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Key Words. Stem cell factor • Hemopoiesis • Erythropoietin • Mathematical model

Abstract. The aim of this study was to determine how stem cell factor (SCF) modifies hemopoietic cell production. First we determined the effects of a prolonged SCF administration on murine hemopoiesis and analyzed the results by a mathematical simulation model of hemopoiesis in order to explain the data. Subsequently we investigated the effects of simultaneous coadministration of SCF + erythropoietin (Epo), to see how effects of early and late cytokines superimpose. SCF administration during 14 days induced a proliferative wave through the hemopoietic system; colony forming units-granulocytge macrophage (CFU-GM), burst forming units-erythroid (BFU-E) and colony forming units erythroid (CFU-E) were the first to be augmented, followed by their respective progeny, ultimately leading to increased blood cell numbers. Despite continued treatment most cell numbers returned to normal values in 14 days. colony forming units-spleen (CFU-S), however, remained elevated.

This wave pattern could be explained within the framework of a previously established mathematical model of hemopoiesis, if it was assumed that SCF affected the cycling status of primitive cells and if regulatory feedback loops of erythroid and myeloid progenitors on these cells were also allowed.

Simultaneous SCF and Epo administration led to synergistic effects on CFU-E numbers and hematocrit values at moderate Epo doses. At high Epo doses, however, this was less pronounced.

We conclude that SCF increases the input into committed hemopoietic lineages, where late acting cytokines can induce further amplification.

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Introduction

Recently a novel hemopoietic growth factor known as stem cell factor (SCF) [1], mast cell growth factor [2, 3] or c-*kit* ligand [4] has been identified. This protein appears to act on primitive hemopoietic cells as suggested by several observations. First, mice deficient for SCF (*Sl/Sl*^{d} mice) show hemopoietic abnormalities in all lineages which can be normalized by the administration of SCF [5]. Second, early hemopoietic cells express the receptors for SCF, c-*kit*, which more mature cells lack [6, 7]. Third, SCF synergizes in vitro with other growth factors to promote myelopoiesis [7, 8], erythropoiesis [8, 9], thrombopoiesis [10] and/or lymphopoiesis [11]. In vivo SCF has been shown to synergize with granulocyte-colony stimulating factor (G-CSF) [12, 13] and to a much lesser extent with granulocyte-macrophage CSF (GM-CSF) [13] in the production of neutrophils.

The mode of action of SCF on primitive hemopoietic cells has not yet been elucidated. Two not mutually exclusive effects can be discussed. First, the SCF-responsive cells may be triggered to multiply (self-renew) themselves, thus causing an increase of early cells. In vitro studies have shown that SCF in combination with other factors produces an increase in pluripotent cells [14–16]. In vivo, treatment with SCF of normal [12] and *Sl/Sl*^{d} [17] mice has also been shown to increase colony forming units-spleen (CFU-S) numbers. Second, SCF could trigger cells into cell cycle and thereby enhance the output of the pluripotent stem cell compartment, which would become evident simultaneously in the erythroid and myeloid cell lineage. In this respect it has

been shown that SCF treatment of normal mice induces an increase of myeloid progenitor cells, most notably in the spleen [12]. The aim of the present study was to discriminate between these possibilities. We quantified CFU-S numbers, which have been shown to express c-*kit* [18], as representatives of the multipotent compartment and the progeny of these cells in both the erythroid and myeloid lineage during a prolonged SCF administration. We subsequently analyzed the experimental data by a mathematical model of stem cell regulation which has previously been published by our group [19–23] in order to deduce statements about the presumptive mode of SCF action.

Finally, we challenged our concept of SCF action in a study where SCF was simultaneously administered with different doses of erythropoietin (Epo). The aim of this experiment was to investigate whether the increased input into the committed lineages, induced by a SCF treatment, could be utilized by a simultaneously administered late acting factor, i.e., whether effects of late and early acting cytokines can superimpose in terms of cell production. Furthermore we analyzed to what extent myeloid compartments were affected by a fully stimulated erythropoiesis. A high Epo dose (50 U/day) was chosen because this dose has previously been shown to maximally stimulate erythrocyte production [24]. Therefore the limiting factor in the production of erythrocytes during this Epo regime should be the input into the Epo-responsive compartment from earlier cell stages, which would be SCF dependent.

Materials and Methods

Mice

Female C57Bl/6 mice, between 8 and 12 weeks of age and weighing 20 to 25 grams, were used in all experiments.

SCF and Epo Administration

Recombinant rat stem cell factor (SCF164) was generously supplied by Amgen (Thousand Oaks, CA) as a solution of 1.56 mg SCF/ml phosphate buffered saline (PBS) + 0.01% bovine serum albumin (BSA). Lyophilized recombinant human erythropoietin was a gift from Boehringer Mannheim (Almere,The Netherlands) and was suspended in sterile saline. Animals (three to six per treatment group) were treated with 2.5 μg SCF/day/mouse and 5 units or 50 units Epo/day/mouse. Both growth factors were mixed in appropriate concentrations and administered by subcutaneously implanted osmotic pumps (Alzet, model 2002), which were kindly provided by Alza Corporation (Palo Alto, CA).

Cell Suspensions and Assays

Femoral and splenic single cell suspensions were made as described previously [24]. Erythroid and myeloid precursors were morphologically identified on May-Gruenwald Giemsa stained cytospin preparations. Progenitors were cultured with the methylcellulose method of *Iscove* and *Sieber* [25]. CFU-GM/BFU-E cultures were supplemented with 100 ng/ml rrSCF, 10 ng/ml rmGM-CSF (a gift from Behringwerke, Marburg, Germany) and 2 U/ml rhEpo. This resulted in optimal colony formation in our assay. CFU-E cultures were supplemented with 500 mU/ml Epo.

