

# Impact of Marrow Cytogenetics and Morphology on In Vitro Hematopoiesis in the Myelodysplastic Syndromes: Comparison Between Recombinant Human Granulocyte Colony-Stimulating Factor (CSF) and Granulocyte-Monocyte CSF

By Arnon Nagler, Christian Binet, Mary Lee Mackichan, Robert Negrin, Charles Bangs, Timothy Donlon, and Peter Greenberg

Marrow cells from 36 patients with myelodysplastic syndromes (MDS) (13 refractory anemia [RA], 14 refractory anemia with excess of blasts [RAEB], 9 RAEB in transformation [RAEB-T]) were evaluated for their in vitro proliferative and differentiative responsiveness to recombinant human granulocyte colony-stimulating factor (G-CSF) or granulocyte-monocyte CSF (GM-CSF). GM-CSF exerted a stronger proliferative stimulus than G-CSF for marrow myeloid clonal growth (CFU-GM) in these patients (44 v 12 colonies per  $10^5$  nonadherent buoyant bone marrow cells [NAB], respectively,  $P < .025$ ). GM-CSF stimulated increased CFU-GM growth in the 16 patients with abnormal marrow cytogenetics in comparison with the 20 patients who had normal cytogenetics (52 and 30 colonies per  $10^5$  NAB, respectively,  $P < .05$ ), whereas no such difference could be demonstrated with G-CSF (11 and 16 colonies per

$10^5$  NAB, respectively). In contrast, granulocytic differentiation of marrow cells was induced in liquid culture by G-CSF in 15 of 32 (47% patients), while GM-CSF did so in only 4 of 18 (22%) patients ( $P < .025$ ) including, for RAEB/RAEB-T patients: 9 of 18 versus 0 of 9, respectively ( $P < .025$ ). For MDS patients with normal cytogenetics, G-CSF- and GM-CSF-induced marrow cell granulocytic differentiation in 12 of 18 (67%) versus 3 of 11 (27%), respectively ( $P < .025$ ), contrasted with granulocytic induction in only 3 of 14 (21%) and 1 of 7 (14%) patients with abnormal cytogenetics, respectively. We conclude that G-CSF has greater granulocytic differentiative and less proliferative activity for MDS marrow cells than GM-CSF in vitro, particularly for RAEB/RAEB-T patients and those with normal cytogenetics.

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**T**HE MYELODYSPLASTIC syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoiesis entailing hypoproliferative and ineffective hematopoiesis associated with morphologic evidence of marrow cell dysplasia resulting in refractory cytopenias.<sup>1-5</sup> During the course of MDS, hematopoietic precursor cell differentiation may become progressively impaired, and in 10% to 40% of cases the disorders culminate in the severe maturational block characteristic of acute myelogenous leukemia (AML).<sup>1</sup>

Human granulocyte and granulocyte-monocyte colony-stimulating factors (G-CSF and GM-CSF) have recently been characterized and produced in recombinant form.<sup>6,7</sup> These CSFs have been shown to enhance proliferation and differentiation of human hematopoietic cells.<sup>8-10</sup> In vitro studies in murine models and human hematopoietic disorders have shown these factors to have potent myelostimulatory effects for normal<sup>6,8,11,12</sup> and leukemic cells.<sup>13-15</sup> G-CSF has been shown to have a predominantly granulocytic differentiative function as well as proliferative effects.<sup>6,10,11,16,17</sup> In vitro leukemic cell clonogenicity and in vivo tumorigenesis were diminished on exposure of murine leukemic cells to differentiation factors, including both G-CSF and GM-CSF.<sup>18-20</sup> Inducing leukemic stem cell differentiation, thereby decreasing neoplastic self-replicative potential, has been proposed as a model for decreasing leukemogenicity.<sup>21-23</sup>

Recently, both GM-CSF and G-CSF have been used clinically for treating MDS, raising peripheral blood leukocyte and neutrophil counts, and stimulating granulopoiesis and myeloid maturation in the bone marrow.<sup>24-27</sup> The biology of the MDS may relate to an uncoupling of hematopoietic cellular differentiative and proliferative programs.<sup>28</sup> In vitro marrow cell myeloid colony growth patterns, cytogenetic abnormalities, and bone marrow morphology are parameters that have been used as possible indicators of disease progression and prognosis in these patients.<sup>3,29-34</sup> In vitro myeloid (CFU-GM) and erythroid (BFU-E) colony growth of MDS marrow cells has previously been shown to generally decrease by using a variety of nonpurified sources of CSF,<sup>3,5,29,35</sup> and

more recently with recombinant human GM-CSF<sup>26,36-38</sup> and G-CSF.<sup>36</sup> Concern has been raised regarding the potential for altering leukemic progression in MDS by in vivo use of these CSFs.<sup>24-27</sup> To determine possible differing effects of G-CSF and GM-CSF in subgroups of MDS that are clinically distinct,<sup>39</sup> we evaluated the in vitro proliferative and differentiative responsiveness to these factors of marrow cells from a relatively large group of these patients with disparate marrow morphologic and cytogenetic features.

## MATERIALS AND METHODS

**Marrow samples.** Bone marrow (BM) samples were obtained from MDS patients according to guidelines established by the Stanford University Human Experimentation Committee. Marrow aspirates were diluted with phosphate-buffered saline (PBS), layered over Ficoll-Hypaque (density 1.077 g/mL), centrifuged at 400g for 20 minutes, and the interface buoyant mononuclear cells (MNC) were collected, washed, and counted.

