Prophylactic Pretreatment of Mice With Hematopoietic Growth Factors Induces Expansion of Primitive Cell Compartments and Results in Protection Against 5-Fluorouracil–Induced Toxicity

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The aim of this study was to expand the primitive and committed hematopoietic cell compartments in vivo in order to confer resistance of the blood cell forming system against the cytotoxic, cell cycle specific drug 5-fluorouracil (5-FU). Possible chemoprotective effects of such a pretreatment could result from increased numbers of hematopoietic cells, present before 5-FU administration. In addition, we hypothesized that an enhanced number of primitive and progenitor cells would result in a reduced cycling activity, ie, 5-FU sensitivity, of these same cells, due to normal physiological feedback loops. Administration of stem cell factor (SCF) plus interleukin-11 (IL-11) to mice was shown to result in expansion of the various immature cell compartments in marrow and, in particular, spleen. The total body content of the primitive cobblestone area forming cells (CAFC)-day 28 was increased to 140%, whereas the more committed cells (CAFC-day 7, erythroid and granuloid progenitors) were increased to 500%. This in vivo expansion resulted in a decreased 5-FU sensitivity of the hematopoietic system. In particular, mice that had received 5-FU 24 hours after discontinuation of growth factor pretreatment showed significantly less toxicity of committed cell stages. Compared with mice not pretreated, it appeared that in pretreated mice, 24 hours after 5-FU administration, the absolute number, but also the fraction of surviving CAFC, was much higher in both marrow and spleen. This was caused by a decrease in the cycling activity of all primitive cell subsets. To explore the possible use of this finding in a chemotherapeutic setting, we determined the interval between two subsequent doses of 5-FU (160 mg/kg) that was required to prevent drug-induced mortality. When control mice received a second dose of 5-FU 7, 10, or 14 days after the first, respectively 0%, 20%, and 80% survived. In contrast, 40% and 100% of mice that received SCF + IL-11 before the first dose of 5-FU, survived a second dose of 5-FU given respectively after 7 or 10 days. To assess whether chemoprotection in this setting could be ascribed to protection of the hematopoietic system, we transplanted a high number of normal bone marrow cells (sufficient to compensate for any hematopoietic deficiency) to normal and pretreated mice after they had been administered 2 doses of 5-FU, given 7 days apart. Bone marrow transplantation (BMT) could only rescue 50% of mice not pretreated, showing that a significant part of the mortality was because of nonhematologic toxicity. However, a BMT given to growth factor pretreated mice saved all mice, indicating that in this setting SCF + IL-11 had additional protective effects on cell systems other than hematopoiesis. In conclusion, our study showed fundamental knowledge about the behavior of primitive cells in vivo and has shown that manipulation of these and other cell compartments with appropriate growth factors may confer resistance against cytotoxic drugs.
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HEMATOLOGIC CYTOPENIAS that result from chemotherapy or irradiation belong to the major dose-limiting factors of tumor therapy. Reduction of cytopenic periods may lead to escalation of the dose of cytotoxic treatment, which should result in increased tumor cell kill. It is generally accepted that several hematopoietic growth factors, when administered to patients, have the potency to shorten these cytopenic intervals. Therefore, numerous clinical trials are currently assessing the efficacy of these growth factors. In almost all of these trials, the effect of cytokines administered after cytotoxic treatment is determined. The obvious aim is to fasten the recovery of peripheral blood (PB) cells from surviving hematopoietic stem and progenitors cells. It is evident that it would be more beneficial if growth factors could be used to prepare patients for chemotherapy to increase the number of surviving stem cells, rather than using these factors only after the damage has been imposed.

In the present study, we wanted to pursue such a prophylactic strategy to reduce hemotoxicity. It has been shown in mice that reduction of cycling activity of hematopoietic progenitors by inhibitory molecules, like the tetrapeptide AcSerAspLysPro, macrophage inflammatory protein-1α, or transforming growth factor-β, results in improved hematologic recovery and survival, after administration of cytotoxic drugs.[1-4] In the experiments described in this report, we wanted to achieve similar chemoprotective effects by pretreating mice with early acting growth factors before they were treated with 5-fluorouracil (5-FU). The aim of this pretreatment was dual: first, the primitive and progenitor cell compartments were to be expanded, which by itself, might already result in more cells surviving 5-FU administration. In addition, we assumed that expansion of these cell compartments in vivo would result in a reduced cycling activity of these same cells. We favor the hypothesis that normal hematopoietic cell production is partly regulated by feedback loops from the various stem cell and immature erythroid and granuloid cell compartments to their immediate ancestors. Thus, increased numbers of committed progenitors would have a negative effect on the cycling activity of more primitive cells. Or conversely, as shown by Harrison et al,[5] a reduction of progenitor cells (eg, as a result of administration of 5-FU) would rapidly increase the cycling activity of primitive cells. This idea has been incorporated in a mathematical

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computer simulation model of murine hematopoiesis, which was previously developed in our group.[6] With this model and with the assumptions of the above mentioned feedback loops, we have been able to simulate data on the effects of the cytostatic drug thiamphenicol[7,8] and of prolonged stem cell factor (SCF) administration to mice.[9] In the present study we wanted to further investigate and exploit the existence of these hypothetical feedback loops.