CFU-S day 8 numbers were determined as reported by *Till* and *McCulloch* [26].

Calculations

Total body cell numbers (bone marrow and spleen) were calculated with the assumption that femoral cellularity represents 6% of total marrow nucleated cells [19].

The data as shown in Figures 1, 2 and 3 are the mean +1 SE of three to six mice per timepoint, assayed individually. We show the number of cells in marrow and spleen and calculate significant differences of the total cell numbers (bone marrow + spleen) compared to normal mice with Student's *t*-test.

Data Analysis

The SCF and Epo combination experiments were conducted according to a 2×3 factorial design (2 doses SCF, 3 doses Epo). The appropriate statistical evaluation involves a regression analysis. This strategy is far more powerful and reliable than pairwise comparisons of particular treatment combinations. As linear dose-response relationships could not be expected on grounds of previous biological knowledge, the statistical model had to be specified in a meaningful way. The regression was performed for the log-values of each cell stage. The log-transformation was chosen for its variance stabilizing property on one side. In addition log-normal cell count distributions are biologically plausible in exponentially growing cell populations like the

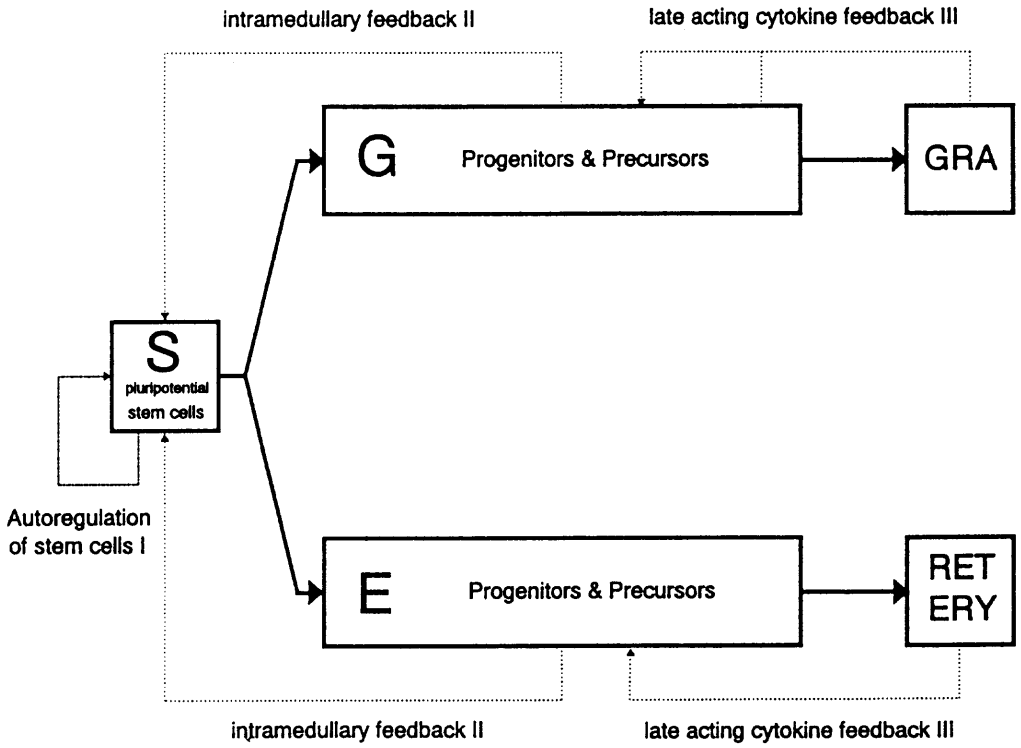

Fig. 1. Simplified structure of the simulation model. Granuloid (G) and erythroid (E) progenitors and precursors descend from a common primitive compartment (S). Mature blood cell production is regulated by three feedback loops: 1. autoregulation of primitive cells (I), 2. regulatory effects of myeloid and erythroid progenitors and precursors on primitive cells (II), and 3. effects of mature blood cells on their respective progenitors and precursors (III).

hemopoietic system. The regression models included terms for the presence of SCF (0 = absent, 1 = present) and for Epo (0 = absent, 1 = 5 U/day, 2 = 50 U/day). This coding of the