*From the Departments of Medicine and Pathology, Stanford University Medical Center, Stanford; and Veterans Administration Medical Center, Palo Alto, CA.*

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*Address reprint requests to Peter Greenberg, MD, Division of Hematology, Stanford University Medical Center, Stanford, CA 94305.*

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### Monoclonal Antibodies (MoAbs) and CSFs

Goat antimouse immunoglobulin G (IgG) unconjugated and fluorescein-conjugated antibodies were purchased from Tago Inc (Burlingame, CA). Monoclonal mouse antihuman antibodies anti-My8, 1 mg/mL protein (which recognizes myeloid cells more mature than promyelocytes<sup>40,41</sup> and antiglycophorin A, 1 mg/mL protein (which recognizes erythroid precursors more mature than BFU-E),<sup>41</sup> were kindly provided by Dr J. Griffin (Dana Farber Cancer Center, Boston, MA). Recombinant human G-CSF, derived from *Escherichia coli*, not glycosylated, specific activity of approximately 10<sup>8</sup> U/mg was provided by AMGen (Thousand Oaks, CA). Recombinant human GM-CSF derived from CHO cells, glycosylated, (2 × 10<sup>6</sup> U/mg) was provided by Genetics Institute (Cambridge, MA).

**Immune adsorption ("panning").** As previously described,<sup>42</sup> after depletion of adherent cells nonadherent buoyant (NAB) cells were exposed to My8 and glycophorin A antihuman MoAbs before immune adsorption on tissue culture dishes coated with goat antimouse IgG. The nonbound (My8 [-] glycophorin A [-]) cells obtained were a relatively enriched immature marrow cell population (EIMCP), consisting of approximately 90% myeloblasts and promyelocytes and 10% lymphocytes and hematopoietic progenitor cells (CFUs).

### Clonogenic Assays for Colony Formation of Hematopoietic Progenitor Cells (CFU-GM and BFU-E)

To assess colony formation, NAB marrow cells were plated in clonogenic culture either with 5 nmol/L G-CSF or GM-CSF. For clonal assays the NAB cells (1 × 10<sup>5</sup> cells/mL) were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 15% fetal calf serum (FCS), 0.9% bovine serum albumin (Armour Pharmaceutical, Tarrytown, NY), 50 μmol/L 1-mercaptoethanol (2ME), 1% penicillin-streptomycin, 1% l-glutamine, and methylcellulose (final concentration 1.1%) with or without growth factors as indicated. Cultures with 0.375 × 10<sup>5</sup> cells were seeded in duplicate in 0.25-mL vol (ie, 1.5 × 10<sup>5</sup> cells/mL) in Mark II microwell tissue culture plates (Costar, Cambridge, MA). CFU-GM colonies stimulated with G-CSF or GM-CSF were scored on days 10 and 14, respectively, of incubation at 37°C in humidified air/5% CO<sub>2</sub>. To evaluate BFU-E, cells were cultured as described above but with 1% Mo conditioned medium (MoCM, a T-cell line CM kindly provided by Dr David Golde, UCLA Medical Center) as a burst promoting activity (BPA) source and 0.5 U/mL recombinant human erythropoietin (Ep), 10,000 U/mL (kindly provided by Ortho Pharmaceutical Corp, Raritan, NJ). BFU-E colonies were scored on day 14.<sup>36,42</sup>

### Differentiation Assay

The EIMCP were plated at 2 × 10<sup>5</sup> cells/mL in suspension culture in 5 to 10 mL IMDM supplemented with 15% FCS, and incubated for 7 days in a fully humidified air/5% CO<sub>2</sub> in 50-mL polypropylene tubes with either 5 nmol/L GM-CSF, 5 nmol/L G-CSF, or no

stimulus. The virtual absence of mature myeloid, monocytic, and erythroid cells from this EIMC population permitted assessment of induced differentiation. After incubation, cell counts were made and were demonstrated to be greater than 90% viable by trypan blue dye exclusion. To assess morphologic differentiation, cells were cytocentrifuged (Shandon cyto-centrifuge, Sewickley, PA), stained with Wright-Giemsa stain, and examined microscopically, performing 100 cell differential counts, as previously described.<sup>36,43</sup>

**Cytogenetics.** Chromosome analyses were determined for native marrow mononuclear cells and (if native cytogenetic abnormalities were present) for EIMCP cells incubated for 7 days in liquid suspension culture without or with G-CSF or GM-CSF. Synchronized chromosome preparations using G banding were obtained on native marrow using a modification of the methods of Yunis and Chandler<sup>44</sup> and Seabright.<sup>45</sup> Suspension culture chromosome preparations were prepared similarly, but without cell synchronization. Briefly, 6 mL of hypotonic solution<sup>46</sup> was added to each culture, along with colcemid (0.05 μg/mL) and incubated for 25 minutes at 37°C. After this time 5 drops of Carnoy fixative was added and the remainder of the harvest technique was performed as previously described.<sup>44</sup>

### Patients

Marrow specimens were obtained from 36 MDS patients (27 men and 9 women) (median 68 years, range 54 to 86) at the time of their initial clinical evaluation (Table 1), and from 8 normal elderly individuals. Diagnostic criteria for MDS have previously been described and include refractory cytopenias in patients with characteristically abnormal marrow morphology. We classified patients according to the FAB (French-American-British) Group classification.<sup>39</sup> Thirteen patients had refractory anemia (RA), 14 had RA with excess of blasts (RAEB), and 9 had RAEB in transformation (RAEB-T) (Table 1). Twenty patients had no cytogenetic abnormality in their native BM cells (NN), 9 had both normal and abnormal karyotypes (AN), and 7 had only abnormal karyotypes (AA). In 12 patients the nonrandom cytogenetic abnormalities involved the no. 5 and/or no. 7 chromosomes (Table 2).

