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Influence of Timing of Administration of 5-Fluorouracil to Donors on Bone Marrow Engraftment in Nonmyeloablated Hosts

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Abstract

We evaluated the engraftment and the cell cycle status of marrow cells at various times after 5-fluorouracil (5-FU) administration. 5-FU (150 mg/kg) was given to donor male BALB/c mice at 1, 2, 6, or 12 days prior to marrow harvest. The donor cells were then assessed in host nonmyeloablated female mice. Bone marrow engraftment of marrow treated with 5-FU was evaluated and compared to marrow treated with diluent (phosphate-buffered saline) at 3 and 10 weeks after marrow infusion. Our data show a rapid induction of an engraftment defect 1 day after 5-FU, persistence of this defect through day 6, and a recovery by day 12. Experiments using hydroxyurea (which selectively kills cells in the S phase) to determine the cell cycle status indicated that cells that engrafted in post–5-FU marrow were noncycling at days 1, 2, and 12 but cycling at day 6. Post–5-FU bone marrow was also analyzed in vitro by colony assays and its cycling status determined by ^{3}H-thymidine suicide assay. High–proliferative-potential colony-forming cells (HPP-CFCs) and low–proliferative-potential colony-forming cells (LPP-CFCs) decreased rapidly 1 day after 5-FU, with a nadir observed at day 6 for HPP-CFCs and day 2 for LPP-CFCs. By day 12, LPP-CFCs showed a total recovery, but HPP-CFCs were still defective. Significant numbers of HPP-CFCs were cycling, mostly at days 6 and 8 after 5-FU, whereas LPP-CFCs appeared quiescent except at day 2. These results emphasize the importance of timing if post–5-FU marrow is used for gene therapy or marrow transplantation. *Int J Hematol.* 2001;74:79-85.

Key words: 5-Fluorouracil; Hydroxyurea; Hematopoietic stem cell; Cell cycle

1. Introduction

A dogma in hematology is that myeloablation is required to open space for marrow stem cell engraftment. This dogma was challenged as early as 1968 by Micklem et al [1]. They demonstrated an 8.5% bone marrow engraftment of T6T6 cells into nonirradiated CBA mice 3 months after transplantation of 20 million bone marrow cells. This observation was confirmed in the 1980s by Brecher et al and Saxe et al. Brecher et al [2] showed that engraftment levels of 16% to 25% were achieved at 2 to 13 weeks when 40 million cells per day were transplanted for 5 days. Saxe et al [3] demonstrated 0% to 16% engraftment in marrow or spleen at 1 to 6 months after transplantation. Cells were tracked using Y chromosome–marked syngenic marrow or mice congenic for subtypes of phosphoglycerate kinase. More recently, after injecting 40 million male BALB/c marrow cells per day for 5 days into nonirradiated female BALB/c mice, Stewart et al [4] reported high levels of persistent engraftment from 15% to 42%, over 2 years, in thymus, spleen, and bone marrow. They also showed that when donors were treated with 5-fluorouracil (5-FU) 6 days before bone marrow harvest, the level of engraftment dropped dramatically. Later, Ramshaw et al [5] confirmed the engraftment defect induced by administering 5-FU to the donor 6 days prior to bone marrow harvest and showed a complete restoration of bone marrow engraftability 35 days after 5-FU administra-

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tion. Using hydroxyurea (HU), they also demonstrated that about 70% of engrafting cells were quiescent. They hypothesized that 5-FU induces stem cells to begin to cycle, which in turn causes an engraftment defect.

5-FU is a chemotherapy drug consisting of a fluorine-substituted analog of uracil. It acts preferentially on cells in G1 or S phase and spares the hematopoietic stem cell compartment. Therefore, marrow harvested during the recovery phase after 5-FU administration is widely used to obtain an enriched hematopoietic stem cell population as a target for gene therapy [6,7]. The 2 aims of our study were, first, to analyze the influence of the timing of the 5-FU administration on bone marrow engraftment and, second, to determine if the effect of 5-FU on bone marrow engraftment was related to hematopoietic stem cell cycle induction. To do so, we carried out both in vivo (bone marrow transplantation) and in vitro (colony assay and thymidine suicide assay) experiments.

