Mutual Inhibition of Murine Erythropoiesis and Granulopoiesis During Combined Erythropoietin, Granulocyte Colony-Stimulating Factor, and Stem Cell Factor Administration: In Vivo Interactions and Dose-Response Surfaces

By Gerald de Haan, Christoph Engel, Bert Dontje, Willem Nijhof, and Markus Loeffler

We investigated the in vivo effects of erythropoietin (EPO) on granulopoiesis and, conversely, the effect of granulocyte colony-stimulating factor (G-CSF) treatment on erythropoiesis. Recombinant human EPO at four different doses in combination with recombinant human G-CSF also at four different doses was simultaneously administered for 7 days to splenectomized mice. In total, 16 different combinations of growth factors were thus tested. G-CSF administration increased granulocyte production as expected, whereas immature colony-forming unit granulocyte-macrophage numbers were decreased. EPO analogously increased late erythroid cell numbers. Both EPO and G-CSF dose-dependently inhibited late cell stages of the opposite lineage, with EPO abrogating G-CSF–stimulated granulopoiesis and, conversely, G-CSF inhibiting EPO-stimulated erythropoiesis. In a subsequent experiment, we tested whether these lineage-competitive effects could be prevented by coadministering stem cell factor (SCF). In these three factor-treated mice, all granuloid and erythroid cell stages increased, thereby reducing the effect of the mutual inhibition. We conclude that EPO-stimulated erythropoiesis and G-CSF–stimulated granulopoiesis inhibited each other at a late level. Simultaneous SCF administration increased the input into both the erythroid and granuloid compartment and thereby compensated the mutual inhibition. This study shows that intricate dose-response relationships exist between various growth factors that should be carefully analyzed before combinations of these factors are used in humans.

THE BALANCED PRODUCTION of blood cells is regulated by a network of different growth factors acting from the early stem cells to the late precursor cells. In the normal steady-state, hematopoietic cells from all lineages are produced sufficiently to meet peripheral requirements. However, little is known if and how the production of one cell lineage is affected when others are stimulated, although the concept of competition between the different hematopoietic lineages has been reported since the late 1960s.[1] Such competitive conditions could be met in the present day treatment of patients with combinations of hematopoietic growth factors. Several reports show that administration of lineage-specific growth factors can lead to side-effects in other lineages. Treatment of children with aplastic anemia with granulocyte colony-stimulating factor (G-CSF) resulted in reduced erythroid/myeloid ratios, whereas the marrow cellularity remained constant, indicating a suppressed erythropoiesis.[2] In mice, G-CSF treatment reduces marrow erythropoiesis severely.[3-7] Stimulated erythropoiesis by erythropoietin (EPO) administration to newborn rats[8] and premature children[9] induced a reduction of the absolute neutrophil count. EPO treatment of adult rats resulted in a marrow myeloid hypoplasia.[10] EPO treatment of mice has been reported to result in thrombopenia as well.[11] When G-CSF and EPO were administered to acquired immunodeficiency syndrome (AIDS) patients simultaneously, the increase in the absolute neutrophil count was significantly less than with a G-CSF treatment alone.[12]

These in vivo studies confirm in vitro experiments that showed that EPO suppressed granulocyte-macrophage colony formation in mice and rats[13,14] and, conversely, colony-stimulating factors reduced both the number of EPO-induced burst-forming units erythroid (BFU-E) and the subsequent hemoglobin synthesis.[14,15] The suppressive effect of EPO on human neutrophil and macrophage production in vitro is controversial.[16,17]

From the experiments mentioned above it is clear that the mutual suppression of erythropoiesis and granulopoiesis only becomes evident under certain stimulatory conditions. In the present study, we wanted to study competition between these two lineages by administering the committed growth factors G-CSF and EPO (each in 4 concentrations, thus 16 combinations) to splenectomized mice. We removed the spleen to prevent the shift of erythropoiesis to this organ as was seen during a G-CSF treatment of normal mice.[4,17] Also, administration of EPO to intact mice leads to a high involvement of the spleen in total erythroid cell production.[18] The statistical analysis of this multifactorial designed combination experiment involved nonlinear regression analysis to evaluate the shape of the dose-response surfaces. The two-dimensional projections of these dose-response surfaces, which were obtained by the regression analysis, generated so-called "equi-response curves" and can be used to define optimal strategies for the production of erythroid and granuloid cells.

In a subsequent experiment, we investigated whether coadministration of SCF could change the competitive effects of granulopoiesis and erythropoiesis. SCF has been shown to act synergistically in vivo with both G-CSF[19] and EPO[20] alone in the production of granulocytes and erythrocytes. Therefore, we assumed that stem cell factor (SCF) would change the EPO/G-CSF dose-response relationship

From the Groningen Institute for Drug Studies, Department of Hematology, University of Groningen, Groningen, The Netherlands; and the Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany.

