Evidence That Stem Cell Factor Is Involved in the Rebound Thrombocytosis That Follows 5-Fluorouracil Treatment

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The mechanisms responsible for 5-fluorouracil (5FU)-induced rebound thrombocytosis are not completely understood. SI/SI^d mice, which do not undergo rebound thrombocytosis in response to 5FU, provide a genetic approach to the study of this phenomenon. Recent reports by several groups that the SI locus encodes a protein known as stem cell factor (SCF), mast cell growth factor, or kit ligand, suggests the possibility that the lack of wild-type SCF in SI/SI^d mice is responsible for their defective response to 5FU-induced thrombocytopenia. It is shown in this report that SCF-treated SI/SI^d mice are as capable as their wild-type littermates in undergoing rebound thrombocytopenia. W/W^* mice, mutated at the locus encoding the SCF receptor, also do not undergo rebound thrombocytosis, but are not responsive to SCF treatment. In normal mice, it is shown by RNA solution hybridization that SCF mRNA expression is increased during the 5FU-induced platelet nadir period. It is also shown by autoradiography that maturing megakaryocytes express SCF receptors, and that in vivo administration of SCF significantly raises the numbers of megakaryocytes, as well as circulating platelet counts. Taken together, these data indicate that SCF may be an important regulator of platelet production under both normal and physiologically disturbed situations.

The regulation of platelet production occurs at several levels. First, megakaryocyte progenitor cell growth and development is regulated within the bone marrow microenvironment such that the pool of megakaryocyte precursors is physiologically suitable. Second, megakaryocyte differentiation is responsive to signals induced by the circulating platelet mass. That is, conditions of thrombocytopenia signal upregulation of platelet production, whereas conditions of thrombocytosis signal down-regulation of platelet production. The upregulation of platelet production can be studied with schemes that experimentally induce thrombocytopenia. One method involves the use of antiplatelet antisera (APS), which, when administered into experimental animals, rapidly and dramatically depletes platelet levels. Within days of the platelet nadir, platelet counts recover, climb to supranormal levels (the “overshoot” effect), then stabilize back to normal. The hypothesis that humoral factors are directing this platelet recovery and overshoot stems from observations that plasma collected from animals during the platelet nadir will, when transferred into normal animals, cause an increase in the peripheral platelet count. This activity from thrombocytopenic animal plasma has been termed thrombopoietin.

Another experimental model for the study of platelet regulation uses the drug 5-fluorouracil (5FU). Administration of this cytotoxic compound depletes the marrow of cycling hematopoietic cells signaling the upregulation of megakaryocyte progenitor cells. 5FU treatment has the added effect of causing a significant reduction in circulating platelet numbers, itself possibly a signal to increase platelet production. The ultimate result of 5FU treatment is a dramatic and prolonged thrombocytosis observed 10 to 15 days after treatment.

Stem cell factor (SCF), a recently identified cytokine with multiple biological properties, has been shown to be the ligand for the tyrosine kinase encoded at c-kit. Two mouse strains have been invaluable in the molecular and biological characterization of SCF. SI/SI^d mice are mutated at the locus encoding SCF with a deletion of the transmembrane and intracellular domains. These mice exhibit hematological abnormalities that can be corrected by in vivo administration of SCF. W/W^* mice, mutated at the locus encoding the SCF receptor, express an altered c-kit protein with reduced tyrosine kinase activity. SI/SI^d and W/W^* mice are similarly hematologically impaired.

One of the hematological abnormalities of SI/SI^d mice is in their response to either APS or 5FU. Platelet counts decrease in response to either agent, increase to normal levels, but in neither case does a thrombocytotic overshoot occur. Given that the defect in SI/SI^d mice is in the expression of SCF, it is reasonable to suspect that SCF is involved in the thrombocytosis that normally follows APS or 5FU treatment. This study addresses this question in the 5FU model and also examines the involvement of SCF in regulating platelet levels in normal mice.

MATERIALS AND METHODS

Mice, 5FU, and SCF treatment. WCB6F1-(SI/SI^d), WCB6F1-(+/+), WBB6F1-(W/W^*), WBB6F1-(+/+), and adult Balb/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). 5FU was administered in a single intravenous injection at 150 mg/kg body weight. Recombinant rat SCF was prepared as previously described and was subcutaneously administered to mice in a sterile vehicle of 150 mmol/L NaCl, 0.1% bovine serum albumin (fraction V, fatty acid-free; ICN Immunobiologials, Lisle, IL) according to the dosing schedule indicated in the figure legends. Control mice were injected with vehicle alone.