2. Materials and Methods

2.1. Mice

BALB/c H-2D mice were purchased from Charles River Laboratories (Wilmington, MA, USA) and housed in a conventional clean facility for at least 1 week before experimental use. All mice received mouse chow and acidified water ad libitum.

2.2. Transplantation of Murine Marrow From 5-FU–Treated Donors to Normal Untreated Hosts

In the first experiment, BALB/c female mice received transplants for 2 consecutive days either of intravenous injections of cells from 1 tibia and 1 femur from male BALB/c mice or of phosphate-buffered saline (PBS) (GIBCO-BRL, Bethesda, MD, USA) alone. In the second experiment, BALB/c female mice received transplants for 2 consecutive days either of intravenous injections of cells from 2 tibiae and 2 femurs from male BALB/c mice or of PBS alone. Donor male mice were injected via the tail vein with 5-FU (150 mg/kg) (Hofmann-Laroche, Nutley, NJ, USA) or PBS on day 0. The bone marrow was then collected 1, 2, or 6 days after treatment for the first experiment and 1, 2, 6, or 12 days after treatment for the second experiment. Volumes for injections were 0.5 mL. Recipient mice were killed at 3 and 10 weeks posttransplantation in the first experiment and at 10 weeks posttransplantation in the second experiment. The percentage of male cells in bone marrow was analyzed by Southern blotting.

2.3. Transplantation of Murine Marrow From HU-Treated Donors to Normal Untreated Hosts

BALB/c female mice received transplants via tail vein injection for 2 consecutive days of cells from 1 tibia and 1 femur in the first experiment and 2 tibiae and 2 femurs in the second experiment from male BALB/c mice that had been treated with 5-FU (150 mg/kg) 1, 2, and 6 days previously in the first experiment or on 1, 2, 6, and 12 days previously in the second experiment. Either HU (900 mg/kg) (Sigma Chemical, St Louis, MO, USA) or PBS was injected into donor mice 2 hours before killing [8]. Recipient mice were killed at 3 and 10 weeks posttransplantation in the first experiment and at 10 weeks posttransplantation in the second experiment. The percentage of male cells in bone marrow was analyzed by Southern blotting.

2.4. Transplantation of Murine Marrow From 5-FU–Treated Male Donors Mixed With Normal Female Marrow Cells to Normal Untreated Hosts

In this experiment, BALB/c female mice received transplants on 2 consecutive days with intravenous injections of cells from 2 tibiae and 2 femurs from male and female BALB/c mice. Donor male mice were injected via the tail vein with 5-FU (150 mg/kg) or PBS and the bone marrow was collected 6 days after treatment. Female marrow cells were added to male 5-FU–treated marrow cells to bring the cell number up to that obtained from males treated with PBS. Volumes for injections were 0.5 mL. Recipient mice were killed at 10 weeks posttransplantation. The percentage of male cells in bone marrow was analyzed by Southern blotting.

2.5. Southern Blot Analysis

DNA extracts of bone marrow were prepared by lysis in 0.15 mol/L NaCl, 0.02 mol/L tris(hydroxymethyl)aminomethane, 0.02 mol/L EDTA, and 1% sodium dodecyl sulfate followed by purification using organic extraction with proteinase K, RNAse, and phenol-chloroform and precipitation in ethanol. The presence of Y chromosome–specific DNA sequences was assessed using a pY2-cDNA probe (donated by I. Leminschka, Princeton University, NJ, USA) [9]. Five micrograms of each DNA sample was digested with the restriction enzyme *Dra* I and separated by gel electrophoresis in 0.8% agarose (GIBCO-BRL). DNA fragments were transferred onto Zetaprobe nylon membranes (Biorad, Richmond, CA, USA) according to established Southern blotting techniques. Sample loading variability was assessed and adjusted for by reprobing membranes with a cDNA for interleukin-3 (IL-3) (donated by J. Ihle and DNAX, Palo Alto, CA, USA). Probes were labeled with ^{32}P using a random primed labeling kit (Boehringer Mannheim, Mannheim, Germany), and autoradiography carried out using Kodak XRP x-ray film (Eastman Kodak, Rochester, NY, USA). Blots were exposed to photostimulatable storage phosphorimaging plates (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of male and female DNA was quantified after the plates were scanned with a GS-363 molecular imager system (Molecular Dynamics).