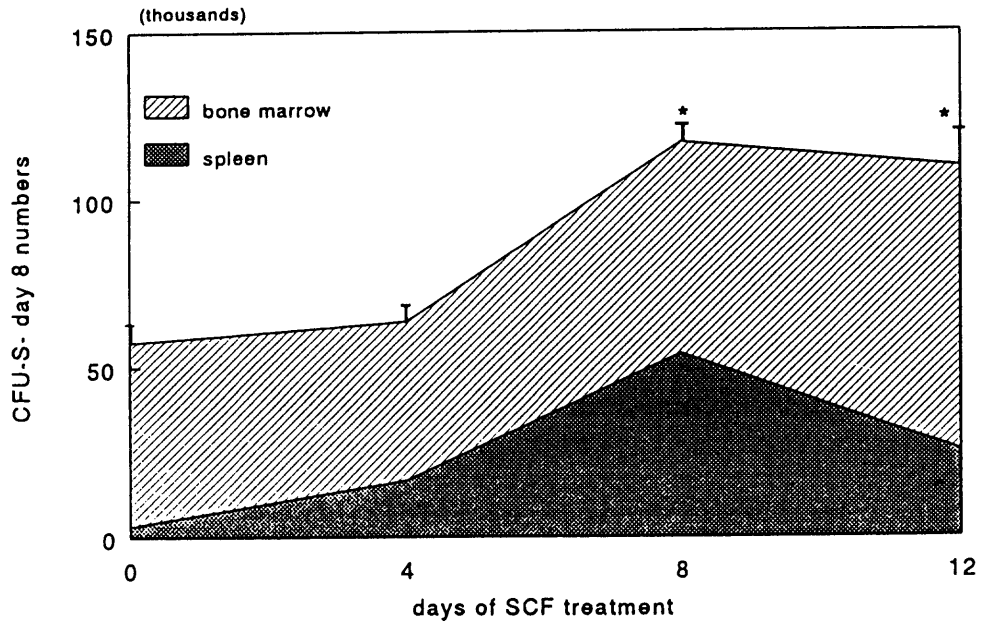

Fig. 2. Effect of SCF treatment on CFU-S day 8 numbers in marrow and spleen. Significant differences were calculated for total (marrow + spleen) CFU-S numbers with Student's *t*-test, * = $p < 0.05$.

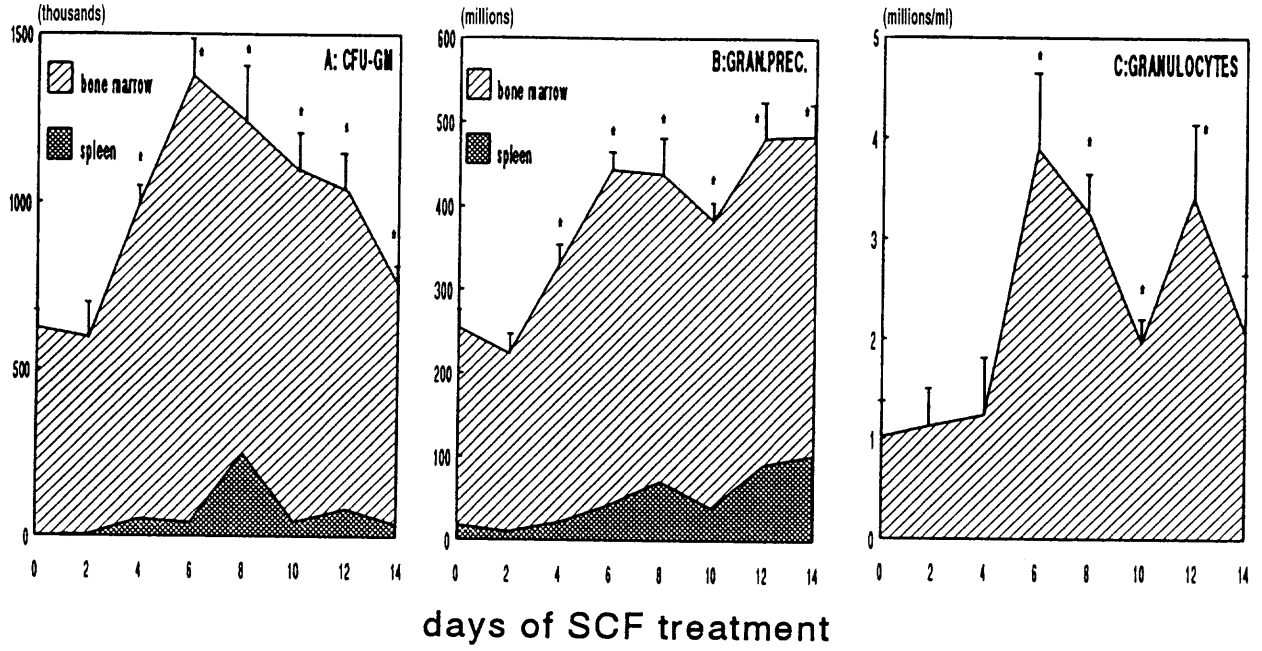

Fig. 3. Effect of SCF treatment on CFU-GM (A) and myeloid precursor (B) numbers in marrow and spleen and on neutrophilic granulocytes (C) in peripheral blood. Significant differences were calculated for total (marrow + spleen) cell numbers with Student's *t*-test, * = $p < 0.05$.

Epo dose is based on a previous experimental observation that the hemopoietic changes are of a similar magnitude in the range from 0 to 5 as from 5 to 50 U/day [24]. Furthermore the experiments reported by *Nijhof et al.* showed a saturation phenomenon with increasing Epo-doses [24]. To allow for such nonlinear dose response characteristics it was decided to incorporate all main and second order effects into the regression model. Hence we included two linear terms (Epo, SCF) and two quadratic terms for Epo (Epo × Epo) and interaction between Epo and SCF (Epo × SCF). Thus, with this analysis we were able to determine whether there existed a significant effect on different cell stages of Epo alone (Epo term) and SCF alone (SCF term). In addition we analyzed whether at high Epo doses saturation of the response took place (quadratic term for Epo) and also whether the stimulating effect of SCF on a certain cell stage was abrogated at high Epo doses (SCF × Epo interaction term). The full model was fitted to the data, and estimated regression coefficients and their standard errors are given in Table I if they were significantly different from 0 ($p < 0.05$, two-sided). All analyses were performed using SAS PROC REG software.