### Statistical Analyses

Statistical analyses were performed using the student's *t*-test, the paired student's *t*-test, and the Wilcoxon rank test.

## RESULTS

### Effects of G-CSF and GM-CSF on Myeloid Colony Formation (CFU-GM)

We assessed the proliferative effects of G-CSF and GM-CSF on myeloid colony formation (CFU-GM) of NAB marrow cells. As can be seen from the titration curves of CSFs for CFU-GM (Fig 1), optimal clonogenic responses were demonstrated for normal subjects and MDS patients

**Table 1. Morphologic and Cytogenetic Features of Patients With Myelodysplastic Syndromes**

Morphology		Cytogenetics				
		Normal	Abnormal		Abnormality	
FAB Classification		NN	AN	AA	5 and/or 7 Chromosomes	Others
RA	13	8	4	1	4	1
RAEB	14	9	2	3	3	2
RAEB-T	9	3	3	3	5	1
Total	36	20	9	7	12	4

Marrow karyotypes: AA, abnormal; NN, normal; AN, mixture of normal and abnormal cells.

Table 2. Cytogenetic Analyses of Native and Cultured Marrow Cells From Myelodysplastic Patients With Abnormal Karyotypes

Patient No.	Native		Cultured				Dominant Abnormality
	N	A	Media		G-CSF		
			N	A	N	A	
<b>Abnormal cytogenetics (AA)</b>							
15	0	14	8	12	1	19	-7,+8,+der(7),t(1;7),del(20)
19	0	21	0	8	0	30	+1,-5,12q-,-14,-17
26	0	19	2	20	0	23	-7,-19
31	0	24	0	20	0	20	-5,-7,-16,-20,-22
<b>Mixture of abnormal and normal cytogenetics (AN)</b>							
2	1	20	15	6	6	14	21q+
25	1	9	2	13	1	49	-22,+dic(21;22)
30	7	4	ND	ND	11	1	-7,+der(7),t(1;7)
34	2	21	0	20	0	11	+8
35	4	16	0	20	0	20	-3,-5,-7,-17,-20,22q+

Giemsa banding of chromosomes from native BM and BM cells cultured for 7 days in the presence or absence of 5 nmol/L G-CSF. Numbers of normal (N) and abnormal (A) metaphases are depicted. Patients with greater than 10 analyzable metaphases are included.

Abbreviation: ND, not done.

with approximately 5 nmol/L G-CSF and GM-CSF. These dose-response curves indicated that at lower concentrations of CSF, GM-CSF was generally a more potent stimulus for MDS patients than was G-CSF.

The median marrow CFU-GM colony growth from the total group of MDS patients with 5 nmol/L GM-CSF was 44 colonies per  $10^5$  NABs (range 0 to 620). These values for the group of patients were significantly lower than those obtained from normal marrow cells ( $61 \pm 7$  per  $10^5$  NAB marrow cells,  $P < .025$ ). GM-CSF stimulated increased CFU-GM colony growth in the subgroup of MDS patients with cytogenetic abnormalities (52 per  $10^5$  NABs [range 8 to 620]), in comparison to those with normal cytogenetics (30 per  $10^5$  NABs [range 0 to 227] [ $P < .05$ ]). We compared the effects of GM-CSF on CFU-GM colony growth from MDS

patients morphologically categorized as RA versus those with RAEB or RAEB-T. Median colony growth values were 47 (range 3 to 227), 42 (range 0 to 176), and 28 (range 7 to 549) per  $10^5$  NABs, for RA, RAEB, and RAEB-T, respectively, and did not differ significantly from each other.

The median marrow CFU-GM colony growth from the MDS patients with G-CSF (5 nmol/L) as a stimulatory source was 12 (range 0 to 693) per  $10^5$  NAB marrow cells. As a group, these values were significantly lower than those obtained from normal marrow cells ( $42 \pm 10$  per  $10^5$  NAB marrow cells,  $P < .025$ ). G-CSF demonstrated similar CFU-GM proliferative effects for patients with either abnormal or normal cytogenetics (median 11 [range 4 to 693] and 16 [range 0 to 248] per  $10^5$  NAB marrow cells, respectively). Similarly, no significant differences in CFU-GM growth