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Address reprint requests to Gerald de Haan, MSc, Groningen Institute for Drug Studies, Department of Hematology, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands.

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and would interfere with competitive effects between granulopoiesis and erythropoiesis.

MATERIALS AND METHODS

Treatment of Animals

C57bl/6 mice between 8 and 12 weeks of age, weighing 20 to 25 g, were splenectomized at least 2 months before they entered in the experiment. Lyophilized recombinant human EPO, a gift from Boehringer Mannheim (Almere, The Netherlands), was suspended in sterile saline. Animals were treated with 0, 0.5, 5, or 50 U EPO/day for 7 days. Recombinant human G-CSF, a gift from Amgen (Thousand Oaks, CA), was delivered in an aqueous buffer at a concentration of 300 μg/mL. G-CSF was diluted in sterile saline. Animals were treated with 0, 0.25, 0.625, or 2.5 μg G-CSF/d for 7 days. In total, $4 \times 4 = 16$ different combinations of growth factors were tested. The experiment was repeated once. Recombinant rat SCF (SCF^{164}), a gift from Amgen, was delivered as a solution of 1.56 mg/mL SCF + 0.01% bovine serum albumin (BSA). Animals were treated with 2.5 μg SCF/d (this dose was also used in previous studies[19,20]). Growth factors were mixed in appropriate concentrations and were administered by subcutaneously implanted osmotic pumps (Alzet model 1007D) that were kindly provided by Alza Corporation (Palo Alto, CA).

Blood Values

Blood was obtained from the orbital plexus. Hematocrit and white blood cell counts were determined according to standard procedures.

Progenitor and Precursor Cell Assays

Single-cell bone marrow and spleen cell suspensions were obtained according to standard procedures. Cytospin preparations were made and stained with May Grünwald-Giemsa to determine erythroid and granuloid precursor numbers. Precursor cells refer to morphologically recognizable erythroid or granuloid cells. Progenitor cells (colony-forming unit granulocyte-macrophage [CFU-GM], BFU-E, and CFU-erythroid [CFU-E] were cultured with the methylcellulose method of Iscove and Sieber.[21] CFU-GM/BFU-E cultures were supplemented with 2 U EPO (Boehringer), 10 ng/mL recombinant murine granulocyte-macrophage–CSF (rmGM-CSF; a gift from Behringwerke, Marburg, Germany), and 100 ng/mL rrSCF. CFU-E cultures were supplemented with 500 mU EPO.

Data Analysis

The EPO and G-CSF combination experiment was conducted in a 4×4 factorial design (4 doses of EPO and 4 doses of G-CSF). The appropriate statistical evaluation of this type of experiments involves a regression analysis. This strategy is highly informative and far more powerful than a pairwise comparison of selected datapoints. It does not only result in information on the significance of a certain effect, but, in addition, it provides a quantatitive description of the dose-response surfaces. This can be used in predicting the response of a certain cell type for any dose combination of growth factors tested. Furthermore, it reduces the number of mice necessary to achieve statistical significance. The present experiment was designed to be performed with 32 mice. One group (5 U EPO) was not used. The regression model had to be specified in a meaningful way. Based on previous experience, we could not expect simple linear dose-response relationships in this study. For both EPO and G-CSF, we have published data showing dose-response saturation characteristics on a logarithmic scale, with 50 U EPO and 2.5 μg G-CSF having close to maximal effects.[7,18] Because we were interested in analyzing the biologic effect of a certain dose, this implied that, in the regression model the administered, pharmacologic EPO and G-CSF doses were logarithmically transformed. Thus, EPO doses were coded as follows: 0 U and 0.5 U = 0 (having no effect), 50 U = 2 (showing maximal effect), and 5 U = 1 (having half-maximal effects). EPO doses 0 U and 0.5 U were pooled because no biologic difference between both doses was observed. The rationale for this coding is based on previous findings, which were confirmed in this study, that the biologic "distance" (ie, response) from 0 to 5 U is equal as that from 5 to 50 U.[18] Similarly, G-CSF doses were coded as follows: 0 μg = 0, 0.25 μg = 1, 0.625 μg = 1.4, and 2.5 μg = 2. For biologic reasons, we consider this coding most plausible; however, different coding scenarios were tested. The qualitive message of the dose-response patterns was never changed by choosing a different coding.