In contrast to the in vivo expansion of mature PB cells, which can readily be induced with the administration of the various lineage-specific growth factors, in vivo expansion of the primitive cell compartments is more difficult to achieve. In murine studies, it has recently been shown that it cannot be obtained by infusing high doses of bone marrow (BM) cells in normal nonmyeloablated recipients. Although this strategy results in significant engraftment of donor cells, this is established by a replacement of host cells and does not result in expansion.[10] Therefore, in the present study, we wanted to induce expansion by administration of the early acting stromal cell-derived growth factors SCF and interleukin-11 (IL-11). Both cytokines as single factors have been shown to stimulate the production of immature cells in vitro and in vivo[11-15] and thus were potential candidate factors to combine. Furthermore, in vitro studies have shown that SCF + IL-11 in combination act synergistically on the expansion of progenitor cell numbers.[16] Using this approach, we were able to induce in vivo primitive cell expansion, which led to significant protection of the various primitive cell stages against the cytotoxic drug, 5-FU.

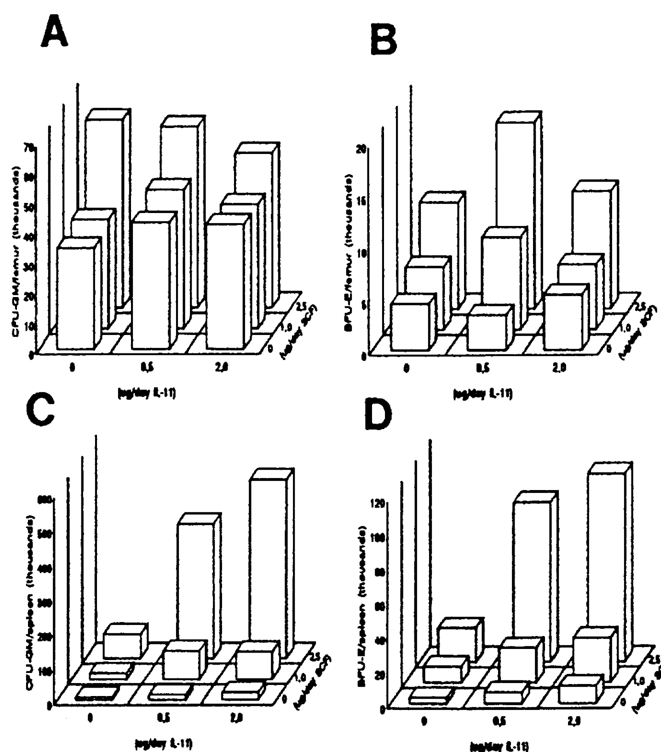

Fig 1. Effect of treatment of mice for 7 days with varying IL-11 plus SCF dose combinations on the number of CFU-GM/femur (A), BFU-E/femur (B), CFU-GM/spleen (C), and BFU-E/spleen (D).

MATERIALS AND METHODS

Treatment of mice. Female C57Bl/6 mice, 12 to 16 weeks old, weighing 20 to 25 g were used. Recombinant rat pegylated SCF was donated by Amgen (Thousand Oaks, CA). Recombinant human IL-11 was a gift from Genetics Institute (Cambridge, MA). Growth factors were appropriately diluted, mixed, and administered by subcutaneously implanted osmotic mini pumps (type Alzet 1007D; Alza Corp, Palo Alto, CA) to avoid high variation in serum cytokine levels. In an initial dose finding study, mice were treated for 7 days with 0, 0.5, or 2.0 μg IL-11/d in combination with 0, 1, or 2.5 μg SCF/d.

Hematological recovery after a single dose of 5-FU. Mice were divided in three groups: A, B, and C. Mice in group A received only 5-FU (Fluracedyl; Pharmachemie B.V., Haarlem, The Netherlands) as an intraperitoneal injection, at a dose of 160 mg/kg. Mice in groups B and C were pretreated with 2.0 μg IL-11 plus 2.5 μg SCF for 7 days. Osmotic pumps were removed at day 7 and immediately thereafter, 5-FU was administered in group B and after 24 hours, at day 8, in group C (see Fig 3).

Determination of time interval between two doses of 5-FU required to prevent drug-induced lethality. The drug was administered twice, at a dose of 160 mg/kg intraperitoneally, 3, 7, 10, 12, or 14 days apart. SCF + IL-11 pretreatment was given only before the first dose of 5-FU (see Fig 9). Survival was monitored for 20 days. To assess to what extent lethality was caused by hemotoxicity, in another set of experiments survival was monitored in pretreated and control mice that received two doses of 5-FU (160 mg/kg) 7 days apart, and additionally received a BM transplantation (BMT) after the second dose of 5-FU. These mice were transplanted 4, 24, and 48 hours after the second dose of 5-FU, with 1 to 1.5 $\times$ 10^7 syngeneic BM cells at each day (according to the protocol of Damia et al[17]). This cell dose has been shown to compensate for hematopoietic defiencies.[17]