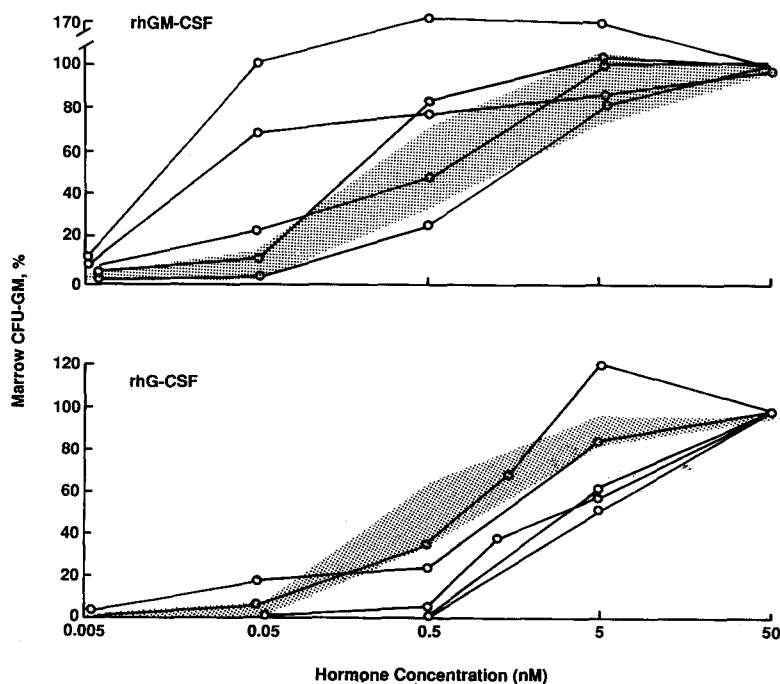
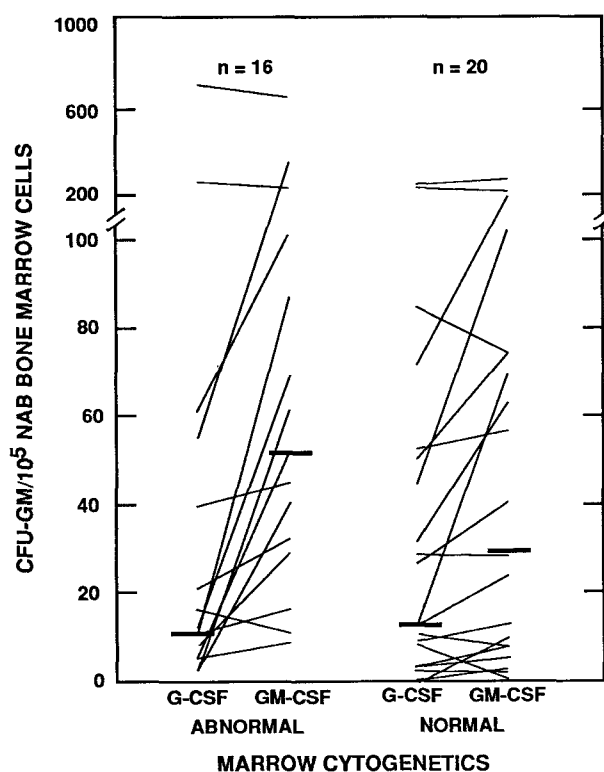


Fig 1. Myeloid clonogenic responsiveness of marrow cells from normal subjects (gray area) and individual MDS patients to recombinant human G-CSF and GM-CSF. NAB marrow cells,  $1 \times 10^5$ , were plated in duplicate with various concentrations of G-CSF or GM-CSF; colonies were scored on days 10 and 14, respectively, and calculated as percent of maximal growth. Gray area represents mean  $\pm$  SE of values for six normal individuals.

were noted for differing MDS morphologic categories with G-CSF (21 [range 0 to 242], 12 [range 0 to 253] and 5 [range 0 to 693] per  $10^5$  NAB marrow cells for RA, RAEB, and RAEB-T, respectively).

Comparative analysis indicated that GM-CSF was more potent than G-CSF as a stimulatory source for marrow CFU-GM growth in 19 of 36 MDS patients (median 44 [range 0 to 620]  $\nu$  12 [range 0 to 693], respectively [ $P < .05$  for all patients]). GM-CSF was also a stronger stimulus than G-CSF for CFU-GM colony growth in MDS patients with abnormal cytogenetics (median 52 [range 8 to 620]  $\nu$  11 [range 4 to 693]) CFU-GM per  $10^5$  NAB marrow cells, respectively ( $P < .025$ ) (Fig 2). Although marrow CFU-GM values for MDS patients with normal marrow cytogenetics were higher with GM-CSF than with G-CSF stimulation (median 30  $\nu$  16 per  $10^5$  marrow cells), these differences were not significant (Fig 2). Comparison of myeloid colony growth in morphologic subgroups of MDS indicated that GM-CSF was also a stronger proliferative stimulus than G-CSF for CFU-GM colony growth for patients with RAEB and RAEB-T. Median colony growth was 42 (range 0 to 176) and 28 (range 7 to 549) CFU-GM per  $10^5$  NAB marrow cells with GM-CSF versus 12 (range 0 to 253) and 5 (range 0 to 693) with G-CSF, respectively ( $P < .05$ ) (Fig 3). Although



**Fig 2. Relationship between marrow cytogenetics and myeloid clonogenic growth in MDS: comparison of stimulation with G-CSF and GM-CSF.** NAB marrow cells,  $10^5$ , were plated in duplicate with either 5 nmol/L G-CSF or 5 nmol/L GM-CSF. CFU-GM colonies were scored on days 10 and 14, respectively. (—), Median CFU-GM colony growth. Our normal values for CFU-GM growth with 5 nmol/L G-CSF and 5 nmol/L GM-CSF are  $42 \pm 7$  and  $61 \pm 10/10^5$  NAB marrow cells, respectively. Individual patient responses are connected by the solid lines.

marrow CFU-GM values for RA patients were higher with GM-CSF than with G-CSF stimulation (median 47  $\nu$  21 per  $10^5$  cells), these differences were not statistically significant (Fig 3).

