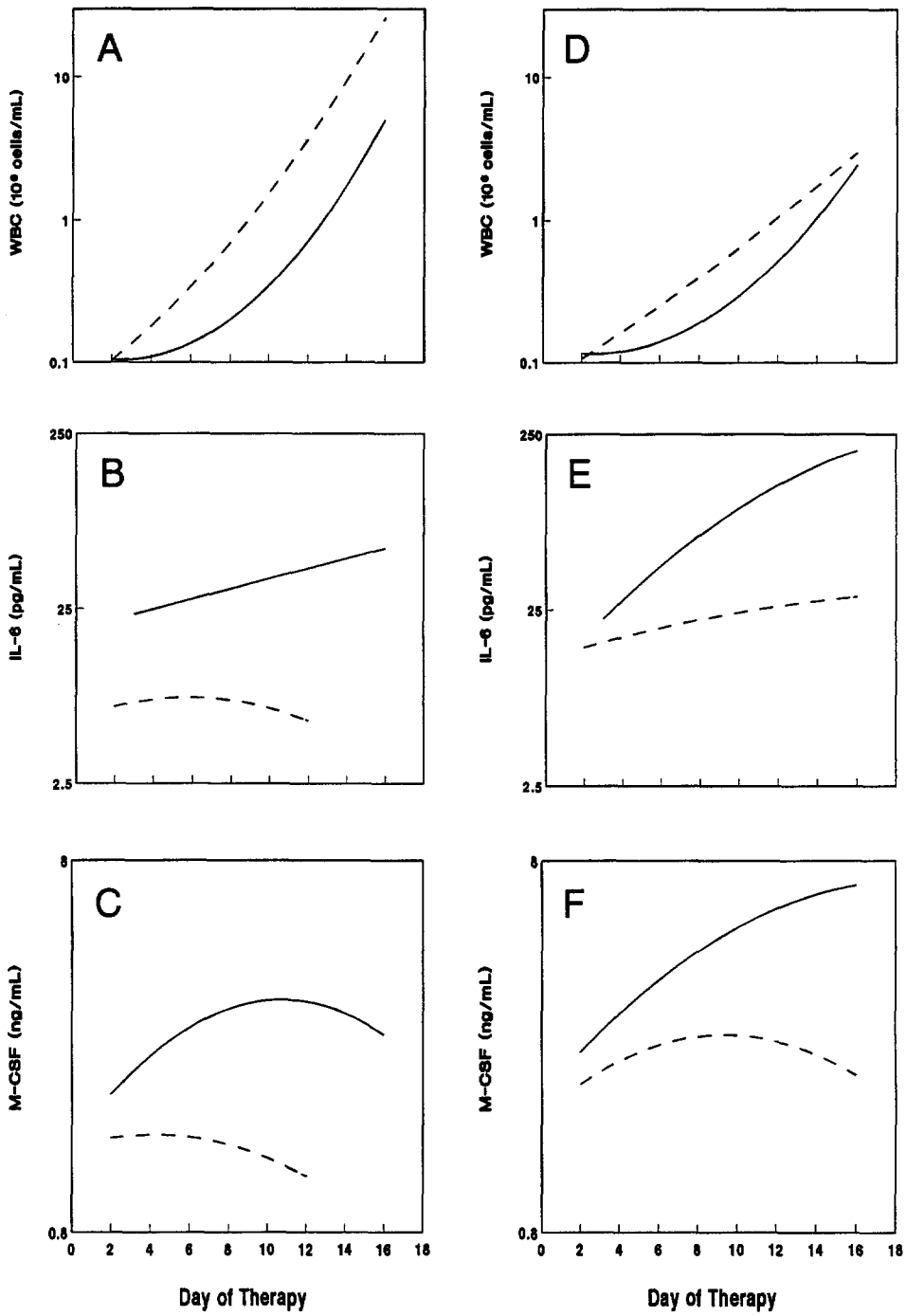

Fig 1. A quadratic model was fit to concentration-time points to yield kinetic curves for G-CSF PBPC (n = 14) and G-CSF (n = 17) patients (A through C) and for GM-CSF (n = 17) and GM-CSF PBPC (n = 20) patients (D through E). Data were available for each patient no less frequently than every 4 days over the displayed duration. Dashed lines represent patients on PBPCs. Solid lines represent patients receiving BM alone.

over 10 times more IL-6 than patients with no detectable TNF-α (median 218 pg/mL *v* 21.6 pg/mL; $P < .0001$). IL-6 concentrations were also greater in toxic patients than in nontoxic patients ($P = .0007$).