Simulation Model of Murine Hemopoiesis

An attempt was made to provide a systematic explanation of the dynamic hemopoietic development observed during continuous SCF administration. This was examined by formulating a specific hypothesis about the mode of SCF action within the framework of a mathematical model of murine hemopoiesis and calculating the consequences. A comparison of model calculations (i.e., simulations) and experimental data can give insight into the validity of such hypotheses.

The model used has previously been developed by our group [19–23]. The model is schematically depicted in a simplified manner in Figure 1. The model consists of different compartments, each containing cells of a defined stage of cell differentiation. The regulation of cell production is governed by three interrelated feedback loops: autoregulation of primitive cells (loop I), feedback loops from erythroid and myeloid progenitors and precursors to primitive cells (loop II), and feedback from mature (circulating) cells to their respective progenitors and precursors (loop III). The molecular mechanisms which govern these loops should be found in levels of hemopoietic growth factors, inhibitory factors, cell-cell contact, apoptosis, etc.

Table I. Statistical evaluation of SCF + Epo combination experiment (Figs. 5 and 6).

Parameter Estimates (s.e.)	a (SCF)	b (Epo)	c (Epo × Epo)	d (SCF × Epo)	Variation Explained
BFU-E spleen	1.56** (0.42)	n.s.	n.s.	–0.78* (0.32)	73%
BFU-E marrow	0.86* (0.42)	n.s.	n.s.	n.s.	36%
BFU-E total	0.99*** (0.21)	0.66* (0.30)	n.s.	–0.34* (0.16)	71%
CFU-E spleen	3.24*** (0.42)	4.0*** (0.58)	–0.51* (0.25)	–1.63*** (0.30)	94%
CFU-E marrow	n.s.	1.66*** (0.23)	–0.45** (0.11)	n.s.	92%
CFU-E total	0.71** (0.21)	1.88*** (0.29)	–0.27* (0.13)	n.s.	95%
Hematocrit	0.035* (0.015)	0.03* (0.016)	n.s.	n.s.	93%
CFU-GM spleen	3.41*** (0.36)	2.73*** (0.51)	n.s.	–1.57*** (0.27)	94%
CFU-GM marrow	0.78** (0.17)	n.s.	n.s.	–0.74*** (0.12)	78%
CFU-GM total	0.83*** (0.17)	n.s.	n.s.	–0.64*** (0.13)	72%
Myeloid precursors spleen	0.96** (0.25)	n.s.	n.s.	–0.39* (0.91)	52%
Myeloid precursors marrow	0.55*** (0.10)	n.s.	n.s.	–0.32** (0.08)	77%
Myeloid precursors total	0.59*** (0.10)	n.s.	n.s.	–0.32** (0.075)	76%

This table summarizes whether or not an effect on a certain cell stage is significant (* = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$). Nonlinear regression was performed for the log-measurements of the cell numbers: log (cell count) = constant + a × SCF + b × Epo + c × Epo^2 + d × SCF × Epo. The estimated regression coefficient for each cell stage and their standard errors (brackets) are given. The positive terms for SCF and Epo indicate the contribution of these factors to a certain cell number. A negative value for Epo^2 indicates that at high Epo concentrations saturation of the response takes place. The negative signs for SCF × Epo reflect the finding that at high Epo doses the SCF effects are diminished.

Compartments are characterized by regulated parameters like transit time, cell cycling activity, amplification coefficients and self-renewal probability. For the values and functional dependencies of these parameters we refer to previous publications [19, 20]. Of particular interest for the present study is the cycling activity (which is termed "a_s") of primitive cells (S), which is described by the formula $a_s = a_s[X(S, E, G)]$. This formula expresses that a_s depends upon the weighted sum (X) of the numbers of primitive cells (S), erythroid (E) and myeloid (G) progenitors and precursors present (for more details see chapter

4, vol. 1 [19]). The lower this sum is, the higher the value of a_s (i.e., the fewer erythroid and myeloid progenitors present, the higher the cell cycling activity of the primitive cells will be, and the other way around).

Simulation of SCF Action

Two model assumptions were made with respect to the mode of SCF action: 1) SCF has a stimulatory effect on the cell cycling of primitive cells; 2) this effect is competitive with any other stimulatory and inhibitory signal otherwise acting on the primitive cells by loop I and/or loop II. In model terms these criteria were met by modifying the formula for the cycling activity a_s by adding a constant term C_{SCF} to the argument X(S, E, G). Thus the expression for the proliferative status of the primitive cells was modified as follows: $a_s = a_s[X(S, E, G)+C_{SCF}]$. The additional stimulatory action of SCF on the primitive cell compartment is described by choosing a negative value for the constant C_{SCF} (in the simulations we used three different values to cover a reasonable range of responses: –0.8, –1.5 and –2.2), while the standard model would work with a value of 0 (i.e., no increased SCF levels). In the simulations a two-day transitory phase was used until the full values of C_{SCF} were adopted.

Results

Effects of a 14-day SCF Administration

In the time course experiment we determined the effects of SCF on different cell stages.

Effect on Marrow and Spleen Cellularity

Femur cellularity increased in time from 22.2×10^6 nucleated cells/femur at day 0 to 29.5×10^6 at day 14. Spleen cellularity rose from 89×10^6 nucleated cells/spleen at day 0 to 227×10^6 at day 14 (n = 6).