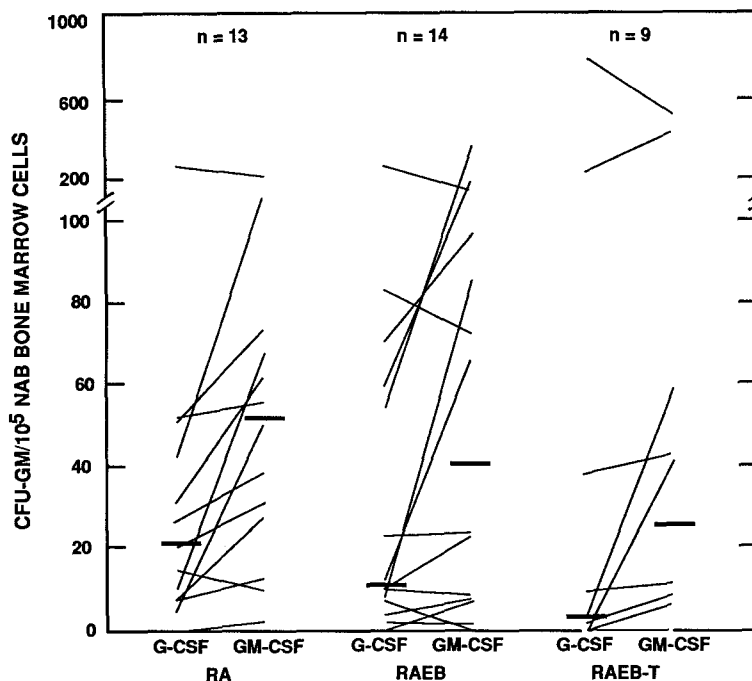
#### Erythroid Colony Growth (BFU-E)

In marrow cells from 33 of 36 patients, subnormal BFU-E growth was noted (1 to 45 per  $10^5$  NAB marrow cells). Our normal BFU-E values are  $103 \pm 20$  per  $10^5$  NAB marrow cells, using 1% MoCM and 0.5 U/mL Ep as a stimulus. No BFU-E growth occurred when marrow cells were plated in the absence of added Ep. Neither marrow morphologic classification (FAB) nor cytogenetic abnormalities were associated with differences in BFU-E colony growth. BFU-E colony growth was 5 and 13 per  $10^5$  NAB marrow cells for MDS patients with abnormal and normal cytogenetics, respectively. Similarly, median BFU-E colony growth for RA, RAEB, and RAEB-T patients were 11, 5, and 8 per  $10^5$  NAB marrow cells, respectively.

#### Effects of CSFs on Myeloid Cell Differentiation

The differentiative effects of G-CSF and GM-CSF on the EIMCP of MDS marrow cells in liquid culture were assessed by comparing induced granulocytic and monocytic differentiation at day 7 with that at day 0. EIMCP recovery after 7 days of culture was similar for G-CSF, GM-CSF, and medium only ( $72\% \pm 34\%$ ,  $68\% \pm 24\%$ , and  $58\% \pm 27\%$ , respectively). These values of cell recovery are similar to those we reported previously for normal subjects.<sup>36</sup> Dose-response curves (with concentrations of 0.5 to 50 nmol/L) for myeloid differentiation of normal ( $n = 3$ ) and MDS ( $n = 3$ ) marrow EIMCP in liquid culture indicated that optimal (plateau) effects occurred at 5 nmol/L for both G-CSF and GM-CSF. Seven days of liquid culture had greater myeloid differentiation demonstrated than did 4 days of liquid culture. Thus, these culture conditions were used for further studies. On culture, both CSFs at 5 nmol/L concentration induced an increase in the absolute and relative number of mature granulocytes compared with medium alone (Figs 4 and 5). For the MDS patients, G-CSF induced greater granulocytic differentiation than did GM-CSF (10%  $\nu$  1%,  $P < .025$ ) (Table 3, top). The granulocytic differentiative effect of G-CSF was less potent for the MDS patients than for normals (10%  $\nu$  32%, respectively,  $P < .05$ ) (Table 3, top). In the 32 patients studied, G-CSF induced granulocytic differentiation in 12 of 18 (67%) MDS patients with normal marrow cytogenetics compared to this effect in only 3 of 14 (21%) patients who had abnormal cytogenetics ( $P < .025$ ) (Table 3, bottom). G-CSF and GM-CSF induced granulocytic differentiation in 67% versus 27% patients with normal cytogenetics ( $P < .025$ ) in comparison to only 21% versus 14% patients with abnormal cytogenetics, respectively ( $P < .005$ ) (Table 3, bottom).