Cobblestone area forming cell-assay (CAFC). The CAFC assay was essentially performed as described by Ploemacher et al.[18] This assay is based on a limiting dilution type long-term BM culture. A stromal cell layer was grown in 96-wells-microtiter plates (Costar Europe Ltd, Badhoevedorp, The Netherlands) in Dulbecco's modified Eagle's medium (DMEM) (GIBCO Nederland, Breda, The Netherlands), 10% fetal calf serum (FCS), 5% horse serum, 10^{-5} mol/L hydrocortison, 3.3 mmol/L L-glutamine, 80 U/mL penicillin, 80 μg/mL streptomycin, 10^{-4} mol/L β-mercaptoethanol, 10 mmol/L HEPES, and 25 mmol/L $NaHCO_3$. Instead of using fresh marrow cells as a source of the stromal layer, we used FBMD-1 cells (a preadipocyte cell line, derived from C57B1/6 mice), which have been reported by Neben et al[16] to result in similar CAFC-frequencies as fresh marrow. Stromal cell layers were allowed to grow confluently for 14 days. Cell layers were then overlaid with BM or spleen cells in six dilutions, each dilution threefold apart. For the CAFC assay the medium was switched to 20% horse serum. All marrow cell suspensions and SCF + IL-11 spleen cell suspensions were overlaid in 81000 → 27000 → 9000 → 3000 → 1000 → 333 cells/well dilution series. Normal and post−5-FU spleen cells were overlaid in a 729000 → 243000 → 81000 → 27000 → 9000 → 3000 dilution serie. SCF + IL-11/post−5-FU spleen cells were used in a 243000 → 81000 → 27000 → 9000 → 3000 → 1000 serie. Each dilution was plated 15-fold. Twice a week half of the medium in a well was replaced with fresh medium. To assay the entire stem cell spectrum, the appearance of cobblestone areas (colonies of at least five small nonrefractile cells, growing underneath the stromal layer) was evaluated at weekly intervals, during 4 weeks. It has been extensively described that the frequency of CAFC-day 7 exclusively correlates with colony-forming unit-granulocyte (erythrocyte) macrophage/colony-forming unit-spleen (CFU-G[E]M/CFU-S)−day 7,

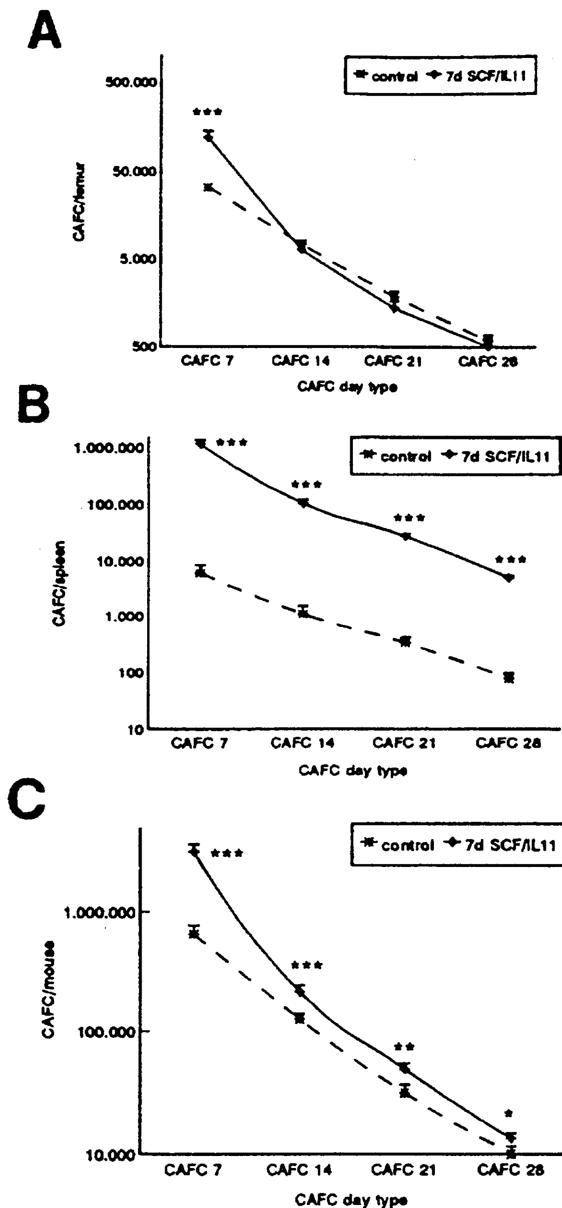

Fig 2. Effect of treatment of mice for 7 days with 2.5 μg SCF/d + 2.0 μg IL-11/d on the number of CAFC subsets in marrow (A), spleen (B), and total mouse (C). * = $P < .05$, ** = $P < .025$, *** = $P < .01$, different from control mice.

CAFC-day 14 correspond to CFU-S–day 12, and CAFC-day 28 coincide with cells that have long-term repopulating ability (LTRA).[18-22] The limiting dilution analysis to determine the actual CAFC frequency was performed as described by Ploemacher et al.[18] In short, individual wells were scored for the presence or absence of a cobblestone area. The percentage of negative wells as a function of the number of cells/well overlaid was used to calculate the absolute frequency of the various stem cell subsets, using the maximum likelihood solution.

Determination of cycling activity of CAFC subsets. The cycling activity of the CAFC subsets was determined with the in vitro hydroxyurea method, as described by Wierenga et al.[23] BM and spleen cell suspensions were diluted in DMEM in the presence of 10% FCS, to 1 and 2×10^7 nucleated cells/mL, respectively. Hydroxyurea (200 μg/mL; Sigma, St Louis, MO) was then added (a total volume of 10 μL), and cells were incubated for 60 minutes in 37°C. Cells were then washed twice, counted, and the appropriate cell suspension was made.