Effect on CFU-S

Total CFU-S numbers were increased to 200% at day 8 and 12 of SCF administration (Fig. 2). Between day 4 and day 8 this change was due to a strong rise of splenic CFU-S. After day 8 however splenic CFU-S decreased again, but total CFU-S numbers remained elevated due to increased marrow CFU-S.

Effects on Granulopoiesis

After two days of treatment CFU-GM numbers were significantly increased. In contrast to CFU-S, this increase was transient. After day 6 a gradual decrease was observed. Although splenic CFU-GM numbers were strongly increased, it is clear that the major part of the increase of total CFU-GM numbers is accomplished by the marrow (Fig. 3A). In contrast to CFU-GM numbers, total myeloid precursor numbers did not decrease after day 6 (Fig. 3B). The increase of immature myeloid cells was also reflected in the peripheral blood where neutrophilic granulocytes were elevated after day 4 (Fig. 3C). Whereas CFU-GM numbers and myeloid precursors were increased for the first time at day 4, the enhanced absolute neutrophil count in the peripheral blood did not become apparent until day 6.

Effects on Erythropoiesis

Total BFU-E numbers were not enhanced before day 6 (Fig. 4A). This occurred somewhat later than the increase in CFU-GM. From day 6 until day 12 BFU-E numbers were increased to 200-300%. At day 14, however, BFU-E values had returned to normal. Both marrow and spleen BFU-E contributed to the increase in total numbers. Like total BFU-E numbers, total CFU-E numbers were not enhanced before day 6 (Fig. 4B). The increase of total CFU-E to 200% at day 6 was primarily located in the spleen. Although marrow BFU-E were twofold increased, marrow CFU-E were not significantly affected by SCF treatment. On the other hand spleen BFU-E numbers were only tenfold increased but spleen CFU-E were fortyfold increased. After day 8 a gradual decrease of CFU-E numbers was observed.

Total erythroid precursor numbers were shortly increased to 150% on day 8 (Fig. 4C). BFU-E and CFU-E were increased at day 6 for the first time, erythroid precursors were significantly enhanced at day 8 and in the peripheral blood, reticulocytes peaked at day 10 (Fig. 4D). At day 14 reticulocyte values returned to a subnormal level. No hematocrit changes were observed during the treatment.

Effects of Simultaneous Administration of SCF and Epo for 6 Days

An experiment was conducted with a six-day simultaneous administration of SCF (none, 2.5 μg/mouse/day) and Epo (none, 5,

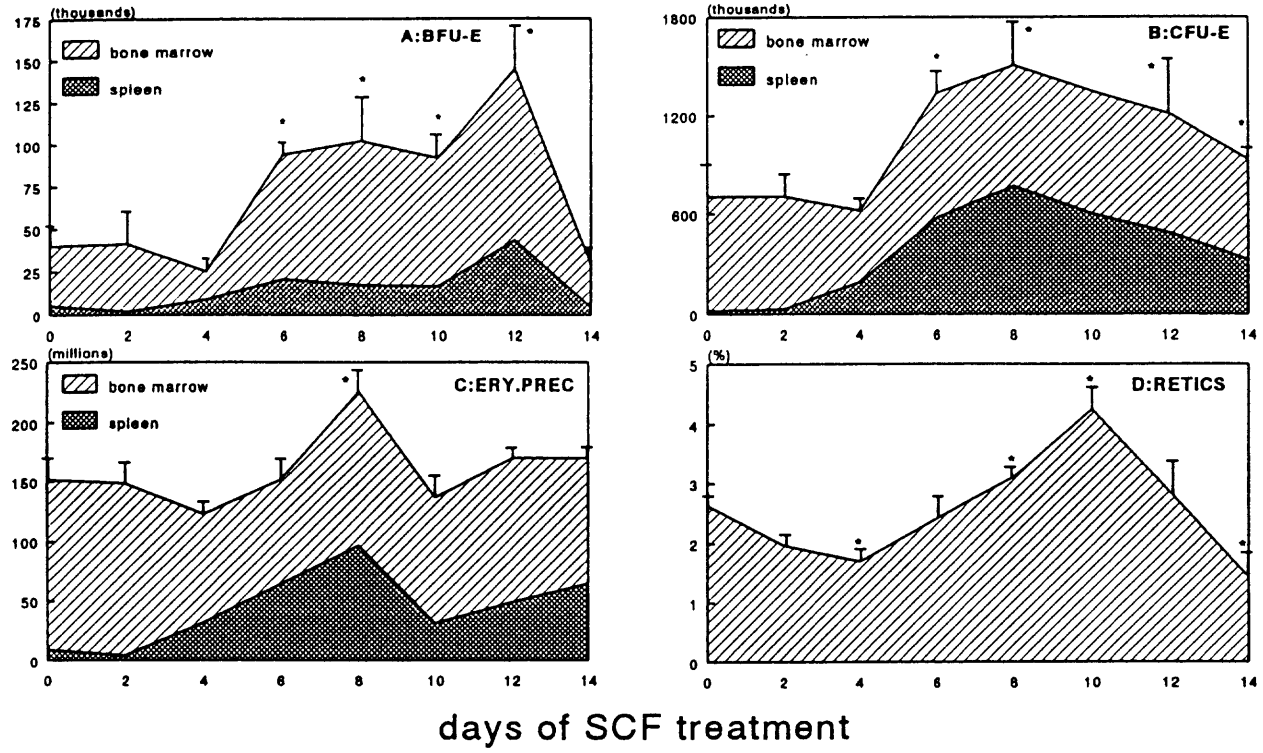

Fig. 4. Effect of SCF treatment on BFU-E (A), CFU-E (B) and erythroid precursor (C) numbers in marrow and spleen, and on reticulocytes (D) in peripheral blood. Significant differences were calculated for total (marrow + spleen) cell numbers with Student's *t*-test, * = $p < 0.05$).