Endogenous M-CSF followed the same general trend as endogenous TNF-α and IL-6, with concentrations being higher among non-PBPC–treated patients and GM-CSF–treated patients. The difference between the two PBPC groups was particularly pronounced, with the median day +12 M-CSF concentration in the GM-CSF with PBPCs group over four times that in the G-CSF with PBPCs group (3.96 ng/mL *v* 0.90 ng/mL; $P = .0018$). M-CSF concentrations were also greater in toxic patients than in nontoxic patients ($P = .0002$).

As with TNF-α, IL-6, and M-CSF, EPO concentrations were highest in the GM-CSF group, but the difference between G-CSF– and GM-CSF–treated patients was not significant. Patients receiving PBPCs did have significantly lower EPO concentrations than patients not receiving PBPCs (median 6.57 mU/mL *v* 19.63 mU/mL; $P = .004$). Serum EPO con-

Table 3. Day +12 Cytokine Concentrations Segregated Based on Treatment Group

	Patient Group	Median	Interquartile Range	Detectable (%)	Compared With	*P*
TNF-α (pg/mL)	G-CSF PBPC	ND	ND-ND	0/13 (0.0)	G-CSF	.0283
	GM-CSF PBPC	ND	ND-ND	4/13 (30.8)	G-CSF PBPC	.1164
	G-CSF	ND	ND-13.71	7/17 (41.2)	GM-CSF	.4010
	GM-CSF	8.54	ND-15.28	11/20 (55.0)	GM-CSF PBPC	.1646
IL-6 (pg/mL)	G-CSF PBPC	ND	ND-ND	3/13 (23.1)	G-CSF	<.0001
	GM-CSF PBPC	27.5	12.2-114	15/17 (88.2)	G-CSF PBPC	<.0001
	G-CSF	33.8	24.5-74.1	15/17 (88.2)	GM-CSF	.0003
	GM-CSF	212	113-448	20/20 (100.0)	GM-CSF PBPC	.0005
M-CSF (ng/mL)	G-CSF PBPC	0.90	0.45-1.42	11/13 (84.6)	G-CSF	.5579
	GM-CSF PBPC	3.96	1.35-5.22	17/17 (100.0)	G-CSF PBPC	.0018
	G-CSF	7.00	ND-13.9	9/17 (52.9)	GM-CSF	.1702
	GM-CSF	9.47	3.62-13.8	18/20 (90.0)	GM-CSF PBPC	.0142
EPO (mU/mL)	G-CSF PBPC	5.01	2.68-10.9	12/13 (92.3)	G-CSF	.0862
	GM-CSF PBPC	8.67	2.37-19.0	13/17 (76.5)	G-CSF PBPC	.4026
	G-CSF	17.9	2.40-55.2	14/17 (82.4)	GM-CSF	.5974
	GM-CSF	20.3	7.40-40.1	17/18 (94.4)	GM-CSF PBPC	.0282

Abbreviation: ND, not detectable.

centrations were elevated, although not very high, in patients experiencing toxicity ($P = .0028$). Thirteen of 21 toxic patients experienced severe hypotension (MAP < 60 mm Hg) at some time before day +22 (six patients on G-CSF, seven patients on GM-CSF). In addition, four patients not suffering major organ toxicity experienced severe hypotension (one patient on G-CSF, three patients on GM-CSF). EPO concentrations for these 17 patients were elevated on day +12 (median 32.2 mU/mL). EPO in the eight toxic patients not experiencing severe hypotension were not elevated (median 7.36 mU/mL).

Platelet transfusions. Over 75% of the patients on G-CSF with PBPCs required fewer than 11 platelet transfusions before day +26, whereas a majority of patients in other groups required approximately twice this number (Table 2). G-CSF PBPC–treated patients also displayed lower endogenous concentrations of TNF-α, IL-6, and M-CSF (Table 3). Treatment group and endogenous cytokine concentrations (TNF-α, IL-6, M-CSF) on day +12 were evaluated by multivariate regression to determine their effect on platelet transfusion requirements. The fourth root of each variable was used to normalize each data set (n = 67; $R^2 = .478$). M-CSF was the best predictor of platelets required ($P = .001$), followed by IL-6 ($P = .019$). High M-CSF and IL-6 both correlated to an increased demand for platelet transfusions. The difference between G-CSF– and GM-CSF–treated patients was not significant. G-CSF PBPC–treated patients required significantly fewer platelet transfusions than other patients, even when controlling for their low M-CSF and IL-6 ($P = .014$).