Progenitor cell assays. CFU-GM/burst-forming unit-erythroid (BFU-E) were grown in α-medium (Life Technologies Ltd, Paisley, Scotland, UK) in 1.2% methylcellulose, supplemented with 10 mmol/L HEPES, 25 mmol/L $NaHCO_3$, and 30% FCS. CFU-GM/BFU-E cultures were stimulated with 100 ng/mL rrSCF, 10 ng/mL rmGM-CSF (supplied by Behringwerke, Marburg, Germany) and 2 U/mL erythropoietin (EPO) (a gift from Boehringer Mannheim, Almere, The Netherlands). These culturing conditions resulted in optimal colony growth.

PB cell stages. The percentage of reticulocytes was microscopically determined after staining the cells with brilliant-cresyl-blue. A minimum of 500 cells was evaluated. The hematocrit was determined with hematocrit capillaries according to standard procedures. Neutrophilic granulocytes were quantified on May-Grünwald-Giemsa stained thin blood films.

Statistics. Results are given as the mean ± 1 standard error of mean (SEM). Significant differences between the various groups were determined with Wilcoxon's test. Each data point shown in Figs 1 through 8 was obtained by analyzing individually three to six mice per group. Survival experiments (Figs 10 and 11) were performed with six to 12 mice per group.

RESULTS

Effects of various SCF + IL-11 dose combinations on expansion of progenitors and CAFC subsets in femur and spleen: Dose finding study. In an initial experiment, it was determined which SCF + IL-11 combination resulted in increased progenitor cell numbers. Figures 1A, B, C, and D show how the number of CFU-GM and BFU-E could be effectively increased in femur and particularly in spleen, when mice were treated for 7 days with various combinations of SCF and IL-11. For further studies, we selected the combination of 2.5 μg SCF + 2.0 μg IL-11, as this induced highly increased cell numbers. Figure 2 shows how the number of the various CAFC subsets were increased after this treatment. In the femur, the most committed CAFC-day 7 were enhanced (fivefold) (Fig 2A). More primitive cell stages were not affected by the treatment. In the spleen, all CAFC subsets were increased about 100-fold (Fig 2B). Figure 2C shows the numbers of each CAFC subset per total mouse (marrow + spleen). For this calculation, we have assumed that one femur represents 6% of total marrow.[24] Total CAFC-day 7 were expanded fivefold, CAFC-day 14, twofold, and CAFC-day 21, 1.6-fold, and 28 increased 1.4-fold.

Effects of SCF + IL-11 pretreatment on hematologic recovery after a single dose of 5-FU. Three groups of mice were treated with 5-FU (160 mg/kg) (Fig 3). Group A served as a control group. Group B was pretreated for 7 days with SCF + IL-11 and received 5-FU at day 7, immediately after the pretreatment. Group C was similarly pretreated, but received 5-FU at day 8, thus 1 day after growth factor treatment. The recovery of all three groups was followed during 10 days. Figures 4A and B show the number of CFU-GM per femur and spleen, respectively. In both organs, a beneficial effect of the pretreatment was observed. Mice in group C had by far the least profound nadir, mice in group B were intermediate, and control mice (A) behaved worst. Mice in group C also showed the highest number of circulating neutrophilic granulocytes in the PB 10 days after 5-FU, approxi-

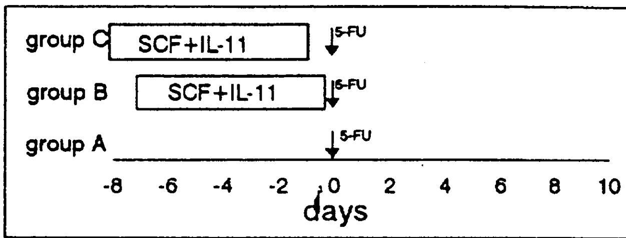

Fig 3. Schematic representation of the treatment protocols. (A) 5-FU administered to normal mice, (B) 5-FU administered immediately after SCF + IL-11 pretreatment, (C) 5-FU administered 24 hours after pretreatment with SCF + IL-11.

mately 10-fold higher than mice in group A (Fig 5A). No significant difference in absolute neutrophil count could be detected between groups A and B at day 10 (Fig 5A), which is in accordance with the number of CFU-GM present in the femur at day 7 and was comparable in group A and B. Figures 5B and C show the values for reticulocytes and hematocrit after 10 days of recovery. At day 7, no reticulocytes were detected in either group (data not shown). At day 10, mice in group A and B had similar reticulocyte values, still below control. However, mice in group C not only had significantly more reticulocytes than groups A and B, but also more than untreated control mice. At day 7, the mean hematocrit value for all three groups was 36% (data not shown). At day 10, however, the hematocrit in both groups A and B had dropped to 30% (Fig 5C). Mice in group C, which also showed highest reticulocyte values, had hematocrits significantly higher than mice in groups A and B (37%).

Acute stem cell toxicity after 5-FU. To be able to interpret these data, we next wanted to assess the effects of SCF + IL-11 in conjunction with 5-FU on the more primitive cell compartments. In these and following experiments, we only evaluated effects of the most beneficial treatment proto-