For each cell stage (Y), a regression was performed based on the model: $Y = a + b[EPO] + c[EPO^2] + d[G\text{-}CSF] + e[G\text{-}CSF^2] + f([EPO] \times [G\text{-}CSF])$. Estimates for the regression coefficients and the 95% confidence intervals were determined. The results of the regression analysis are given in Table 1. For each cell stage, the regression coefficient of a term is given and it is indicated (underlined) whether it differed significantly from 0 ($P < .1$ and .01). The regression model obtained is able to determine whether there is a significant linear dose-response effect on a specific cell stage of low EPO ("b" term) and low G-CSF ("d" term) doses. Furthermore, it is able to indicate whether at high EPO ("c" term) or high G-CSF ("e" term) doses saturation or further stimulation of these linear effects occurs. Finally, it detects whether there exists an interaction of EPO and G-CSF ("f" term). Positive values should be interpreted as stimulatory activity; negative values indicate inhibitory effects. All analyses were performed using SAS PROC REG (SAS Institute Inc, Cary, NC).

Visualizing the Regression Surfaces

The development of a regression model not only enabled us to detect significant effects, but also to predict, at any EPO + G-CSF combination, the response of each cell stage by simply filling in the (coded, log-transformed) EPO and G-CSF doses of interest in the formula of which the coefficient estimates are given in Table 1. Thus, Table 1 can be graphically depicted, resulting in smooth 3-dimensional dose-response surfaces, showing the prediction by the regression model of the behavior of each cell stage for any growth factor combination. As an example, we have plotted the dose-response surfaces for granulocytes and hematocrit obtained by the regression analysis using the GNUPLOT program (see Fig 3). The projections of the equi-response curves on the ground base of the coordinate system are also shown. Figure 3A explains how these curves were created. At distinct Y-values, cross-sections were made through the dose-response curves (shown is the 20×10^6/mL cut off). The intersection of the dose-response surface with this cross section was then redrawn on the ground base of the figure. To fascilitate interpretation of the experimental results and to enable us to predict a response at EPO + G-CSF doses that were not tested, this procedure was performed for all evaluated cell stages. The results of this statistical exercise can be found in Fig 4 and will be discussed below.

RESULTS

Splenectomy

We determined steady-state erythroid and granuloid parameters of mice that had been splenectomized at least 2 months before the experiments were performed. The only differences in splenectomized mice compared with normal mice were an increased hematocrit (47.5% ± 0.6% *v* 44.2%

Table 1. Statistical Evaluation of the G-CSF + EPO Combination Experiment

Y-Parameter	a	EPO b	EPO² c	G-CSF d	G-CSF² e	(EPO × G-CSF) f
CFU-GM/femur ($\times 10^{-3}$)	51.8	−13.92	0.39	3.20	−10.73	8.38
ANC/mL ($\times 10^{-6}$)	0.57	15.76	−7.06	0.03	8.73	−5.67
Ratio of ANC/GM (fraction of normal)	−1.46	24.87	−10.03	−14.97	23.02	−11.15
BFU-E/femur ($\times 10^{-3}$)	3.68	1.33	−0.99	2.32	−2.08	0.45
CFU-E/femur ($\times 10^{-3}$)	93.81	87.90	13.89	−65.04	9.84	−57.06
Ratio of CE/BE (fraction of normal)	0.86	1.20	0.40	−0.86	0.29	−0.95
Hematocrit (%)	44.09	2.66	0.85	0.68	−1.06	−1.11

This table shows for each cell stage (Y) the result (coefficient estimate) of the regression analysis, based on the model Y = a + b[EPO] + c[EPO²] + d[G-CSF] + e[G-CSF²] + f([EPO] × [G-CSF]). Underlined estimates are significant from 0 (single underscore, $P < .1$; double underscore, $P < .01$). Apart from the analysis of significance, this table allows to predict for any (coded, log-transformed) EPO + G-CSF dose the response of a certain cell type. As an example, the hematocrit after a treatment with 25 U (1.7 coded) EPO + 1 μg (1.6 coded) G-CSF is calculated as follows: HCT = 44.09 + (1.7)2.66 + (1.7^2)0.85 + (1.6)0.68 − (1.6^2)1.06 − (1.7 × 1.6)1.11 = 46.4%.

± 0.1%) and an increased absolute neutrophil count (1.9 ± 0.1 × 10^6 *v* 1.0 ± 0.2 × 10^6, n = 10, $P < .001$).

EPO + G-CSF Combination Experiment

Data analysis. The experimental data obtained in the EPO + G-CSF experiment are shown in Figs 1 and 2 and will be discussed in detail below. These results were statistically evaluated using a nonlinear regression analysis (which is explained in the Materials and Methods). The results of this analysis are given in Table 1, which gives for each cell stage the coefficient estimates of the regression model and its significance. The coefficient estimates for the regression models given in Table 1 were then used to create Figs 3 and 4, from which predictions of the behavior of the various cell stages can be obtained.