2.6. Colony Assays

Bone marrow cells at 1, 2, 6, 8, or 12 days after 5-FU (150 mg/kg) administration and untreated cells were harvested from 1 to 3 mice per time point; 4 bones were flushed per mouse. Cells were counted and cultured at a cell density

of 20,000 per plate in the double-layer nutrient agar system using 35-mm plastic tissue culture dishes. Underlays were α-minimal essential medium with 15% heat-inactivated fetal calf serum (HI-FCS) (Hyclone, Logan, UT, USA) supplemented with vitamins and glutamine. Seven growth factors (colony-stimulating factor 1, 10,000 U/dish; granulocyte-macrophage colony-stimulating factor, 5 ng/dish; granulocyte colony-stimulating factor, 10 ng/dish; IL-1α, 500 U/dish; IL-3, 200 U/dish; recombinant rat stem cell factor, 200 ng/dish; and basic fibroblast growth factor, 10 ng/dish) were included in a 1-mL underlayer with a final agar concentration of 0.5%. Cells were included in 0.5-mL overlays with a final agar concentration of 0.33%. Culture dishes were incubated at 37°C for 14 days in the presence of 5% O_2, 10% CO_2, and 85% N_2. Three to 6 plates were set up for each group of subjects in an experiment, and the experiment was repeated 5 times. Plates were scored on a dissecting microscope for high–proliferative-potential colony-forming cells (HPP-CFCs, highly dense colonies > 0.5 mm in diameter) and low–proliferative-potential colony-forming cells (LPP-CFCs, all cell clusters with more than 50 cells and not fulfilling HPP-CFC criteria) [10].

2.7. Thymidine Suicide Assay

The in vitro ^{3}H-thymidine suicide technique was used as previously described [8,11]. Concentrations of 1×10^6 marrow cells/mL were incubated in the presence of 5% CO_2 at 37°C for 30 minutes in Hanks' balanced salt solution (HBSS) containing 200 μCi/mL ^{3}H-TdR (Dupont New England Nuclear, NET-027X) (specific activity, 20.0 Ci/mM) or a concentration of unlabeled "cold" thymidine (Sigma Chemical, T-9250) equivalent to that in the ^{3}H-TdR incubation. Following this incubation, the cell suspension was diluted by the addition of 30 mL of cold HBSS containing 10% HI-FCS and 100 μg/mL of unlabeled thymidine. Cells were then centrifuged and resuspended in 10 mL of cold HBSS containing 10% HI-FCS and 100 μg/mL of unlabeled thymidine for a second wash. Cells were resuspended in 0.5 mL of cold HBSS containing 10% HI-FCS and cultured at an equivalent cell density of 20,000 per dish (based on the original count). Cells were plated in the double-layer nutrient agar system using 35-mm plastic tissue culture dishes as described above. Three to 6 cultures with ^{3}H-TdR or unlabeled thymidine were set up for each group in an experiment. The experiment was repeated 5 times. The number of cells in cycle was determined by the percentage reduction of LPP-CFCs or HPP-CFCs induced by ^{3}H-TdR exposure.