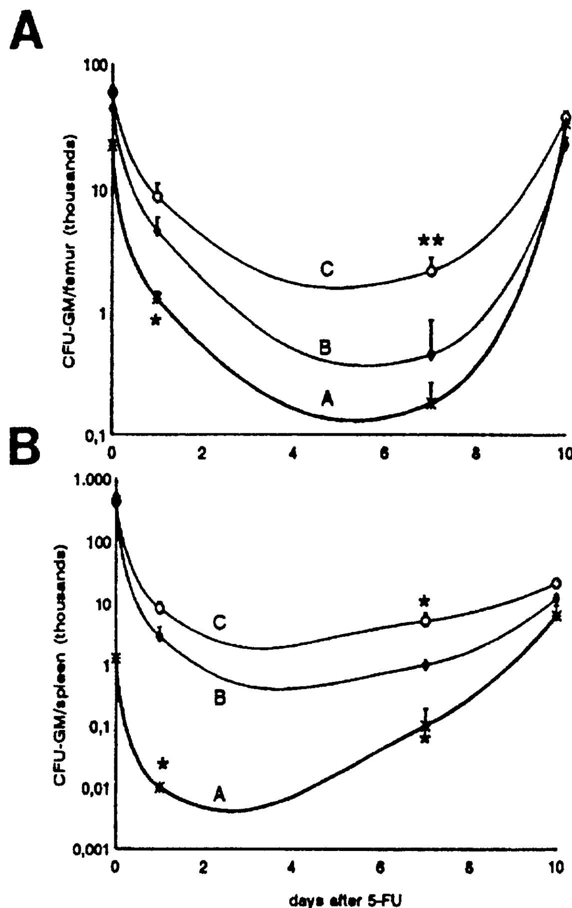

Fig 4. Effect of pretreatment on CFU-GM recovery in femur (A) and spleen (B) after 5-FU administration on day 0. A = control mice, B = SCF + IL-11 from day -7 to day 0, C = SCF + IL-11 from day -8 to day -1. * = $P < .05$, ** = $P < .025$. Levels of significance indicate whether at a certain time point A was different from B, or C was different from B.

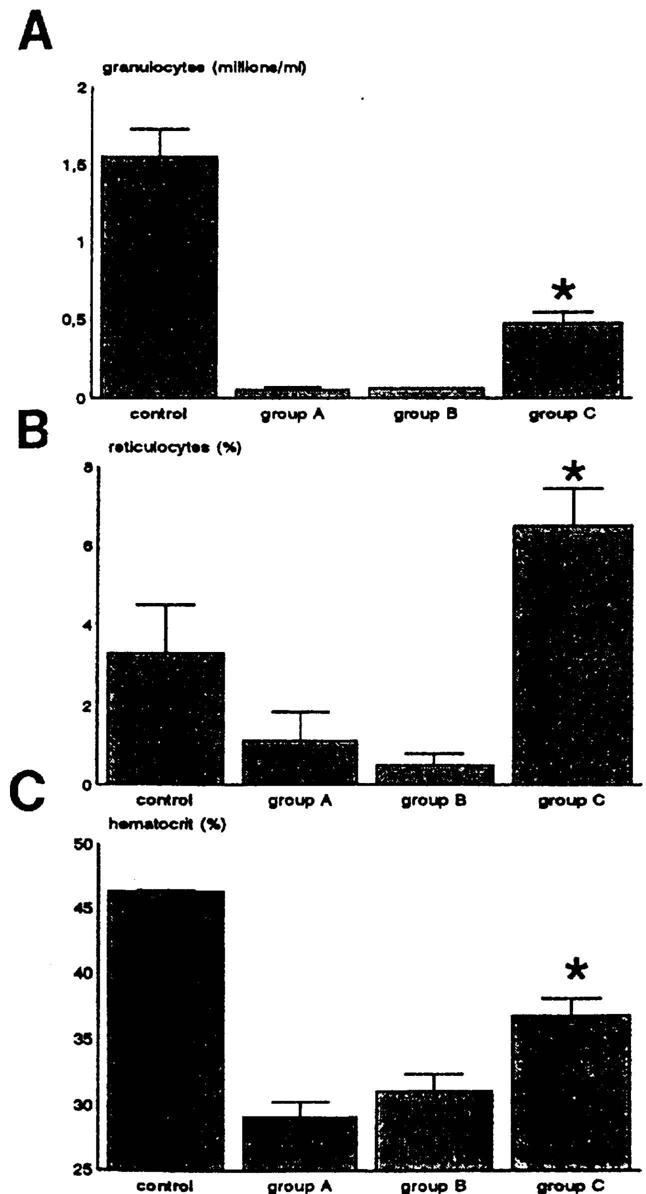

Fig 5. Effects of pretreatment on peripheral blood values 10 days after 5-FU administration. For further details, see Fig 4. For comparison, control values of untreated mice are shown.