Effects of EPO and G-CSF on granulopoiesis. To give an illustration of how to read Table 1 in conjunction with Figs 1 and 2, the behavior of CFU-GM (Figs 1A and 4A) is explained in detail. EPO reduced CFU-GM numbers (negative EPO term). At high EPO doses, no significant saturation of this inhibition was observed (nonsignificant [NS] EPO^2 term). Low G-CSF doses did not significantly influence CFU-GM numbers (NS G-CSF term). At high G-CSF doses, however, CFU-GM numbers were significantly reduced (negative $G\text{-}CSF^2$ term). Finally, the superposition of effects on CFU-GM at high EPO and G-CSF doses was significantly increased (positive EPO × G-CSF term), indicating that G-CSF was able to increase CFU-GM numbers at high EPO doses.

Figure 1B shows that EPO-induced suppression of granulocyte production occurred at high EPO doses and was most clearly shown by reducing the efficacy of medium and high G-CSF doses to increase granulocyte numbers. Whereas 2.5 μg G-CSF increased granulocytes to about 40 × 10^6/mL, the coadministration of 50 U EPO reduced this to only 10 × 10^6/mL (Figs 1B, 3A, and 4B). To give an impression of the altered production of granulocytes produced per CFU-GM, we calculated the ratio of granulocytes/CFU-GM (normalized values) for the different groups. Figure 4C shows that, at high EPO doses, this ratio is reduced.

Effects of EPO and G-CSF on erythropoiesis. BFU-E numbers were increased at low G-CSF doses, but G-CSF at high doses decreased BFU-E similarly as CFU-GM numbers. EPO, alone or in combination with G-CSF, mildly affected BFU-E numbers. There was a tendency for reduced BFU-E frequencies at the higher EPO doses, but this was not significant (Figs 2A and 4D).

In contrast to BFU-E, CFU-E numbers were strongly influenced by both EPO and G-CSF. Whereas G-CSF dose-dependently reduced CFU-E numbers to 3%, EPO could increase CFU-E numbers to 360% of normal. Simultaneous treatment with G-CSF and EPO showed that EPO could prevent the erythroid inhibition at low G-CSF doses, but at increasing G-CSF doses, EPO proved to be less capable of increasing CFU-E numbers (Figs 2B and 4E). To give an

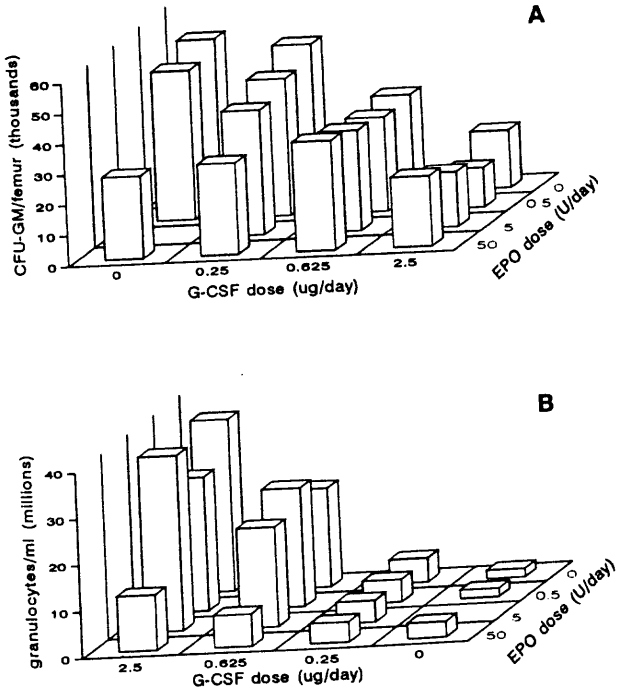

Fig 1. Experimental results of the effects of a combined 7 days of G-CSF and EPO administration on CFU-GM numbers in femur (A) and granulocytes in peripheral blood (B).