Blood cell analyses. Twenty microliters of blood, drawn into micropipettes through a 2- to 3-mm incision made by a scalpel in the lateral tail vein, were immediately diluted into manufacturer’s diluent for the Sysmex Cell analyzer (TOA Medical Electronics, Kobe, Japan). Bleeding was stopped immediately with pressure so that blood loss was minimal (usually < 50 μL per bleed). Platelet counts, mean red blood cell volume (MCV), and red blood cell...
counts (RBC) were obtained on that instrument. Care was taken to dilute samples appropriately so that read out would be within the linear range of the instrument. Data are expressed as the mean of the indicated number of samples, error bars are the SEM. Statistical significance is assigned to P < .05 between control and experimental groups (t test, Statview software package; Abacus, Berkeley, CA). In experiments where mice were serially bled, a group of control mice was serially bled with the test groups in order to observe effects of mild bi-weekly to triweekly phlebotomy. No adverse effects were ever noted on platelet or RBC parameters.

RNA solution hybridization. Probes for mouse SCF or human actin mRNA (which detects the corresponding murine mRNA) were generated by runoff transcription of cloned gene regions in vectors containing SP6 or T7 promoters using 35S-UTP according to standard protocols (Promega Biotech, Madison, WI), or from synthetic oligonucleotide partial duplexes.29 RNA sense strand standards for quantitation of the hybridization assays were produced by runoff transcription of the same region in the direction opposite to the direction of probe synthesis using tracer quantities of 35S-UTP and 0.2 mmol/L unlabeled UTP.

SCF or actin mRNA levels were quantitated as previously described30 with some modifications. Briefly, bone marrow cells were explanted from animals at the given time post-5FU, enriched for low-density cells by centrifugation on 65% Percoll (Pharmacia, Piscataway, NJ), and lysed at 3 × 10⁶ nucleated cells/mL in 0.2% sodium dodecyl sulfate (SDS), 10 mmol/L Tris, pH 8, 1 mmol/L EDTA, 20 mmol/L dithiothreitol, and 100 μg/mL proteinase K (Boehringer Mannheim, Indianapolis, IN). Samples (30 μL) were added to 70 μL of hybridization mix consisting of 30 μg/mL yeast tRNA, 30 μg/mL carrier DNA, and 145,000 cpm/mL 35S-labeled probe in 4.8 mol/L sodium phosphate, pH 7.2. Samples were incubated at 84°C for 2 to 3 hours then cooled to room temperature before addition of RNase A to 0.03 mg/mL and RNase T1 to 5,000 U/mL. Samples were incubated at 37°C for 20 minutes before addition of 120 μL of 0.0025% bromophenol blue in formamide. The entire sample was then loaded onto a 3.8-mL Sephacryl S200 Superfine (Pharmacia) gel filtration column (0.7 cm × 10 cm) and eluted with 2.0 mL of 10 mmol/L Tris, pH 8, 1 mmol/L EDTA, and 50 mmol/L NaCl. Effulents containing hybridized RNA duplexes were collected directly into scintillation vials. After addition of 5 mL Liquiscint (New England Nuclear, Boston, MA), samples were counted for 20 minutes or to 3% error. The cpm were converted to molecules mRNA by comparison to the linear portion of the standard curve (correlation coefficient, 0.97). The data point for each sample is the mean of duplicate determinations from samples taken from three animals. Error bars represent the SEM. Statistical significance is assigned as described above.

To calculate the amount of RNA used in the solution hybridization experiments, a portion of the explants were lysed in guanidinium thiocyanate and total RNA prepared according to the procedure of Chomczynski and Sacchi.31 Since preliminary experiments showed that the amount of RNA recovered from cells from normal mice or from mice treated with 5FU was equivalent and reproducible (ranging from 5.7 to 6.3 μg/3 × 10⁶ cells), this determination was not regularly performed and the assumption was made that the lysates prepared from bone marrow explants of equal cell numbers contained equivalent amounts of RNA.

Acetylcholinesterase staining of bone marrow or splenic megakaryocytes. Bone marrow and spleens were harvested from individual mice, and viable cells were enumerated by trypan blue exclusion and resuspended to 10⁶ nucleated cells/mL. One hundred microliters of each cell suspension was aliquoted to triplicate wells of a flat-bottomed 96-well microtiter plate. Cells were centrifuged to a monolayer and tested for acetylcholinesterase activity by the procedure of Burstein et al.22 After 24 hours, the mean percentage of acetylcholinesterase-positive cells in the triplicate wells was determined. This value was determined for three animals at each time point of an experiment. The data are given as the mean ± SEM of the three values.