Sequence of events in toxic patients. "Elevated" concentrations of endogenous cytokines were defined in the following manner for kinetic analysis. TNF-α > 7.5 pg/mL was defined as a significant elevation, because day +12 TNF-α was not detectable in over 80% of nontoxic patients. Likewise, IL-6 concentrations >100 pg/mL, M-CSF concentrations of >7 ng/mL, and EPO concentrations of >20 mU/mL were defined as significant elevations, because fewer than 25% of nontoxic patients had such concentrations on day +12 compared with a majority of toxic patients.

The time sequence of events in toxic patients (n = 21) is summarized in Fig 2. M-CSF concentrations were >7 ng/mL in 15 of 21 patients before the first toxic event (serum creatinine >1.8 mg/dL or serum total bilirubin >3.6 mg/dL). M-CSF preceded the onset of toxicity by a median of 7 days ($P = .002$). TNF-α increased before the first toxic event in 18 of 21 toxic patients. The TNF-α increase preceded the toxic onset by a median of 3 days ($P = .001$).

Cytokine concentrations were higher in the subset of toxic patients who died, owing in part to hepatic or renal failure, than in other toxic patients experiencing less severe outcomes. TNF-α, IL-6, and M-CSF concentrations were detectable before the onset of toxicity in each of these five patients and remained elevated for multiple days during therapy in each case. Maximum TNF-α concentrations followed the first detectable TNF-α concentration by at least 3 days in each patient.

DISCUSSION

Endogenously produced cytokines are thought to be important components in the regulation of normal hematopoietic activity, although little information is available regarding their disposition following myeloablative therapy. Schneider et al[27] have previously studied the ability of peripheral blood mononuclear cells from 27 BM transplant patients to produce interferon-γ, IL-4, and IL-2 and found a pattern of lymphokine production similar to the pattern observed during immune ontogeny.

In this study, we have measured concentrations of four cytokines at multiple time points following BM transplantation in 68 patients given the same high-dose chemotherapy regimen. Day +12 was selected as the best time for comparisons of cytokine concentrations, because kinetic curves displayed near maximal values at this time point. The optimal

Table 4. Day +12 Cytokine Concentrations Segregated Based on Patient Toxicity

	Patient Group	Median	Interquartile Range	Detectable (%)	P
TNF (pg/mL)	Nontoxic	ND	ND-ND	7/46 (17.8)	
	Toxic	9.91	ND-16.8	15/21 (71.4)	<.0001
IL-6 (pg/mL)	Nontoxic	24.2	ND-81.1	34/46 (73.9)	
	Toxic	160	40.0-273	19/21 (90.5)	.0007
M-CSF (ng/mL)	Nontoxic	1.52	0.16-6.44	35/46 (76.1)	
	Toxic	10.1	2.93-16.5	20/21 (95.2)	.0002
EPO (mU/mL)	Nontoxic	8.34	2.45-19.5	38/46 (82.6)	
	Toxic	20.8	7.50-60.8	18/19 (94.7)	.0028

Abbreviation: ND, not detectable.

sampling times for cytokine monitoring may be dependent on the chemotherapy and supportive care regimens used; thus, caution should be exercised in extrapolating our methods to other patient populations.

Our red blood cell transfusion regimen that maintained the hematocrit >42% was generally successful in reducing EPO concentrations compared with other reports in which concentrations >100 mU/mL were evident on multiple days post-transplant.[7-10] Only 6 of 65 (9.2%) patients described in this study had peak EPO concentrations >100 mU/mL. All six of these patients experienced severe hypotension (MAP < 60 mm Hg) at or near the time of peak EPO concentration. We hypothesize that the etiology of these elevations was a reduction in renal blood flow.

Elevations in M-CSF concentrations have been described in response to neutropenia,[28] in patients with ovarian neoplasms,[29,30] and in mice following bacterial and fungal infections.[31] M-CSF concentrations in our patients were higher in those receiving rHuGM-CSF and not receiving PBPCs. Differences between rHuG-CSF– and rHuGM-CSF–treated patients may result from rHuGM-CSF stimulation of macrophage cytokine production in addition to other mechanisms (eg, differences in WBC response). Patients with organ toxicity exhibited significantly higher M-CSF concentrations compared with other patients. We were able to detect substantial elevations in serum M-CSF approximately 7 days before the clinical manifestation of toxicity in the majority of cases. This report is in agreement with in vitro studies suggesting that GM-CSF may stimulate M-CSF secretion, and the latter may trigger a cascade of events as part of an acute-phase response. Macrophages incubated with M-CSF in vitro produce 12 times more TNF than control macrophages.[32]