2.8. Statistical Analysis

For the analysis of engraftment data, the percentage of male cells engrafted was calculated by considering a male control as 100% and a female control as 0%. When treated samples were compared to controls of the same day (eg, day 1, 5-FU versus PBS), the level of statistical significance was set at .05. In cases where pair-wise, between-day comparisons were made among subjects of the same treatment (eg, 5-FU treated, day 1, 2, and 12 each compared to day 6), the level of statistical significance was adjusted for the number of multiple comparisons and set at .05/3, approximately .02. For the analysis of colony assays, HPP-CFCs and LPP-CFCs are presented as the number of colonies per 4 bones divided by number of colonies of untreated marrow and expressed as a percentage. Samples from each time point were compared to the nadir (day 6 for HPP-CFCs, day 2 for LPP-CFCs). The level of statistical significance was set at .05/4, approximately .01. For the analysis of the in vitro ^{3}H-TdR suicide assay, results are presented as the percentage of kill induced by ^{3}H-TdR. Nine pair-wise comparisons were made (5 comparisons to controls and 4 comparisons to day 6), and the level of statistical significance was set at .05/9, approximately .005. Descriptive statistics are presented in median and range. Graphs display the mean and the standard error of the mean. The non-parametric Wilcoxon rank-sum test was used for comparison. All *P* values are 2-sided.

3. Results

In an exploratory experiment, we administered 5-FU (150 mg/kg) to male BALB/c mice, killed them 1, 2, or 6 days later, and compared the capacity of these bone marrow cells to engraft in normal untreated female BALB/c hosts with cells from mice treated with PBS. Global levels of engraftment were low. In female hosts analyzed at 3 and 10 weeks after transplantation, engraftment was decreased at 1, 2, and 6 days after 5-FU compared with controls that received PBS (Table 1). 5-FU is thought to induce cycling of the hematopoietic stem cell. Because the cell cycle status of the stem cell may be related to its capacity to engraft, we assessed this cell cycle status by administering HU (900 mg/kg), which selectively kills cells in S phase, 2 hours before killing the male donors. In female hosts analyzed at 3 and 10 weeks after undergoing transplantation, the effect of HU on the number of male cells engrafting in female marrow was difficult to interpret due to the low-to-absent engraftment levels (data not shown).

Table 1.
Percentage of Male DNA in Bone Marrow of Recipient Female Mice at Weeks 3 and 10*

	Day 1		Day 2		Day 6	
Treatment	Week 3	Week 10	Week 3	Week 10	Week 3	Week 10
5-FU (range)	1 (0-1)	1 (1-1)	0 (0-0)	0 (0-0)	1 (0-2)	0 (0-2)
PBS (range)	5 (3-8)	4 (3-5)	5 (5-8)	3 (2-4)	3 (2-7)	5 (3-8)

*Recipient female mice were given 2 transplants of male bone marrow treated with 5-fluorouracil (5-FU) or phosphate-buffered saline (PBS) (control). They received cell equivalents of 1 tibia and 1 femur per day for 2 consecutive days and were analyzed by Southern blot at 3 and 10 weeks after transplantation. Each group consisted of 6 mice.

Table 2.
Percentage of Male DNA in Bone Marrow of Recipient Female Mice at Week 10*

Treatment	Day 1	Day 2	Day 6	Day 12
5-FU (range)	10† (4-12)	4† (0-37)	3† (0-7)	18 (7-79)
PBS (range)	25 (13-28)	28 (6-57)	23 (4-57)	15 (9-18)

*Female recipient mice were given 2 transplants of male bone marrow treated with 5-fluorouracil (5-FU) or phosphate-buffered saline (PBS) (control). They received cell equivalents of 2 tibiae and 2 femurs per day for 2 days and were analyzed by Southern blot at 10 weeks after transplantation. Each group consisted of 6 mice.

†Statistically significant ($P \leq .02$) compared to controls.

Although unlikely, the possibility exists that 5-FU treatment, rather than directly affecting the engrafting stem cells, was eliminating or modulating accessory cells, which could in turn affect homing or engraftment. Accordingly, on 2 consecutive days, we transplanted the equivalent of 2 tibiae and 2 femurs' worth of male cells from day 6 after 5-FU administration mixed with cells from normal female mice. Engraftment was evaluated at 3 and 10 weeks after transplantation. This experiment, which included 6 mice per group, showed that adding normal female marrow cells to male cells treated with 5-FU did not significantly increase the percentage of engraftment (data not shown).