Cell surface binding of 125I-SCF. Cell-surface binding of 125I-rat SCF was performed according to the procedure of Broudy et al.33 Normal Balb/c bone marrow enriched for light density cells as above was resuspended at 2 × 10⁶ cells in 100 μL RPMI plus 1% fetal calf serum (FCS), 1.3 ng 125I-rat recombinant SCF, plus or minus an 1,000-fold excess of unlabeled rat SCF. 125I-SCF was prepared by ICN (Irvine, CA) to a specific activity of 73.8 μCi/μg. Cells were incubated for 1 hour at 4°C then spun through a layer of 30% bovine serum albumin (BSA) to remove unbound SCF. Slides of each preparation were prepared with a Cytospin centrifuge (Shandon, Pittsburgh, PA), processed for autoradiography, and stained with Wright-Giemsa. It was not possible to analyze the biological activity of 125I-SCF; however, the radiolabeled material was active in a receptor-binding assay where 2 ng of unlabeled SCF could displace 0.4 ng of labeled material.

Megakaryocyte enumeration and staging. Bone marrow cells were harvested, and viable cells were enumerated by trypan blue exclusion and spun onto slides with a Cytospin centrifuge at low speed (800 rpm, 3 minutes, low acceleration) to preserve cellular morphology. Slides were stained with May-Grünwald Giemsa. All of the megakaryocytes in 6 × 10⁶ nucleated bone marrow cells were enumerated and staged according to the scheme of Ebbe and Stohlman.34 Briefly, stage I megakaryoblasts are characterized by a high nuclear to cytoplasmic ratio and an intensely basophilic, nongranular cytoplasm. Stage II megakaryocytes are basophilic with (occasionally) azurophilic granules. The nucleus is usually lobulated and the nuclear to cytoplasmic ratio is reduced. Stage III megakaryocytes have azurophilic granulation throughout the eosinophilic cytoplasm. The nucleus appears to be either single or segmented and the nuclear to cytoplasmic ratio is very low.

RESULTS

Effect of SCF treatment on 5FU-induced rebound thrombocytosis in SI/SI² and W/Wv mice. Before injection of 5FU, SI/SI² mice (n = 9) were treated daily with 30 μg/kg SCF for 48 days. This treatment ameliorated the macrocytic anemia of these mice by decreasing the MCV and increasing the RBC count to within 85% and 70% of normal littermate values, respectively. Platelet counts increased after 12 days of SCF then readjusted to normal levels by day 35, when they remained stable even with continued SCF administration (Table 1). These data are consistent with those of Zsebo et al.20 Six SCF-treated SI/SI² mice, along with a control group of six +/+ littermates and six SI/SI² mice not pretreated with SCF, were then injected with 5FU. The mice pretreated with SCF continued to receive SCF daily, while the other two groups received vehicle. As shown in Fig 1, the platelet counts in all three groups decreased to roughly 30% of initial values within 7 days of 5FU injection. Within 12 to 16 days, the platelet counts in the wild-type mice increased to 250% to 350% of initial counts, whereas the platelet counts in SI/SI² mice, although returning to normal levels by day 21, did not undergo a rebound thrombocytosis. Two of the animals in this group died without recovering normal platelet counts, one on day 7, the other on day 19. In contrast, SI/SI² mice treated with SCF behaved more like +/+ mice in that a rebound
thrombocytosis was observed through days 12 to 24, peaking after day 17. All of the mice in this treatment group survived the experiment.

The effect of SCF on W/Wv mice was also evaluated. W/Wv mice (n = 7) were treated daily with 30 µg/kg SCF for 35 days. This treatment had no effect on the MCV, RBC counts, or platelet counts of these mice (Table 1).

The seven SCF-treated W/Wv mice, seven +/+ littermates, and seven untreated W/Wv mice were then injected with 5FU. The SCF-treated mice continued to receive SCF daily, while the other two groups received vehicle. As shown in Fig 2, platelet counts in all three groups decreased to a nadir within 5 to 7 days after 5FU treatment. Throughout days 9 to 18, the +/+ group entered a state of rebound thrombocytosis. In contrast, the platelet counts in W/Wv mice and W/Wv mice treated with SCF, although returning to normal, did not exhibit a rebound thrombocytosis.