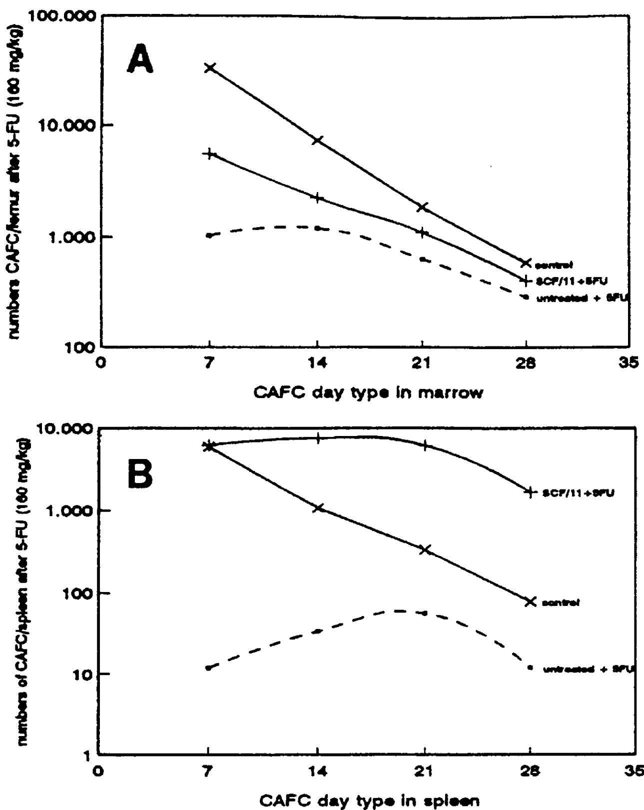

Fig 6. Numbers of surviving CAFC subsets in marrow (A) and spleen (B) in untreated or SCF + IL-11 pretreated (treatment protocol C) mice, 24 hours after 5-FU administration. For comparison, values for control mice (no 5-FU) are plotted.

col C (Fig 3). Pretreated and control mice were treated with 5-FU (160 mg/kg), and 24 hours later femur and spleen were assessed for surviving CAFC subsets. The results of this experiment are given in Figs 6 and 7. Figure 6 shows the absolute numbers of the various CAFC subsets in femur (A) and spleen (B) that survived 5-FU treatment. For comparison values, mice that were not 5-FU treated are also plotted. Figure 7 shows the same data, but now plotted as the surviving fraction, allowing a comparison between the absolute numbers of CAFC present after 5-FU administration and their relative 5-FU sensitivity.

As expected, administration of 5-FU to control mice resulted in an almost complete elimination of the most committed (CAFC-day 7 and 14) cells (Fig 6A). In agreement with literature data,[20,25] the more primitive CAFC-day 21 and 28 were increasingly less vulnerable to the drug (Figs 6A and 7). Interestingly, all splenic stem cell subsets were approximately 10-fold more sensitive to 5-FU as their marrow counterparts. In the marrow, pretreatment with SCF + IL-11 resulted in more surviving cells of all CAFC subsets (Fig 6A). When the surviving fraction of these cells was calculated, it was demonstrated that all cells had become less sensitive to 5-FU (Fig 7). In the spleen, pretreatment had even more drastic effects; the spleens of pre- and 5-FU treated mice contained far more, in particular primitive, CAFCs than 5-FU treated control mice or even untreated normal animals (Fig 6B). Again, also in this organ, it became apparent that all cell subsets became less sensitive to 5-FU after growth factor pretreatment (Fig 7).

Effects of SCF + IL-11 pretreatment on cycling activity of various CAFC subsets. To explain the apparent decreased 5-FU sensitivity of the various CAFC cell subsets, the cycling activity (% hydroxyurea kill) of these cells was assessed 24 hours after the SCF + IL-11 pretreatment (Fig 8). Both in marrow and in spleen, the cycling activity of particularly the CAFC-day 7 and 14 was decreased, compared with normal control mice.

Effects of SCF + IL-11 pretreatment on the time interval between two doses of 5-FU required to prevent mortality. In an attempt to verify whether 5-FU dose escalation could be achieved by the SCF + IL-11 pretreatment, we determined at which day a second high dose (160 mg/kg) of 5-FU could be administered after a first one, without resulting in drug-induced mortality. The experimental design is shown in Fig 9. The first dose of 5-FU was administered to normal or pretreated mice at day 0. A second equal dose of 5-FU was then administered at day 3, 7, 10, 12, or 14, and survival was monitored during 20 days (Fig 10). When the interval between two doses was 3 or 7 days, all control mice died. In the SCF + IL-11 pretreated group, however, 40% of the mice survived two doses of 5-FU given 7 days apart. Similarly, when an interval of 10 days was choosen, only 20% of the control mice survived, while 100% of the pretreated mice survived. Even at an interval of 14 days, some mortality was observed in the control group. Overall, pretreated mice could be treated 4 to 5 days earlier than control mice. In addition, it should be noted that when mortality was observed in the pretreated group, this occurred later than in control mice (data not shown).

To assess whether the observed decrease in mortality could be attributed to reduced hemotoxicity, we transplanted pretreated and control mice that were injected with two doses of 5-FU, given 7 days apart, with high doses of normal BM cells (Fig 11). For comparison, survival data for nontransplanted control and pretreated mice are also plotted. First, it

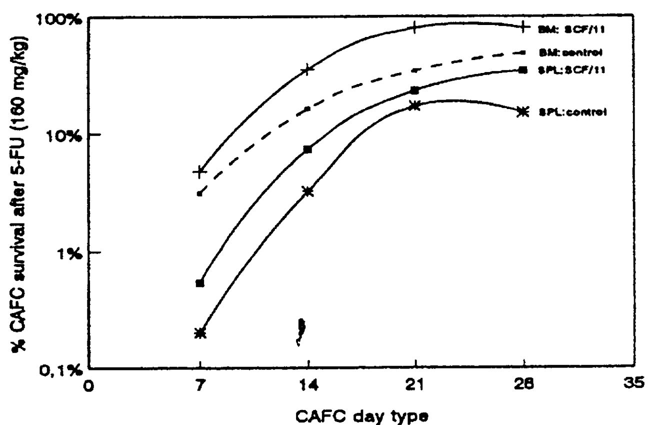

Fig 7. Fraction of surviving CAFC subsets in marrow and spleen in untreated or SCF + IL-11 pretreated mice, 24 hours after 5-FU administration. Data are expressed as percent of pre-5-FU cell numbers, ie, as percent of untreated control mice (for controls not pretreated), or as percent of 7 days SCF + IL-11 treated mice (for pretreated mice).