To further explore the effect of 5-FU, the cell doses administered were doubled to increase the levels of bone marrow engraftment. In addition, to evaluate the timing of bone marrow recovery after 5-FU administration, we analyzed male donors 12 days after 5-FU administration. In female hosts analyzed 10 weeks after undergoing transplantation, there was a significant decrease in engraftment at 1, 2, and 6 days and a recovery at day 12 after 5-FU administration compared with controls (Table 2). The nadir of engraftment was observed 6 days after 5-FU administration. The level of engraftment of day 6 post–5-FU marrow was significantly lower than the levels observed 1 and 12 days after 5-FU administration (Wilcoxon rank-sum test, $P = .016$ and .005, respectively). We then analyzed the effect of HU administration 2 hours before collecting 5-FU–treated marrow. In female hosts analyzed at 10 weeks after undergoing transplantation, HU had no effect on the number of male cells engrafting in female marrow except for at day 6 after 5-FU administration ($P = .006$) (Figure 1). The Southern blot of this last group is presented in Figure 2. These results suggest that marrow cells that engraft are noncycling except at 6 days after 5-FU administration. Each group consisted of 6 mice.

In addition to these in vivo experiments, post–5-FU bone marrow from 1 to 3 mice per time point was also analyzed in vitro by colony assays, and the bone marrow's cycling status was determined by ^{3}H-thymidine suicide assay. Five consecutive experiments were done using the same design; because no difference between experiments was detected, all results were pooled. We observed a rapid decrease in the numbers of both HPP-CFCs and LPP-CFCs compared to untreated marrow 1 day after 5-FU administration, with a significant nadir observed at day 6 for HPP-CFCs and day 2 for LPP-CFCs. At day 8 and 12, LPP-CFCs showed a rebound increase, but HPP-CFCs were still defective (Figure 3). The cycling analysis of these cells using ^{3}H-TdR showed that a significant number of HPP-CFCs were cycling at day 6 after 5-FU administration (59% ± 7%) compared to control mice (11% ± 3%), whereas LPP-CFCs appeared quiescent at the same time point. LPP-CFCs had a relatively low cycling rate except at day 2 (Figure 4), which was consistent with the observed rebound at day 8.

4. Discussion

The dogma that myeloablation is required to open space in bone marrow to allow engraftment has been thoroughly challenged [1-5,12,13]. Different authors have demonstrated high levels of engraftment in bone marrow, spleen, and thymus when high numbers of bone marrow cells were infused into nonmyeloablated hosts. These data have suggested that donor cell engraftment was quantitative at the stem cell level. The final percentage of donor chimerism was determined by the ratio of host to donor stem cells and not by an effect on opening "niches" for stem cell engraftment [14].

Microscopic examination of bone marrow after 5-FU treatment has shown that the extravascular compartment is markedly depleted of cells, with a nadir observed by day 5.

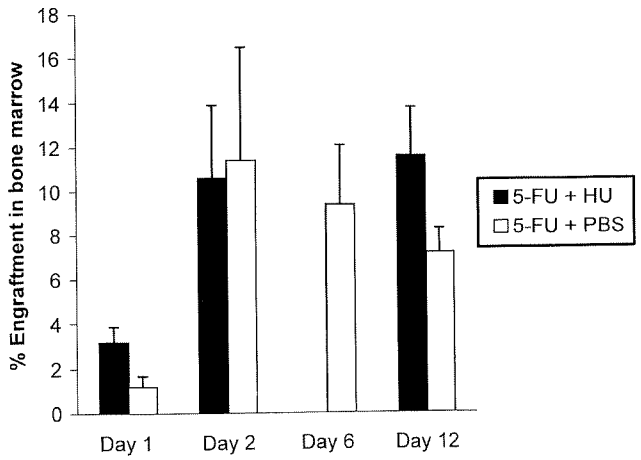

Figure 1. Effect of hydroxyurea (HU) on bone marrow treated with 5-fluorouracil (5-FU). Donor males were injected with 5-FU (150 mg/kg) 1, 2, 6, or 12 days before harvest and with either HU (900 mg/kg) or phosphate-buffered saline (PBS) 2 hours before death. The percentage of male cells in female host marrow was assessed at 10 weeks posttransplantation by Southern blot. No effect of HU was observed on bone marrow engraftment when compared to PBS controls except at day 6 after 5-FU administration ($P < .01$). Each group consisted of 6 mice.