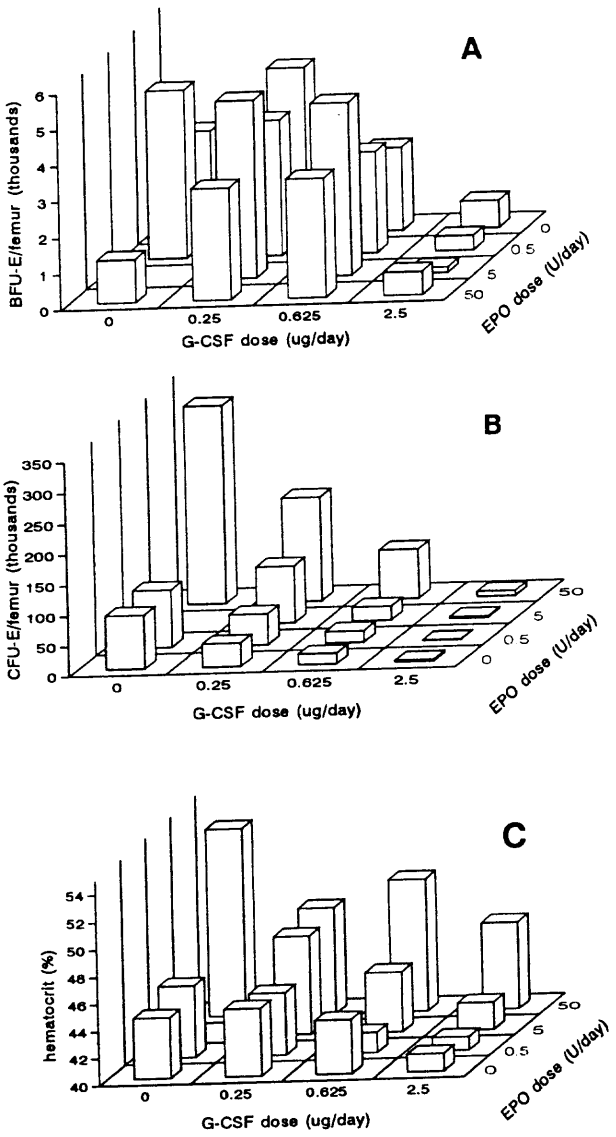

Fig 2. Experimental results of the effects of a combined 7 days of G-CSF and EPO administration on BFU-E (A) and CFU-E (B) numbers in femur and on the hematocrit (C).

impression of the number of CFU-E produced per BFU-E, we calculated the ratio CFU-E/BFU-E (normalized values) for all groups. Figure 4F shows that G-CSF reduced this ratio.

The effects of EPO and G-CSF on the production of erythroid cells in the marrow was also reflected in the hematocrit (Figs 2C, 3B, and 4G). High G-CSF doses induced a mild anemia in 7 days of treatment and, again, the stimulating effect of EPO on erythroid cell production could be reversed by a simultaneous high-dose G-CSF.

SCF + EPO + G-CSF Combination Experiment

In a subsequent experiment, we tested whether simultaneous SCF administration would change the observed EPO and G-CSF dose-response relationships and thus could prevent the inhibitory effects. SCF (2.5 μg/d) was coadministered to 50 U EPO + 2.5 μg G-CSF–treated mice. Figure 5 shows that femur cellularity, which was markedly decreased by EPO + G-CSF treatment, increased when SCF was simultaneously administered. As a consequence, all measured erythroid and granuloid cell stages benefited from this SCF treatment. CFU-GM numbers increased 1.5-fold, BFU-E numbers 3.4-fold, CFU-E numbers 7.2-fold, granulocytes 1.7-fold, and the hematocrit increased from 46.3% to 52.1%.

DISCUSSION

In this study, we investigated how granulopoiesis and erythropoiesis mutually interacted with each other in splenectomized mice during growth factor treatment. In the first experiment, we administered the lineage-specific growth factors G-CSF and EPO in 16 different dose combinations. Our results show that not only did G-CSF treatment inhibit EPO-

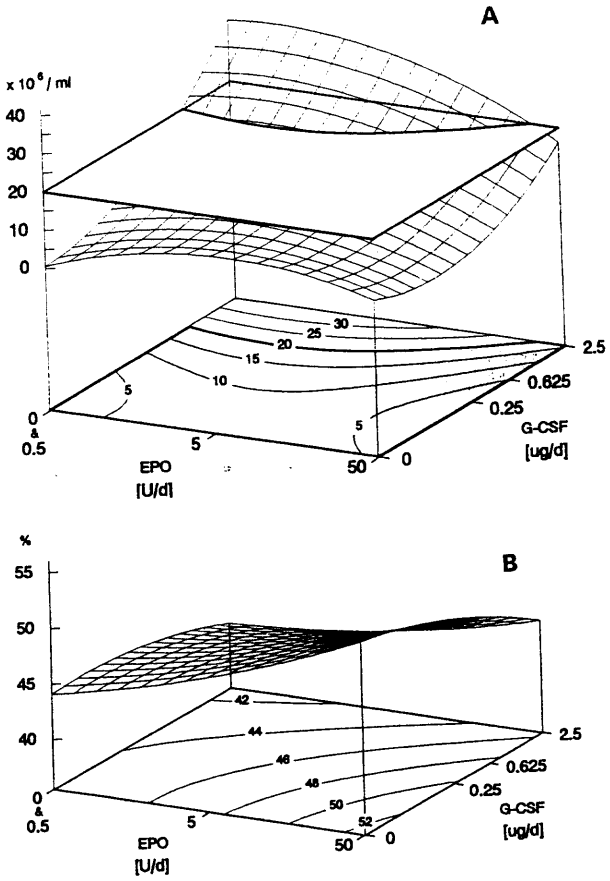

Fig 3. Evaluation of the dose-response surface, obtained by the regression analysis, of G-CSF and EPO administration on granulocytes in peripheral blood (A) and hematocrit (B). (A) A cross-section was made through the D/R surface at 20 × 10^6. The intersection of the surface with the cross-section gives the EPO and G-CSF dose ranges that result in 20 × 10^6 ANC/mL. This equi-response curve was projected at the base of the figure, where other curves are shown. A two-dimensional representation of this same contour plot is shown in Fig 4B. Data obtained at 0 and 0.5 U EPO/d are pooled (see Materials and Methods).