50 U/mouse/day) in all six possible combinations (2 × 3 factorial design). Figure 5 shows the observations for the log values of BFU-E and CFU-E numbers in marrow, spleen and totals, as well as the hematocrit. Figure 6 shows similar data for CFU-GM numbers and myeloid precursors. The figures illustrate the dose-response relationship with respect to Epo and SCF. The statistical analysis of the data using nonlinear regression is given in Table I. For details and motivation of this statistical approach we refer to the Materials and Methods section. The analysis provides estimates of the following effects on log transformed cell numbers: linear effect of effect of SCF alone (SCF term), Epo alone (Epo term), saturation effects of Epo (Epo × Epo term) and effects of interaction of Epo and SCF (SCF × Epo term). Table I provides parameter estimates for these effects and their standard errors if they differed significantly from 0.

An example may illustrate how to read the table in conjunction with Figure 5. Splenic CFU-E (Fig. 5E) show a clear increase with increasing Epo doses, for animals treated without or with SCF (i.e., positive linear Epo effect, Table I). However, at high Epo-levels the curves tend to flatten (i.e., negative quadratic Epo effect). In addition the curves for animals receiving SCF are above those for non-SCF-treated animals (i.e., positive SCF-effect) yet this effect diminishes at high Epo levels (i.e., negative SCF-Epo interaction effect).

Effects on Erythropoiesis

SCF had an enhancing effect on BFU-E numbers in spleen and marrow (Figs. 5A, 5B). There was a tendency for Epo to increase spleen BFU-E numbers, although the changes could not be shown to be significant. The stimulating effect of SCF on total BFU-E numbers was less pronounced in combinations with high Epo doses (Fig. 5C). Epo alone had a stimulatory effect on CFU-E numbers in bone marrow and spleen with a saturation tendency at high doses (Figs. 5D, 5E). The simultaneous administration of SCF had a significant synergistic stimulatory effect (i.e., additive in the log-transformed data) on CFU-E numbers in the spleen and hence in the whole animal (Fig. 5F). This gain was again less pronounced for high Epo levels (negative SCF × Epo

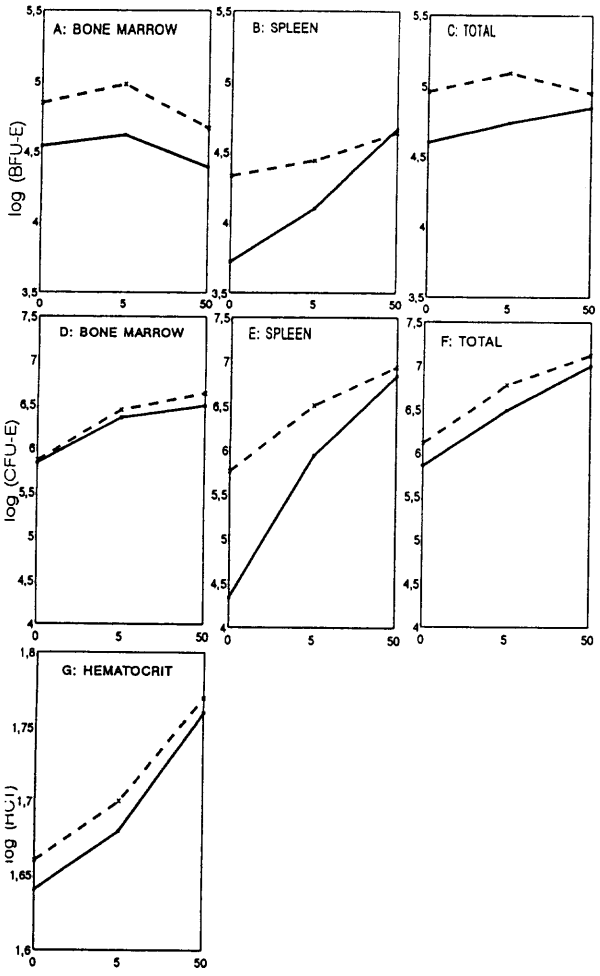

Fig. 5. Effect on marrow, spleen and total BFU-E (A, B, C) and CFU-E (D, E, F) numbers and on the hematocrit (G) of mice, treated for six days with either 0, 5 or 50 units/day Epo in combination with (broken line) or without (solid line) SCF. Data are depicted as the log values of the actual cell numbers. On the x-axis the given Epo dose is indicated. Statistical evaluation was carried out by nonlinear regression analysis. The results of this analysis are shown in Table I.

term in the spleen). Addition of SCF synergistically enhanced the effect of Epo on the hematocrit (Fig. 5G).

Effects on Granulopoiesis

SCF had a significant enhancing effect on both CFU-GM and myeloid precursors in marrow and spleen (Figs. 6A, 6B, 6D, 6E). Epo itself had no effect on granulopoiesis except for CFU-GM numbers in the spleen. It is, however, remarkable that the stimulatory activity of SCF on granulopoiesis, especially in the marrow, was Epo-dose-dependently reversed.