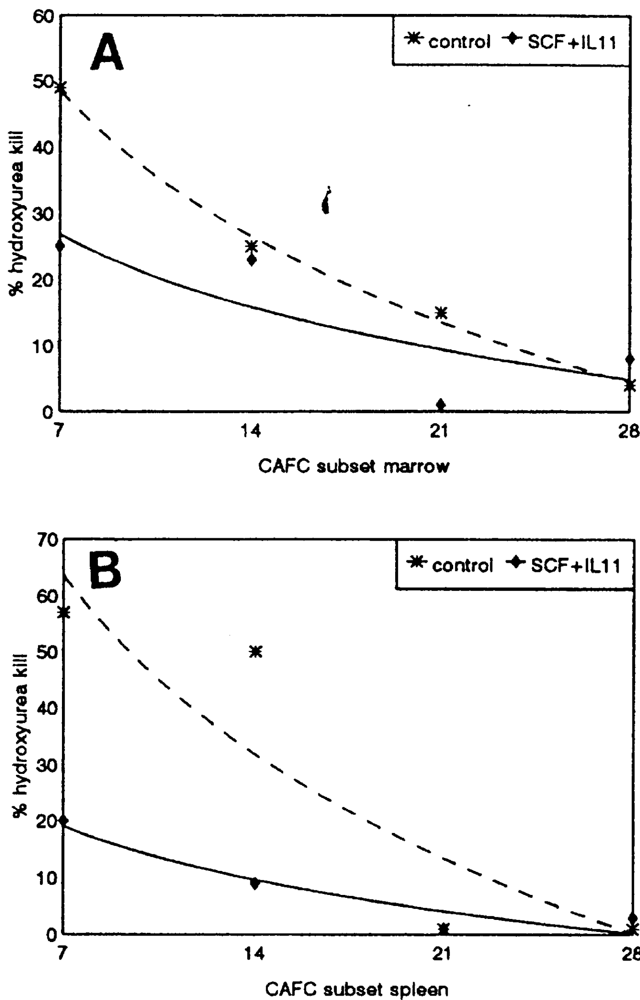

Fig 8. Effect of SCF + IL-11 pretreatment on the cycling activity of the various CAFC subsets in femur (A) and spleen (B), assayed 24 hours after SCF + IL-11 treatment. The cycling activity is given as the fraction of cells killed by in vitro hydroxyurea incubation.

became evident that in the group of control mice that received high-dose BMT, still significant mortality was observed (50%). Survival in this BMT group appeared to be similar to the SCF + IL-11 pretreatment group that did not receive a BMT. However, all mice survived when they were prophylactically pretreated with SCF + IL-11 and additionally received a BMT after the second dose of 5-FU.

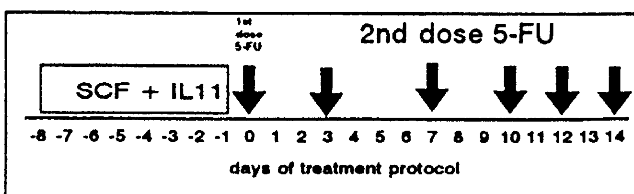

Fig 9. Schematic representation of the treatment protocol. Mice were either not pretreated or treated with SCF + IL-11 from days -8 to -1. At day 0, 5-FU (160 mg/kg) was injected. A subsequent equal 5-FU dose was administered at the various time points indicated.

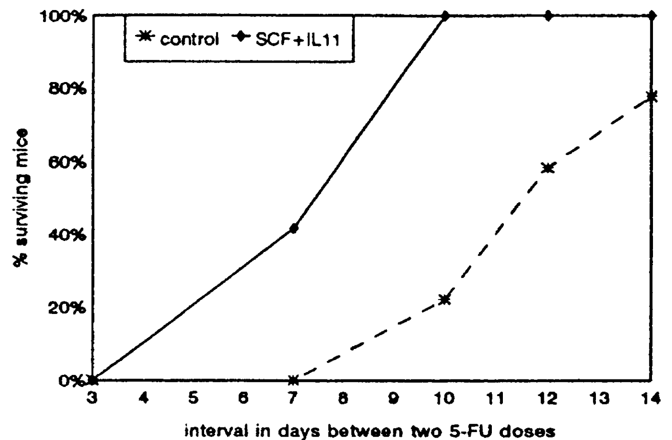

Fig 10. Effect of SCF + IL-11 pretreatment on the interval between two doses of 5-FU (160 mg/kg) required to prevent drug-induced mortality. Survival was monitored for at least 20 days after the second dose of 5-FU.

DISCUSSION

In this study, we aimed to test and exploit the hypothesis that increased numbers of hematopoietic primitive and progenitor cells negatively affect the cycling activity of these cell compartments as an integral part of feedback loops regulating normal hematopoietic cell production. In addition we wanted to show that there may be a novel concept (besides the accepted one of use of growth factors after chemotherapy) of prophylactic cytokine treatment to shorten cytopenic periods. Our strategy was to induce in vivo expansion of the primitive and progenitor cell compartments by prolonged administration of an effective combination of two cytokines, SCF and IL-11, each of which has been shown to stimulate primitive cell stages.[11-15] In combination these two cytokines have been demonstrated to expand progenitor cell numbers in vitro.[16,26] In our study, we subsequently abrogated this stimulatory treatment, allowing the physiological regulatory mechanisms to operate. The effect of growth factor therapy on cycling activity and in vivo 5-FU sensitivity of the various cell types was determined.