Morphologic subgroups of MDS were also evaluated for differences in granulocytic differentiation. G-CSF induced granulocytic differentiation in cells from 6 of 12 (50%) RAEB patients and 3 of 6 (50%) RAEB-T patients, while GM-CSF induced granulocytic differentiation in none of the



**Fig 3. Relationship between marrow morphology and myeloid clonogenic growth in MDS: comparison of stimulation with G-CSF and GM-CSF. NAB marrow cells, 10<sup>5</sup>, were plated in duplicate with either 5 nmol/L G-CSF or GM-CSF. (-), Median CFU-GM colony growth.**

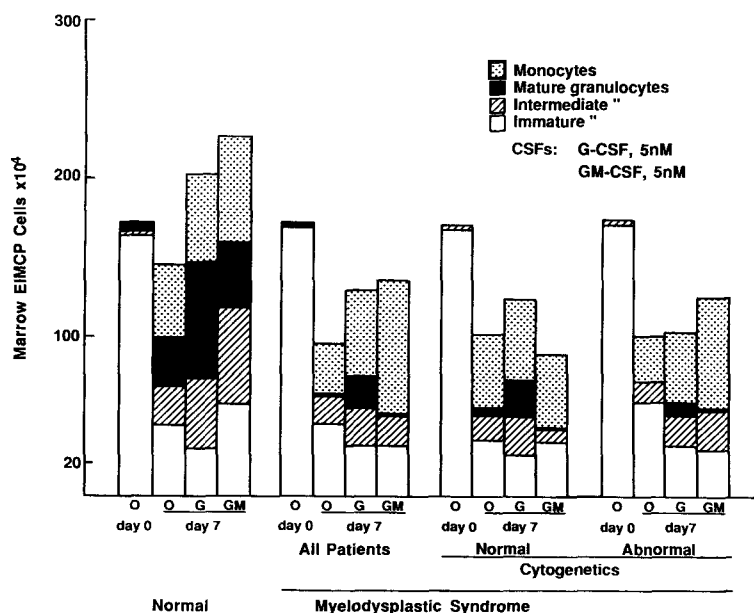
patients tested ( $P < .025$ ) (Table 3, bottom). No difference was observed between G-CSF and GM-CSF granulocytic differentiative capacity for RA patients (50% and 44% of the patients, respectively) (Table 3, bottom). As graphically shown in Fig 6, for marrow cells from both normal subjects and MDS patients, composite data demonstrated that GM-CSF was more potent than G-CSF as a proliferative stimulus, whereas the converse was found for these agents regarding their granulocytic differentiative activity.

Monocytic differentiation after 7-day liquid culture was similar for MDS patients with either G-CSF or GM-CSF, 27% and 33%, respectively (Figs 4 and 5). These values do not differ from the values we reported previously for normals.<sup>36</sup>

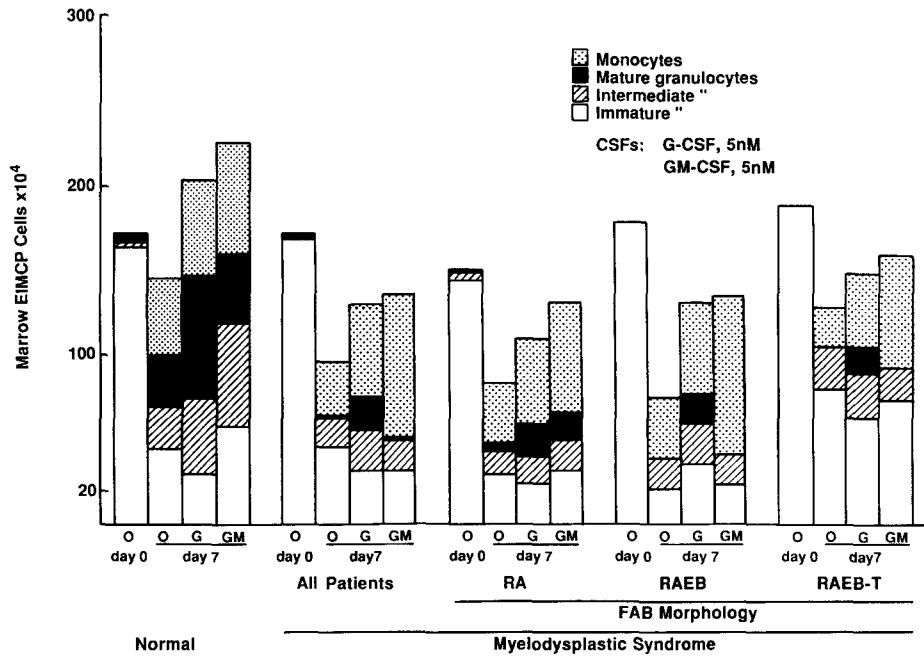
However, as 18% monocytic differentiation also occurred in the liquid cultures without a stimulus (ie, with medium alone), the monocytic differentiative effect of both CSFs for MDS patients appears to be minimal. Rather, MDS cells seem to possess intrinsic potential for monocytic differentiation *in vitro* in these conditions.