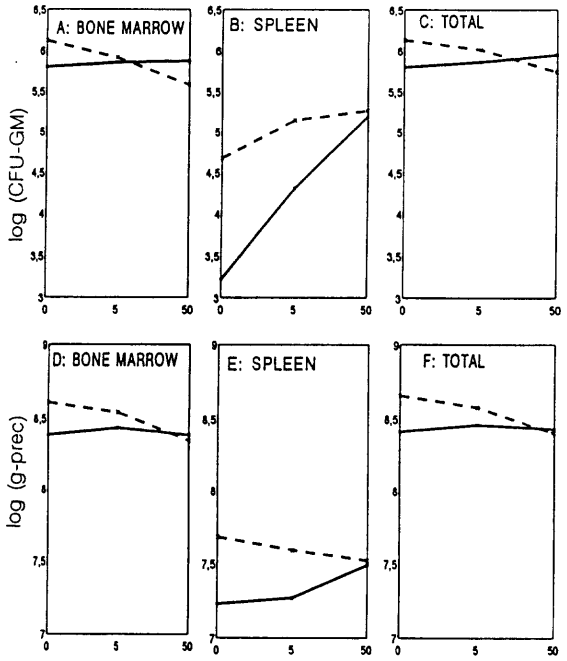

Fig. 6. Effect on marrow, spleen and total CFU-GM (A, B, C) and myeloid precursor (D, E, F) numbers of mice, treated for six days with either 0, 5 or 50 units/day Epo in combination with (broken line) or without (solid line) SCF. Data are depicted as the log values of the actual cell numbers. On the x-axis the given Epo dose is indicated. Statistical evaluation was carried out by nonlinear regression analysis. The results of this analysis are shown in Table I.

Discussion

Our results show that treatment of mice with SCF for 14 days induced a proliferative wave through the erythroid and myeloid cell lineages. The increases of the different cell stages were sequential and were occurring both in the myeloid and erythroid cell lineages to a similar order of magnitude.

In the myeloid lineage CFU-GM and myeloid precursors were increased at day 4 and granulocytes in the peripheral blood at day 6. In the erythroid lineage BFU-E and CFU-E were enhanced at day 6, erythroid precursors at day 8 and reticulocytes in the peripheral blood at day 10. There was a tendency for all these cell types to return to near normal values by day 14. Changes of CFU-S numbers, however, revealed a different pattern. This cell type was initially unaffected but increased towards the end of the treatment. *Molineux et al.* have shown that continuation of

the treatment period increased CFU-S numbers even further [12]. For all cell stages we observed a marked difference between marrow and spleen increases. This is a phenomenon we have observed previously with other growth factor administrations as well [24, 27, 28].

The mechanisms which induce the proliferative wave in the erythroid and myeloid lineages during SCF treatment are not clear. In particular the late decline under persisting SCF application deserves explanation. To obtain some insight into the possible mechanisms we explored various hypotheses about the mode of SCF action within the framework of a mathematical model of murine hemopoiesis previously developed in our group [19–23]. The structure of this model is schematically given in Figure 1 and further explained in the Materials and Methods section. The model describes the erythroid and myeloid cell development from the stage of primitive bipotent progenitor cells (CFU-S d8) to the functional end cells. A key part of the model is a concept about the network of controlling regulatory feedbacks. We assume that three regulatory processes (called feedback loops I, II and III) control hemopoietic cell production dynamically (see Materials and Methods and Fig. 1). The model has so far successfully withstood a large variety of tests and can explain a broad spectrum of experimental observations during erythropoietic manipulations (e.g., hypoxia, red cell transfusion, bleeding) as well as behavior during and following radiation and cytotoxic damage [21–23, 29]. It was therefore tempting to investigate whether a simple manipulation of the model would generate a dynamic behavior similar to the observations made during SCF application.

This is indeed the case if one accepts the following two hypotheses about the mode of SCF action: 1) SCF has stimulatory activity on the cell cycle of the primitive bipotent progenitor cells (compartment S); 2) this stimulatory activity competes with all other stimulatory and inhibitory signals otherwise acting on the primitive cells through feedback loop I and/or loop II. Figure 7 shows simulations with a range of three different stimulatory activities. The initially increased proliferative activity in compartment S (Fig. 7A) leads to an increased input into erythroid and myeloid lineages. The high numbers of erythroid and myeloid cells emerging after day 5 (Figs. 7B, 7C) lead to regulatory counteraction via feedback loop II. Inhibitory signals