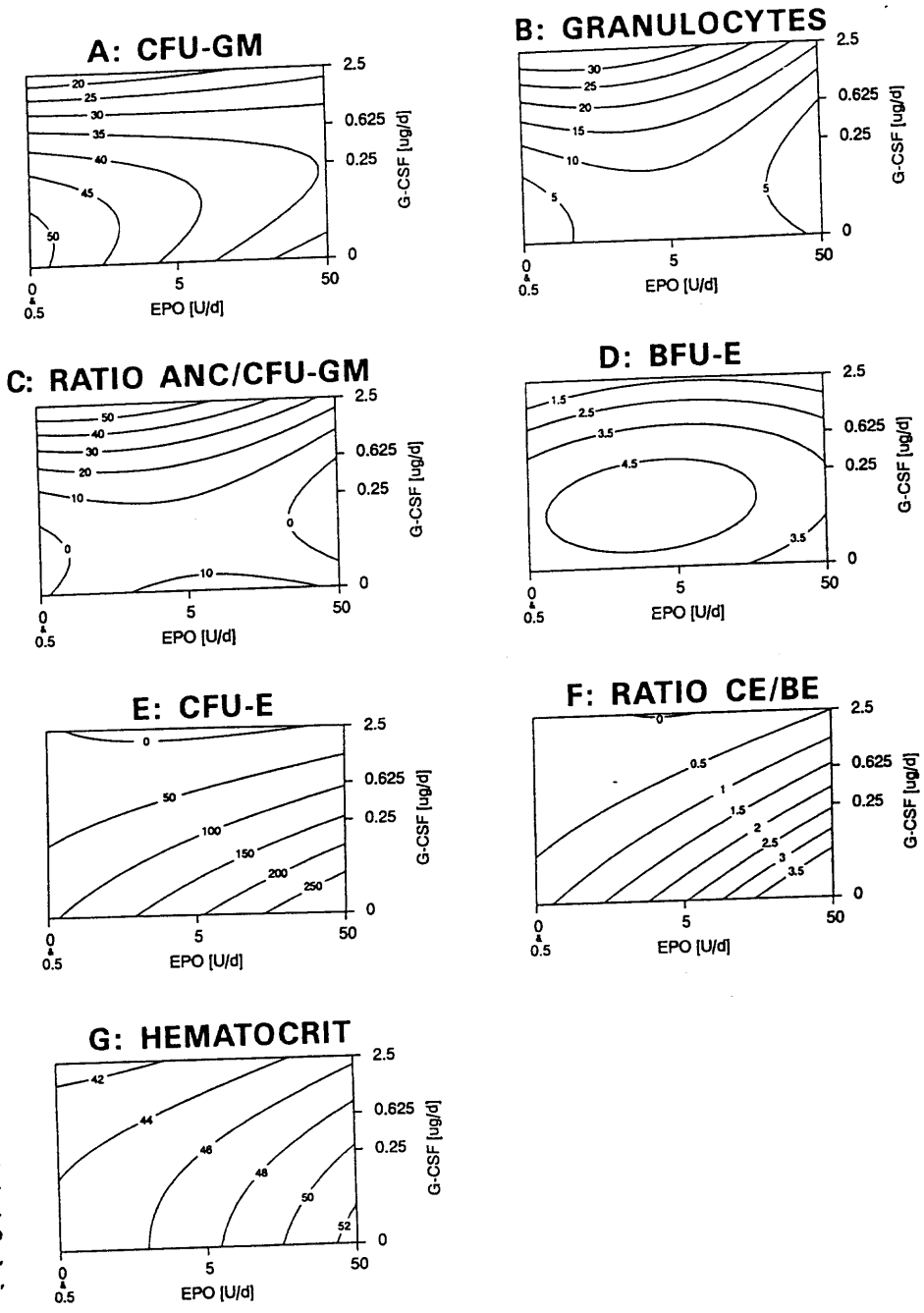

Fig 4. Equi-response curves showing the predicted behavior of CFU-GM (A), granulocytes (B), the ratio of granulocytes/CFU-GM (C), BFU-E (D), CFU-E (E), the ratio of CFU-E/BFU-E (F), and hematocrit (G) for all possible G-CSF and EPO combinations. Numbers in the figures indicate thousands/femur (A, D, and E), millions/mL (B), percentage (G), or fraction of normal (C and F).