*Cytogenetics After Liquid Culture*

Sixteen of the 36 MDS patients had cytogenetic abnormalities in their native BM, 9 had both normal and abnormal karyotypes (AN), and 7 had only abnormal karyotypes (AA) (Table 1). To more directly determine the ability of G-CSF to enhance differentiation of cytogenetically abnormal versus



**Fig 4. Influence of marrow cytogenetics on differentiation and proliferation in liquid suspension culture of MDS enriched immature myeloid cell populations (EIMCP) of marrow cells exposed to G-CSF, GM-CSF, or no stimulus for 7 days compared with the cells initially plated. The histograms represent the absolute number of viable cells. All MDS patients, n = 32; normal cytogenetics, n = 18; abnormal cytogenetics, n = 14; normal subjects, n = 8.**



**Fig 5.** Influence of marrow morphology on differentiation and proliferation in liquid suspension culture of MDS enriched immature myeloid cell populations of marrow cells exposed to G-CSF, GM-CSF, or no stimulus for 7 days compared with the cells initially plated. The histograms represent the absolute number of viable cells. All MDS patients, n = 30; RA, n = 12; RAEB, n = 12; RAEB-T, n = 6; normal subjects, n = 8.

normal MDS cells, we evaluated cytogenetics of their EIMCP cells grown for 7 days in liquid culture in the presence or absence of G-CSF. In nine of these patients adequate numbers of metaphases were present to permit this analysis. In three of four patients who initially had all abnormal

cytogenetics (AA), the same karyotypes were found after culture (Table 2). One of these patients (no. 31) had granulocytic differentiation induced in vitro with G-CSF, indicating possible differentiation of the abnormal cells. In three of the five patients who initially had a mixture of

**Table 3. Neutrophilic Differentiation-Inducing Ability of Recombinant Human CSFs for Normal Subjects and Subgroups of Myelodysplastic Syndromes**

Marrow Cells	G-CSF*		GM-CSF*
<b>Degree of induced neutrophilic differentiation</b>			
<b>Myelodysplastic syndromes</b>			
All patients	10% (0-57)†	‡	1% (0-25)‡
<b>Marrow cytogenetics</b>			
Normal	12% (0-46)	§	1% (0-25)
Abnormal	4% (0-57)	NS	1% (0-15)
<b>Marrow morphology</b>			
RA	18% (0-57)	NS	7% (0-25)
RAEB	9% (1-46)	§	1% (1-9)
RAEB-T	8% (2-17)	§	0% (0-1)
Normals	32% (16-67)	‡	18% (7-41)
<b>No. of patients with induced neutrophilic differentiation</b>			
<b>Myelodysplastic syndromes</b>			
All patients	15/32 (47%)	‡	4/18 (22%)
<b>Marrow morphology</b>			
RA	6/12 (50%)	NS	4/9 (44%)
RAEB	6/12 (50%)	§	0/4 (0%)
RAEB-T	3/6 (50%)	§	0/5 (0%)
<b>Marrow cytogenetics</b>			
Normal	12/18 (67%)	§	3/11 (27%)
Abnormal	3/14 (21%)	NS	1/7 (14%)
Normals	8/9 (89%)	NS	4/6 (67%)

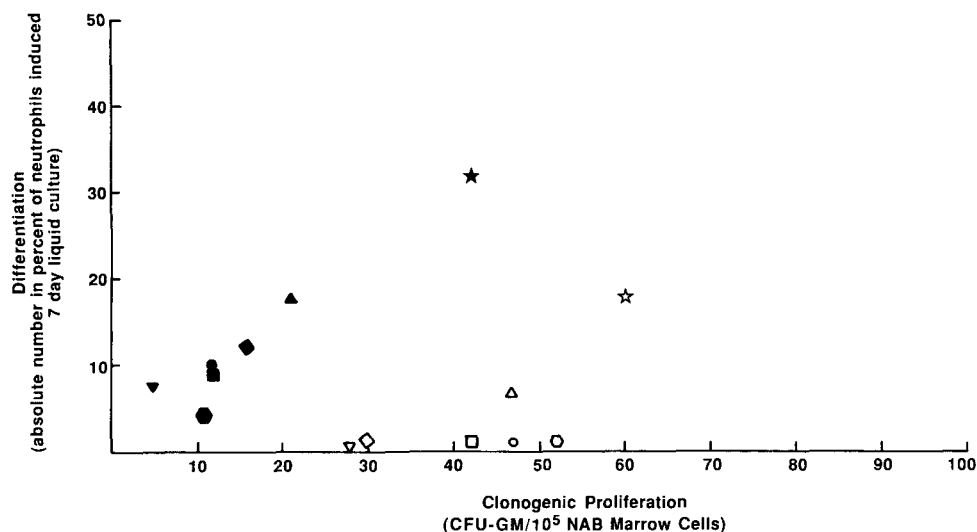
\*5 nmol/L.

†Results are medians (range) of the absolute mature neutrophilic cell number relative to the initial cell inoculum, expressed in percent.

‡P < .025.

§P < .005.

||Proportion of patients with induced differentiation, ie, ≥10% of enriched immature marrow cells developing into mature neutrophils after 7 days of liquid culture.



**Fig 6.** Relationship between proliferation (CFU-GM colony growth) and granulocytic differentiation induced by G-CSF and GM-CSF in marrow cells from myelodysplastic syndrome patients and normal individuals. Cells were stimulated by 5 nmol/L G-CSF (solid symbols) or 5 nmol/L GM-CSF (open symbols). All MDS patients,  $n = 36$  (●, ○); RA,  $n = 13$  (▲, △); RAEB,  $n = 14$  (■, □); RAEB-T,  $n = 9$  (▼, ▽); normal cytogenetics,  $n = 18$  (◆, ◇); abnormal cytogenetics,  $n = 14$  (•); normal subjects,  $n = 8$  (★, ☆). Mean values of CFU-GM proliferation and absolute numbers of mature neutrophil induced differentiation for each patient group is shown.

abnormal and normal cytogenetics (AN), similar proportions of cells with these karyotypes were present after culture (Table 2), indicating in vitro responsiveness or persistence of both the cytogenetically normal and abnormal cells to G-CSF. In two AN patients (nos. 34 and 35) only abnormal metaphases were found after liquid culture, suggesting possible proliferative or survival advantage of the abnormal cells in these patients. Similar persistence of cytogenetically abnormal and normal cells were found in four of these patients (one AA, three AN) after stimulation with GM-CSF (data not shown).