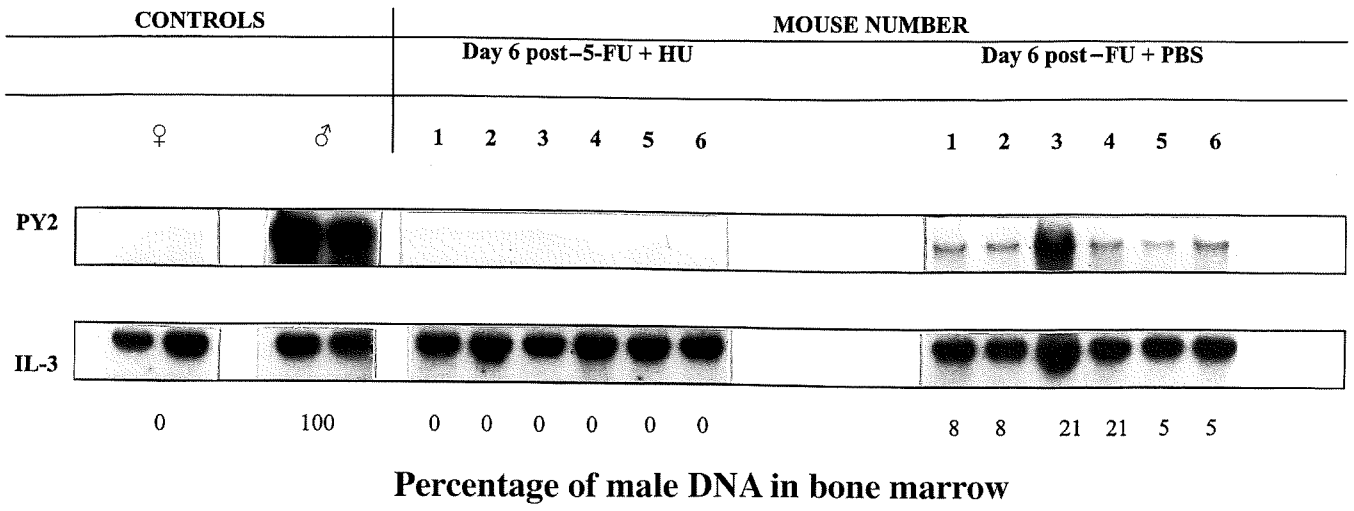

Figure 2. Male DNA in marrow of host female mice receiving day 6 post–5-fluorouracil (5-FU) marrow and either hydroxyurea (HU) (900 mg/kg) or phosphate-buffered saline (PBS) (0.5 mL) 2 hours before the harvest. BALB/c females received transplants on 2 consecutive days with the equivalent of 2 tibiae and 2 femurs per day. DNA was extracted 10 weeks after transplantation and digested with *Dra* I, and a Southern blot was performed using pY-2 as a probe. The interleukin-3 gene was used to correct the DNA loading. Autoradiographs were exposed for 3 days with intensifying screens. One can notice the absence of engraftment in the HU group compared with the control group.

Proliferating hematopoietic cells disappeared within 2 days of treatment, but progressive repopulation was observed by day 6 [15]. However, some studies have suggested that marrow treated with 5-FU (150 mg/kg) at varying time intervals was enriched in HPP-CFCs [16-18]. Our results, which show defective long-term engraftment at various times after 5-FU administration, were interpreted as relating in part to the cell cycle status of the engrafting cells because other studies have suggested that long-term engraftment is lost in proliferative marrow cells at certain points in the cell cycle [19-22]. However, Harrison and Lerner [18,23] have failed under certain experimental conditions to demonstrate a defect in engraftment. The reasons for these discrepancies are not clear, but may be related to the specific model used.