stimulated erythropoiesis but the data also show that EPO treatment suppressed G-CSF–stimulated granulopoiesis. The inhibiting effect of G-CSF on erythropoiesis predominantly took place in the EPO-responsive cell compartment (CFU-E). The negative effects of EPO on granulopoiesis became apparent at the level of the neutrophilic granulocytes in the peripheral blood. High EPO doses reduced the efficacy of G-CSF to produce granulocytes from CFU-GM. Thus, the mutual inhibition took place at a late level of differentiation. The effects on more immature cells were less clear; CFU-GM were decreased by both EPO and G-CSF and BFU-E were stimulated by low G-CSF doses but inhibited by high doses. The reduction of these immature cell stages is probably caused by the mobilization of these cells from the marrow to the blood because both G-CSF and EPO are known to induce mobilization.[4,7,18,22] Also, this reduction will be induced by a strongly stimulated differentiation of BFU-E and CFU-GM into later cell stages.

In summary, EPO and G-CSF mutually antagonized each other, leading to intricate dose-response relationships. We were able to carefully display these interactions by plotting the equi-response curves constructed by the regression analysis. Such contour plots allow us to identify optimal strategies for blood cell production in growth factor combination treatments.

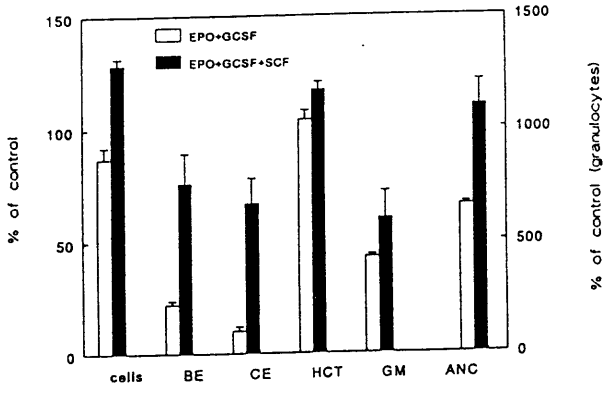

Fig 5. Effect of coadministration of SCF (2.5 μg/d) to EPO (50 U/d) and G-CSF (2.5 μg/d) treated mice. Results are given as percentage of untreated control mice (± 1 SEM). Cells, femur cellularity; BE, BFU-E; CE, CFU-E; HCT, hematocrit; GM, CFU-GM; ANC, absolute neutrophil count. Note that ANC values are given on the right Y-axis.

In a second experiment, we investigated to what extent simultaneous SCF treatment changed the observed mutual inhibition. SCF has been shown to act synergistically in vivo with both G-CSF[19] and EPO,[20] probably because it increases the input from the early compartments into the lineage-restricted cell stages. Our data show that, under high EPO and high G-CSF stimulation, SCF was capable of increasing femur cellularity and thereby all measured erythroid and granuloid cell stages. By increasing the size of the BFU-E and CFU-GM compartments, SCF administration compensated for the mutual inhibition in terms of cell production. Based on these data, we expect that the optimal production of peripheral blood cells of different lineages will be obtained under high SCF, moderate G-CSF, and moderate EPO doses. A more elaborate study to test this hypothesis is presently underway in our laboratory. The experimental design and, in particular, the statistical analysis of the results that we present here allow detailed experiments in which the effects of multiple growth factors can be assessed.

Although such extensive EPO/G-CSF combination experiments were not previously reported, there are several reports that are in agreement with our findings. In vitro EPO inhibited marrow CFU-GM colony growth.[14-16] This effect could not be reproduced in CFU-GM derived from peripheral blood.[17] Conversely, BFU-E colony growth has been reported to be inhibited by colony-stimulating factors.[15]

In vivo data have shown that G-CSF inhibits erythropoiesis.[3-7] Recently, Cronkite et al[6] have shown that EPO was not able to correct the decrease of red blood cells induced by G-CSF. EPO treatment has been reported to lead to reduced granulocyte production.[8,9] In addition, EPO administration has been associated with thrombopenia.[11] These studies provoked the question of whether competition for a common stem cell exists. However, in the present study, we did observe competition at a more mature level. This became most apparent when CFU-E/BFU-E and granulocytes/CFU-GM ratios were calculated (Fig 4C and F). A cause for the observed effects may be located at receptor level because EPO has been shown to downregulate CSF-1 receptors[23]; also, other growth factors appear to be able to modify expression of different receptors.[24] So far, long-term administration of single growth factors has never led to a depletion of stem cells.[4,19,25] Treatment with combinations of (early acting) factors may, however, affect the size of the stem cell pool. We are currently investigating the behavior of different stem cell subsets during such multifactor treatments.