#### DISCUSSION

Our studies describe hematopoietic precursor proliferative and differentiative responsiveness to G-CSF and GM-CSF in vitro of marrow cells from a relatively large group of MDS patients, comparing these effects in differing cytogenetic and morphologic subgroups of these patients. We demonstrated that GM-CSF was a more potent clonogenic myeloid proliferative stimulus than G-CSF for marrow cells from MDS patients. This feature was particularly prominent in cytogenetically abnormal patients and those with RAEB/RAEB-T. Previous studies have shown the abnormal (possibly leukemic) clones within MDS marrow to be more responsive to GM-CSF than to G-CSF.<sup>13,14,47-50</sup> Prior studies have demonstrated stronger proliferative effects of GM-CSF than G-CSF for murine and human leukemic blasts.<sup>12,13,17,51</sup> These findings indicate that biologic parameters such as marrow cytogenetics as well as marrow morphologic features (FAB subtype) correlate with responses to GM-CSF and G-CSF in MDS patients.

Our demonstration of subnormal myeloid clonogenicity for MDS patients with recombinant GM-CSF is in agreement with previous reports with this recombinant material,<sup>26,37,38</sup> and those using nonpurified CSFs.<sup>3,5,29,35,52</sup> In addition, we

have also demonstrated subnormal colony growth for MDS patients with recombinant human G-CSF. Dose-response curves with these agents (Fig 1) showed normal or enhanced myeloid proliferative clonogenic responses in MDS to GM-CSF in contrast to normal or diminished responses with G-CSF. Increased CFU-GM responses of MDS precursors to high GM-CSF concentrations have also been shown in a previous study.<sup>38</sup>

Differing from their effects on clonogenic hematopoietic precursors, granulocytic differentiation induction of MDS marrow cells was more prominent with G-CSF than with GM-CSF. This was particularly evident for cells from RAEB and RAEB-T MDS patients, and patients lacking cytogenetic abnormalities (Figs 4 and 5, Table 3). The stronger granulocytic differentiative effect of G-CSF compared with GM-CSF confirms previous studies indicating that G-CSF is predominantly differentiative, whereas GM-CSF has mainly proliferative effects.<sup>14,51,53</sup> However, this effect was heterogeneous and less than that occurring for cytogenetically normal cells. The diminished differentiative response of MDS cells compared with that for normal marrow cells suggests intrinsic differences in the differentiative potential of normal versus leukemic (preleukemic) cell populations to G-CSF, or that additional growth factors are needed for optimal cell differentiation. Further, both G-CSF and GM-CSF had particularly poor granulocytic differentiation potential in MDS patients with cytogenetic abnormalities. These data suggest that cytogenetically abnormal cells have more defective differentiation ability than those that are cytogenetically normal. To more directly determine the differentiative ability of the cytogenetically abnormal MDS marrow cells to G-CSF we analyzed their karyotypes after cell growth in liquid culture. Our results demonstrated that in the MDS patients with native cytogenetic abnormalities, the same karyotypes were also generally present after culture

with G-CSF. These karyotypic abnormalities noted in culture reflected mitoses of immature and intermediate myeloid precursor cells and suggest induced granulocytic differentiation in vitro by G-CSF. However, direct proof of this point requires analysis of the mature neutrophils themselves by methods such as genetic restriction fragment length polymorphisms (RFLP).<sup>54</sup> RFLP analysis of cells from a responding female patient (whose in vitro marrow culture studies are reported in this article), demonstrated clonality in her resultant peripheral blood neutrophils on in vivo treatment with G-CSF,<sup>55</sup> suggesting induced differentiation of the abnormal clone in vivo. However, in an MDS patient treated with GM-CSF, polyclonality was demonstrated in the responding neutrophils.<sup>56</sup>

Reports in the literature indicate induction of monocytic differentiation by GM-CSF.<sup>15,16</sup> Although our study also showed this effect, we also observed monocytic differentiation of MDS patients on cell exposure to medium alone. This

“spontaneous” monocytic cell differentiation may be attributed to factors present in the FCS or to an intrinsic monocytic differentiation potential of the cells.

Our data indicated that biologic parameters such as cytogenetic abnormalities and morphologic classification contributed to the in vitro proliferative and differentiative responses of MDS marrow cells to G-CSF and GM-CSF. Neutrophil elevations occur in most MDS patients treated with either G-CSF or GM-CSF.<sup>24-27</sup> Although a small proportion of MDS patients treated with either CSF had developed AML,<sup>26,27,55</sup> consistent with our in vitro findings one of the trials using GM-CSF suggested that MDS patients with high marrow blast counts may be more susceptible to blastic responses in vivo.<sup>26</sup> Prospective investigations will be necessary to determine the possible utility of such in vitro studies for designing in vivo clinical trials with these CSFs.

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