In these experiments, we have studied different time schedules of 5-FU administration and analyzed their effect on bone marrow engraftment levels. Our data show that the engraftment defect observed after 5-FU administration varied over time. The defect was observed as early as 1 day after 5-FU administration, with a nadir around day 6 and a total recovery by day 12. The nadir cannot be defined precisely because no time point between days 2 and 6 and between

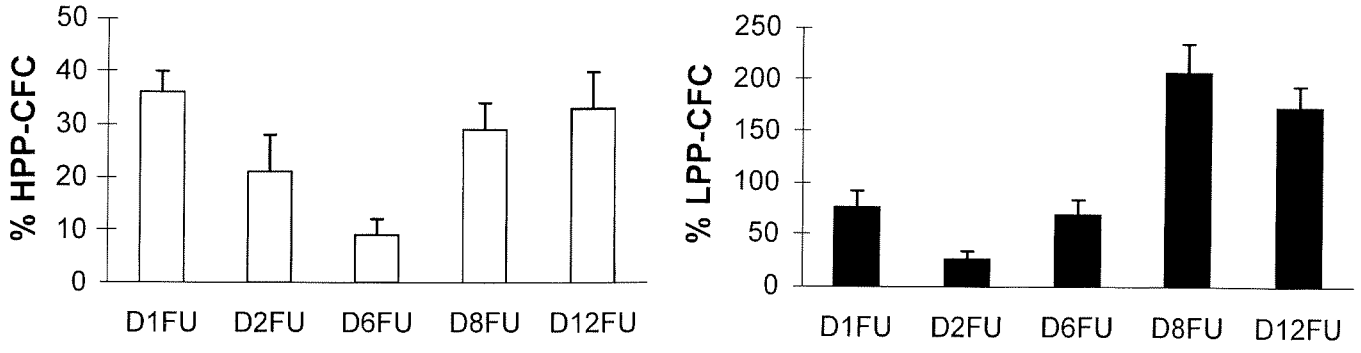

Figure 3. Mean percentage (± SEM) of marrow colonies per 4 bones at days 1, 2, 6, 8, and 12 after 5-fluorouracil (5-FU) injection (150 mg/kg) relative to untreated marrow. The number of colonies per 4 bones in untreated animals was considered to be 100%. The respective absolute control values are 45,050 ± 8741 for high–proliferative-potential colony-forming cells (HPP-CFCs) and 17,359 ± 4092 for low–proliferative-potential colony-forming cells (LPP-CFCs). Pair-wise comparisons using the non-parametric Wilcoxon's rank-sum test were carried out to reveal statistical differences between groups. HPP-CFCs show a rapid decrease on day 1, a significant nadir on day 6 (all $P < .002$), and an incomplete recovery by day 12. LPP-CFCs show a rapid decrease by day 1, a significant nadir at day 2 (all $P < .002$), and significant overshoots on days 8 and 12 (all $P < .003$). Abbreviations on x-axes indicate number of days after 5-FU administration.