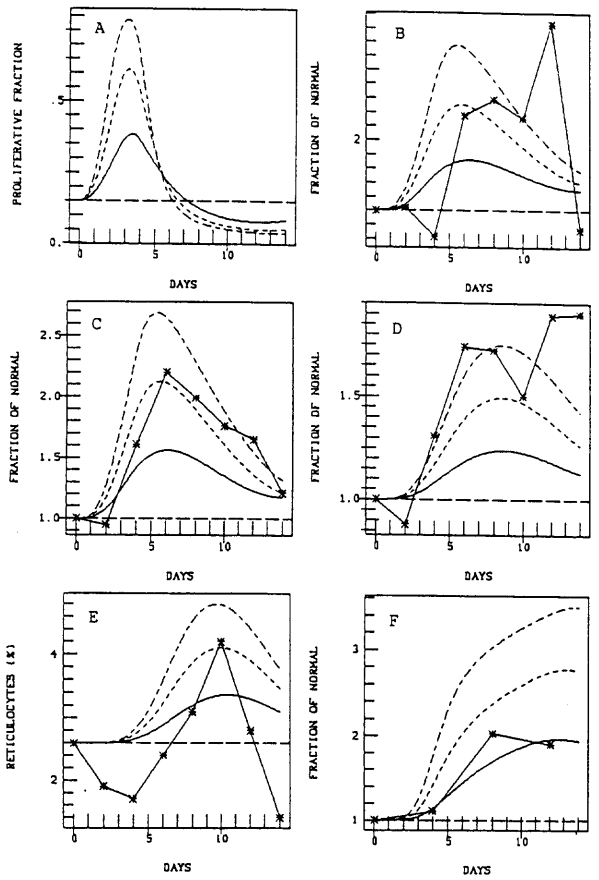

Fig. 7. Computer simulations of SCF effects in the model of erythro/granulopoiesis (for details see Material and Methods). SCF was assumed to increase the cycling activity of primitive cells (A), but normal regulatory feedback loops of erythroid and myeloid progenitors were supposed to still be present. Three different increases of cycling activity were simulated. The increased number of BFU-E (B) and CFU-GM (C), resulting from the increased cycling activity of primitive cells, in time negatively affect the cycling status of these cells (A). As a consequence the output of the primitive compartment is reduced, leading to reduced values of BFU-E, CFU-GM, myeloid precursors (D) and reticulocytes (E). In contrast, this reduced output results in increased numbers of primitive cells (F). The experimental data (reproduced from Figs. 2, 3 and 4) are also plotted to allow comparison with the simulations (*). The data are plotted as percentage of control.

then overrule the stimulatory effects of SCF, leading to a decline in cycling activity (Fig. 7A) and a concomitant increase in number (Fig. 7F) of the primitive cells. Consequently the cell production drops and the wave collapses.

The model calculations are compared with the relative changes of the total cell numbers of

BFU-E (Fig. 7B), CFU-GM (Fig. 7C), myeloid precursors (Fig. 7D), reticulocytes (Fig. 7E) and CFU-S (Fig. 7F) in the mice. The observed changes follow the general pattern exhibited by the model. The discrepancies can partly be related to experimental measurement errors and partly to oversimplifications in the model, e.g., we ignored the differences between marrow and spleen environments and the apparent shift of BFU-E and CFU-GM to the spleen and only simulated total cell numbers. We conclude that the effects observed during continuous SCF administration can be explained by a stimulation of the cycling characteristics of primitive cells at the bipotent stage. All other observations can be explained as a consequence merely reflecting the ability of the system to react dynamically to perturbations due to its regulatory feedbacks. Clearly this conclusion is model-based and requires further experimental validation. Recently *Molineux et al.* demonstrated that short-term administration of SCF, prior to a single dose of 5-fluorouracil, resulted in fatal marrow hypoplasia, which is in full agreement with our conclusion [30]. The model exercise also hints at specific targets for further research. Measurements of primitive cell stages and their cell cycle activity require careful planning as the stimulatory effect may only be restricted to a particular cell stage and interval in time before inhibitory signals overrule.

As SCF increases the input into the lineage committed compartments, it is a good candidate growth factor to be used in combination with late acting lineage specific factors. If the effects of both factors were fully independent of each other, one would expect multiplicative (i.e., synergistic) enhancement. If SCF would double the input for example in the myeloid lineage, the output in terms of production of mature neutrophils would also be doubled. If, however, SCF administration is combined with a late-acting factor, the two factors will superimpose and the output will be synergistically increased. In in vitro cultures these synergistic effects of SCF with other growth factors on cell production have been frequently reported [7, 8, 11, 14, 17]. This synergism has also been observed when mice and rats were treated with SCF and G-CSF [12, 13]. In our report we have focused on erythropoiesis, and also the combination of SCF and Epo has revealed the following features:

1. The effects of Epo alone on BFU-E, CFU-E and hematocrit were in agreement with previous knowledge [24, 31, 32]. A saturation characteristic at high Epo doses on CFU-E numbers was found. In the 6-day treatment period this was not yet reflected in hematocrit values, but it did occur in a 10-day treatment [24].

2. SCF had a strong synergistic (additive on a log-scale) enhancing effect on Epo-stimulated erythropoiesis in the spleen while this effect was less evident in marrow. Overall this led to a significant stimulating effect on the hematocrit.

3. There was a significant negative interaction of SCF and Epo for the number of BFU-E and CFU-E in the spleen. This indicates that the stimulating effect of SCF on BFU-E and CFU-E numbers in the spleen is much stronger at low Epo doses than at the high dose. Remarkably, at high Epo doses there is an inhibitory effect of Epo on almost all SCF increased myeloid cell stages, which is so strong that the gain in myeloid cell numbers obtained by SCF is completely lost.

We can conclude that the effects of SCF and low and moderate Epo doses on BFU-E and CFU-E numbers and hematocrit superimpose in a synergistical manner. At high Epo doses this synergism is less pronounced. Interestingly the stimulatory myeloid effects of SCF were abrogated by high Epo doses. The role of Epo in the regulation of granulopoiesis remains to be investigated. Preliminary model simulations show that this inhibitory effect can be explained by regulatory feedback loops already discussed above, and other inhibitory effects of stimulated erythropoiesis on granulopoiesis (paper in preparation). The potential role for SCF in clinical situations may lie in its ability to lower the effective dose of simultaneously administered late acting factors. Unwanted inhibitory effects on other lineages may so be prevented. It will be interesting to see whether the erythroid inhibition of a prolonged G-CSF treatment [27] can be prevented by a concomitant SCF administration. Further experimental study is needed, however, before clinically meaningful trials can be conducted.

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