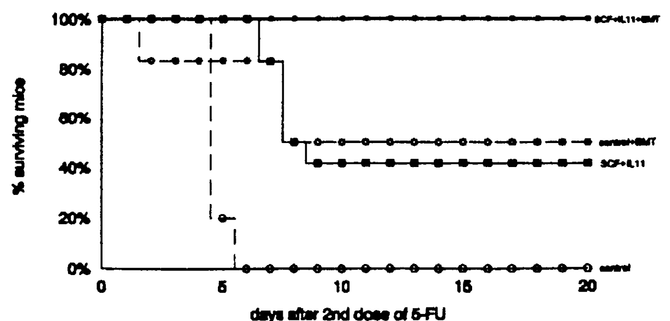

Fig 11. Comparison of survival in mice treated with two doses of 5-FU (160 mg/kg) 7 days apart. Control = 5-FU only, SCF + IL-11 = pretreated before the first dose of 5-FU, control + BMT = bone marrow transplantation (3 times 1 to 1.5 × 10^7 cells) given at 4, 24, and 48 hours after the second dose of 5-FU. SCF + IL-11 + BMT = pretreatment before, and BMT after 5-FU. Survival was monitored for at least 20 days.

Our data show that significant chemoprotection could be achieved using this approach. Part of the reduction of hemotoxicity could be attributed to the presence of increased numbers of the various subsets of primitive cells and progenitors before 5-FU. This was particularly the case for CAFC-day 7. When hematological recovery after a single dose of 5-FU was determined in the PB, an enhanced number of erythroid and granuloid cells could only be shown when 5-FU was administered 24 hours after cessation of growth factor treatment. Also the number of CFU-GM in marrow and spleen were highest using this scenario. The omission of this lag-phase abolished the beneficial effect of the pretreatment on recovery of blood parameters. This strongly suggests that another factor, in addition to the mere increased numbers of primitive cells, is important to explain the reduced hemotoxicity. This was substantiated by the finding that the expansion of cell compartments was associated with a reduced 5-FU sensitivity, ie, cycling activity, of the various more primitive cells. Apparently this endogenous inhibitory effect becomes most pronounced if the stimulating growth factor treatment is stopped.

SCF + IL-11 pretreatment allowed considerable 5-FU dose escalation, as the drug could be administered to pretreated mice 4 to 5 days earlier than control mice. Because 5-FU is known to affect other rapidly turning-over tissues, we wanted to establish whether protection from lethality in this particular setting was because of reduced hematologic damage. Our findings show that although growth factor pretreatment alone was as efficient as a BMT after chemotherapy (both saving 40% to 50% of the mice), combining pretreatment with BMT saved all mice. Since we assume that lethality after the BMT regimen we used is because of nonhematologic toxicity, we conclude that in this experimental setting SCF and IL-11 have potent protective effects on other cell tissues. The beneficial effect that we observed of SCF + IL-11 pretreatment on the numbers of surviving stem cells after one dose of 5-FU, apparently is greatly reduced when the drug is administered twice in a short time interval. If hematopoiesis is protected (with a BMT), other cell tissues become dose limiting, and our study suggests that SCF + IL-11 pretreatment has substantial effects on these tissues. It is interesting to note that both IL-11 and SCF have been shown to affect the proliferation of intestinal epithelia.[27-29]

Studies related to our present data were recently reported by Molineux et al[30] and Harrison et al.[31] Molineux et al showed that when mice were pretreated with SCF for 1.5 days and subsequently were injected with a high-dose of 5-FU, this resulted in fatal marrow aplasia.[30] These results are not in contrast with the explanation of the protective effects we report now on the effects of SCF plus IL-11. We have previously proposed that the cycling activity of stem cells is only transiently increased when SCF alone is administered. Initially the cycling activity of stem cells increases in response to SCF, and this explains why administration of 5-FU at that time is detrimental. If SCF administration is continued, however, as a result of the stimulating effect of SCF, the number of committed progenitors increases and expansion of this compartment results in downregulation of stem cell cycling activity.[9] In a related study Harrison et al[31] have shown that 5-FU is able to abolish the increased numbers of primitive cells in the spleen, induced by SCF administration to mice, if it is given in the middle of a 7-day SCF treatment period. However, if it was administered at day 8, many stem cells had lost vulnerability to 5-FU.[31]

In conclusion, our data are in support of the existence of negative regulatory feedback loops between the progenitor cell compartment and more primitive stem cells. More importantly our study indicates that these loops may be exploited in chemotherapeutic settings. Our results show that SCF plus IL-11 treatment is capable of in vivo expansion of immature cell compartments, which we believe is a prerequisite for effective hemoprotection. Other early acting growth factors may have a similar effect.[32-34] Possibly, this prophylactic use of growth factors is more attractive and may be more effective than treating after chemotherapy, when it is likely that already high levels of endogenous growth factors are present. After we have reported that hematopoietic growth factors may be used simultaneously with cytotoxic drugs,[35] we give in this study another example of an alternative application of hematopoietic growth factors. However, much more information is needed on the effects of growth factors on normal hematopoiesis and also on other cell tissues, before these strategies can be applied in patients. Such studies are currently underway in our laboratory. Specifically, it will be important to assess which growth factor or combination of growth factors can be used prophylactically.

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