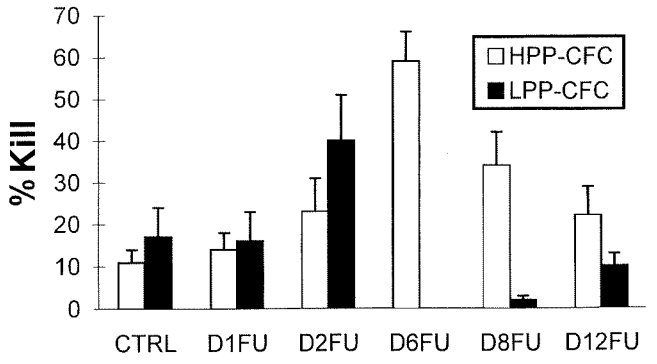

Figure 4. Mean percentage (± SEM) of kill induced by incubation of days 1, 2, 6, 8, or 12 post–5-fluorouracil (5-FU) (150 mg/kg) marrow and untreated marrow in ^{3}H-TdR, reflecting the percentage of cycling cells at the time of marrow harvest. A significant kill was observed for high–proliferative-potential colony-forming cells (HPP-CFCs) at 6 days after 5-FU administration when compared to untreated marrow ($P < .001$) For low–proliferative-potential colony-forming cells (LPP-CFCs), although not reaching statistically significant levels, an increased cell cycle induction was observed at day 2 followed by a negative overshoot at days 6 and 8. CTRL indicates control (untreated) marrow; other abbreviations on x-axis indicate number of days after 5-FU administration.

days 6 and 12 has been assessed. However, our results are in accord with previous results of Stewart et al [4] and Ramshaw et al [5] who reported very low levels of engraftment 6 days after 5-FU administration. In addition, Ramshaw et al showed a complete recovery 35 days after 5-FU administration. Our data indicate that the correction of bone marrow engraftability is more rapid at day 12. Our experiments are also consistent with the theory that marrow engraftment depends on the ratio of host to donor stem cells and rule out the possibility that 5-FU treatment, rather than directly affecting the engrafting stem cells, acts on eliminating or modulating accessory cells, which could affect homing or engraftment.

To evaluate the cell cycle status of engrafting cells, we administered HU to the 5-FU pretreated male donors 2 hours prior to the harvest of their bone marrow for transplantation. HU specifically kills cells in S phase. No difference in engraftment was observed when post-HU and control marrow was compared, except at day 6 after 5-FU administration. This lack of difference suggests that engrafting cells are quiescent except on day 6 after 5-FU. In previous studies, we demonstrated that engrafting cells 6 days after 5-FU administration were also quiescent. This discrepancy is at present unexplained, although the higher levels of engraftment observed in our experiment compared to the data of Ramshaw et al could reveal a small hidden difference.

Our in vitro analysis of bone marrow treated with 5-FU using colony assay and ^{3}H-thymidine suicide assay provides additional information about the effects induced by this drug. A decrease in the number of HPP-CFCs is rapidly observed 1 day after 5-FU administration, with a significant nadir at day 6 and a partial recovery by day 12. The kinetics for LPP-CFCs are slightly different, with a more rapid decrease, a nadir at day 2, and a high overshoot at day 8 persisting through at least day 12. These results are consistent with those of Yeager et al [24], who found a depletion of all hematopoietic progenitors from the bone marrow within 5 days of administration of a single dose of 5-FU. The in vitro assessment of cell cycle status at the time of marrow harvest by ^{3}H-thymidine suicide assay shows a significant induction of HPP-CFCs to cycle at 6 days after 5-FU administration. The concomitant decrease in the number of HPP-CFCs and induction of these cells to cycle at day 6 after 5-FU administration may explain in part the nadir of engraftment we observed when this 5-FU marrow is transplanted into normal hosts. Day 14 HPP-CFCs stimulated by 7 factors is a surrogate stem cell assay that needs to be compared with engraftability. Hodgson et al [25] showed a correlation between the content of HPP-CFCs and the platelet and marrow granulocyte-macrophage colony-forming cell repopulating ability of bone marrow cell suspensions. The data showing a recovery of engraftment at day 12 with depression of HPP-CFCs can be contrasted to in vitro studies in cytokine-stimulated cultures showing high levels of HPP-CFCs with either no or good engraftment depending on the time after cytokine exposure [20,21]. Thus HPP-CFCs and engraftment results may be discordant given the marked fluctuation in engraftment of cytokine-stimulated marrow cells observed over short time intervals in vivo [26].

In conclusion, overall engraftment appears to be defective 1 to 6 days after 5-FU administration, with recovery 12 days after 5-FU. This defective engraftment is in part related to the cell cycle status of engrafting cells, but other important factors may play a role, as reflected by HPP-CFC depletion.

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