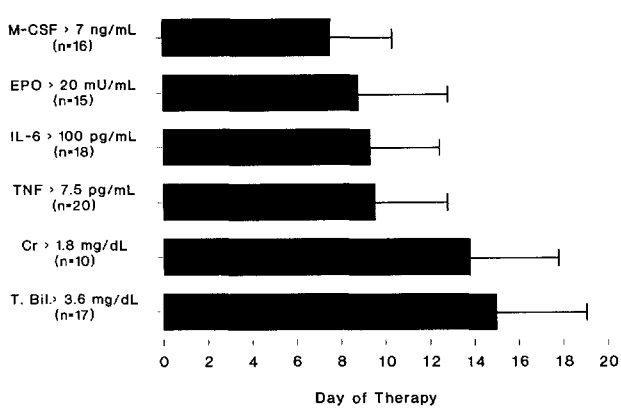

Fig 2. Mean days (with standard deviations) to the first measured occurrence of events after transplantation. Data were derived from 21 patients experiencing renal and/or hepatic toxicity posttransplant. (Cytokine concentrations are from serum/plasma samples; Cr, serum creatinine; T. Bil., total serum bilirubin.)

IL-6 concentrations were also closely associated with organ toxicity. Seventy-nine percent of patients in this report had IL-6 >10 pg/mL on day +12. Concentrations >10 pg/mL have not been reported in normal adults, although elevated IL-6 has been described in patients with multiple myeloma,[33,34] plasma cell leukemia,[35] and ovarian cancer.[36] Extended neutropenia, infection, or rHuGM-CSF infusion could account for the elevated IL-6 concentrations found in this study.[37,38] IL-6 supports the granulocytic differentiation of hematopoietic progenitor cells.[39] Whether endogenous IL-6 in our patients contributes to hematopoietic reconstitution, predominately reflects an acute-phase response, or is a direct mediator of major organ toxicity is unknown.

IL-6 has also been shown to stimulate platelet production in animals.[40-42] Elevated endogenous IL-6 has been reported in patients with reactive thrombocytosis[43] and in those experiencing rapid platelet recovery following high-dose chemotherapy.[44] In contrast, patients treated with recombinant M-CSF[45] and TNF[1,2] have experienced transient, dose-related thrombocytopenia. To evaluate the effect of endogenous cytokines on platelet production, we performed a multivariate regression in an attempt to predict the number of platelet transfusions required based on day +12 TNF-α, IL-6, and M-CSF concentrations and supportive care group. High M-CSF ($P = .001$) and IL-6 ($P = .019$) both correlated with an increased demand for platelet transfusions. IL-6 may simply be a marker of clinical problems or increased TNF-α production that results in thrombocytopenia. Alternatively, thrombocytopenic patients may have increased IL-6 production in response to a low platelet count, although one study has failed to confirm this hypothesis.[46] Despite controlling for the low M-CSF and IL-6 concentrations found in G-CSF PBPC–treated patients, fewer platelet transfusions were required in this group compared with the others ($P = .014$). rHuG-CSF–primed PBPCs have higher levels of CD34

compared with rHuGM-CSF–primed PBPCs.[47] The relative importance of treatment group and endogenous cytokine concentrations cannot be fully discerned using the available data, although these data suggest that each play a role in platelet kinetics.

This study is in agreement with other observations suggesting that endogenous TNF-α is closely associated with toxicities accompanying BM transplantation.[3,4] Phase I studies of rHuTNF have shown a wide range of toxic side effects, including fever, rigors, headaches, fatigue, hypotension, thrombocytopenia, leukopenia, and increases in total bilirubin.[1,2] The patients suffering from major organ toxicity described in this report experienced some clinical symptoms similar to those attributed to rHuTNF administration. Five patients with renal or hepatic dysfunction who died on the BM transplant unit each manifested elevated TNF-α, IL-6, and M-CSF concentrations at multiple time points, with peak concentrations following the first detectable concentration by at least 3 days.

Numerous therapeutic possibilities exist for reducing serum cytokine concentrations in BMT patients, including the use of drugs such as pentoxifylline,[48] ciprofloxacin,[49] dexamethasone,[50] MoAbs or soluble receptors to cytokines; however, the clinical effects of blocking cytokines are unknown. Cytokines such as M-CSF and TNF-α perform many important biologic functions: aiding immune response, wound healing, tissue repair, and possibly providing some antitumor effects.[51]

Intervention with MoAbs or soluble receptors to cytokines in specific instances is an appealing alternative to blocking production/action in all patients. We have noted that elevations in M-CSF concentrations tend to precede elevations in TNF-α concentrations. Therefore, prospective measurement of endogenous M-CSF may enable early identification of those patients who will have subsequent rises in TNF-α concentrations. Further work is needed to explore this possibility.

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