REFERENCES

1. Harris PF, Harris RS, Kugler JH: Studies of the leukocyte compartment of guinea pig bone marrow after acute haemorrhage and severe hypoxia. Evidence for a common stem cell. Br J Haematol 12:419, 1966

2. Kojima S, Fukuda M, Miyajima Y, Matsuyama T, Horibe K: Treatment of aplastic anemia in children with recombinant human granulocyte colony-stimulating factor. Blood 77:937, 1991

3. Molineux G, Pojda Z, Dexter TM: A comparison of hematopoiesis in normal and splenctomized mice treated with granulocyte colony-stimulating factor. Blood 75:563, 1990

4. de Haan G, Loeffler M, Nijhof W: Long-term recombinant human granulocyte colony-stimulating factor (rhG-CSF) treatment severely depresses murine marrow erythropoiesis without causing an anemia. Exp Hematol 20:600, 1992

5. Molineux G, Dexter TM: The suppression of erythropoiesis by rhG-CSF in mice. Exp Hematol 20:268A, 1992 (abstr)

6. Cronkite EP, Burlington H, Shimosaka A, Bullis JE: Anemia induced in splenectomized mice by administration of rhG-CSF. Exp Hematol 21:319, 1993

7. Bungart B, Loeffler M, Goris H, Dontje B, Diehl V, Nijhof W: Differential effects of recombinant human colony stimulating factor (rhG-CSF) on stem cells in marrow, spleen and peripheral blood in mice. Br J Haematol 76:174, 1990

8. Christensen RD, Liechty KW, Koenig JM, Schibler KR, Ohls RK: Administration of erythropoietin to newborn rats results in diminished neutrophil production. Blood 78:1241, 1991

9. Halperin DS, Wacker P, Lacourt G, Felix M, Babel J-F, Aapro M, Wyss M: Effects of recombinant human erythropoietin in infants with the anemia of prematurity: A pilot study. J Pediatr 116:779, 1990

10. Ulich TR, del Castillo J, Yin S, Egrie JC: The erythropoietic effects of interleukin-6 and erythropoietin in vivo. Exp Hematol 19:29, 1991

11. McDonald TP, Clift RE, Cottrell MB: Large chronic doses of erythropoietin cause thrombocytopenia in mice. Blood 80:352, 1992

12. Miles SA, Mitsuyasu RT, Moreno J, Baldwin G, Kirby Alton N, Souza L, Glaspy JA: Combined therapy with recombinant granulocyte colony-stimulating factor and erythropoietin decreases hematologic toxicity from ziduvidine. Blood 77:2109, 1991

13. Koenig JM, Christensen RD: Effect of erythropoietin on granulopoiesis: In vitro and in vivo studies in weanling rats. Pediatr Res 27:583, 1990

14. Van Zant G, Goldwasser E: The simultaneous effects of erythropoietin and colony stimulating factor on bone marrow cells. Science 198:733, 1977

15. Van Zant G, Goldwasser E: Competition between erythropoietin and colony-stimulating factor for target cells in mouse marrow. Blood 53:946, 1979

16. Christensen RD, Koenig JM, Viskochil DH, Rothstein G: Downmodulation of neutrophil production by erythropoietin in human hematopoietic clones. Blood 74:817, 1989

17. Ward CS, Westwood NB, Emmerson AJB, Pearson TC: The in vitro effect of high dose recombinant human erythropoietin on granulocyte-macrophage colony production in premature infants us-

ing a defined serum deprived cell culture system. Br J Haematol 81:325, 1992

18. Nijhof W, Goris H, Dontje B, Dresz J, Loeffler M: Optimalization of murine erythroid cell production by erythropoietin occurs by exploiting the splenic microenvironment. Exp Hematol 21:496, 1993

19. Molineux G, Migdalska A, Szmitkowski M, Zsebo K, Dexter TM: The effects on hematopoiesis of recombinant stem cell factor (ligand for c-*kit*) administered in vivo to mice either alone or in combination with granulocyte colony-stimulating factor. Blood 78:961, 1991

20. de Haan G, Dontje B, Loeffler M, Nijhof W: The effects of stem cell factor on normal and erythropoietin stimulated murine hemopoiesis. Exp Hematol 21:1150a, 1993

21. Iscove NN, Sieber F: Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. Exp Hematol 3:32, 1975

22. Ganser A, Bergmann M, Voelkers B, Gruetzmacher P, Scigalla P, Hoelzer D: In vivo effects of recombinant human erythropoietin on circulating human hemopoietic progenitor cells. Exp Hematol 17:433, 1989

23. Van Zant G, Chen BD-M: Erythropoietin caused down-regulation of colony stimulating factor (CSF-1) receptors on peritoneal exudate macrophages of the mouse. J Cell Biol 97:1945, 1983

24. Walker F, Nicola NA, Metcalf D, Burgess AW: Hierarchical down-modulation of hemopoietic growth factor receptors. Cell 43:269, 1985

25. Pojda Z, Molineux G, Dexter TM: Effects of long-term in vivo treatment of mice with purified murine recombinant GM-CSF. Exp Hematol 17:1100, 1989