Detection and quantitation of SCF mRNA in bone marrow from normal mice injected with 5FU. If SCF is normally involved in 5FU-induced rebound thrombocytosis, then it might be expected that SCF mRNA expression would increase sometime after 5FU injection and before the thrombocytotic peak is observed. To test this notion, Balb/c mice were injected with 5FU and the level of SCF mRNA in their bone marrow cells was measured at various time points thereafter. Figure 3 shows data generated by an RNA solution hybridization technique, which allows for a quantitative estimation of RNA levels. By 7 days after 5FU, SCF mRNA expression had increased to 250% of

![Graph](image1)

**Fig 1.** SCF administered to S/S+ mice allows for a post-5FU thrombocytotic overshoot like that observed in wild-type littermates. Platelet counts (x10^3/µL) were obtained on the indicated days after 5FU injection. (○) Wild-type littermates, n = 6. (■) S/S+ mice, n = 6. (□) S/S+ mice treated with daily administration of 30 µg/kg SCF, n = 6. Data from wild-type mice and S/S+ mice are significantly different (P < .05) on days 11 to 18 post-5FU. Data from SCF-treated S/S+ mice and S/S+ mice are significantly different (P < .05) on days 11 to 24 post-5FU.

![Graph](image2)

**Fig 2.** SCF administered to W/Wv mice does not alter the abnormal response to 5FU. Platelet counts (x10^3/µL) were obtained on the indicated days after 5FU injection. (○) Wild-type littermates, n = 7. (■) W/Wv mice, n = 7. (□) W/Wv mice treated with daily administration of 30 µg/kg SCF, n = 7. Data from wild-type mice are significantly different (P < .05) from data from W/Wv mice or SCF-treated W/Wv mice on days 9 to 18.

| Table 1. Effect of SCF on Hematological Parameters in S/S+ and W/Wv Mice |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                             | Day 0                       | Day 5                       | Day 12                      | Day 20                      | Day 26                      |
| S/S+                        |                             |                             |                             |                             |                             |
| MCV                         | 62.1 ± 5.6                  | 60.0 ± 3.3                  | 68.2 ± 1.2                  | 65.0 ± 1.6                  | 62.0 ± 1.4                  |
| RBC                         | 3.4 ± 0.6                   | 4.0 ± 0.5                   | 5.8 ± 0.6                   | 6.0 ± 0.3                   | 5.8 ± 0.5                   |
| WBC                         | 10.3 ± 1.1                  | 14.3 ± 4.1                  | 15.6 ± 2.0                  | 13.9 ± 0.9                  | 6.9 ± 0.7                   |
| Platelets                   | 1,507 ± 112                 | 1,348 ± 89                  | 2,290 ± 258                 | 2,223 ± 229                 | 2,074 ± 271                 |
| W/Wv                        |                             |                             |                             |                             |                             |
| MCV                         | 69.0 ± 0.7                  | 70.2 ± 0.8                  | 70.7 ± 1.2                  | 70.4 ± 0.9                  | 70.7 ± 0.9                  |
| RBC                         | 6.7 ± 0.2                   | 6.1 ± 0.3                   | 5.7 ± 0.3                   | 5.2 ± 0.4                   | 6.2 ± 0.2                   |
| WBC                         | 13.5 ± 1.1                  | 16.8 ± 0.6                  | 9.8 ± 0.5                   | 16.4 ± 0.8                  | 14.1 ± 0.9                  |
| Platelets                   | 1,253 ± 71                  | 1,370 ± 47                  | 1,154 ± 68                  | 1,312 ± 26                  | 1,379 ± 65                  |

SCF (30 µg/kg/d) was administered to mice daily for 48 (S/S+ n = 9) or 35 (W/Wv n = 7) days. At the indicated day of treatment, MCV (fL), RBC (x10^6/µL), WBC (x10^9/mL), and platelet levels (x10^3/µL) were determined. +/+ refers to the blood cell data collected from wild-type littermates of either S/S+ or W/Wv mice. Data are expressed as the mean ± SEM. Values that differ significantly from pretreatment values (day 0) are italicized.
normal levels. The peak of SCF mRNA expression coincided with the end of the platelet nadir period and preceded the initiation of the thrombocytotic phase by 5 days. To control for the possibility that mRNA expression in general follows this pattern after 5FU injection, changes in actin mRNA were also monitored. Actin mRNA expression decreased sharply by 5 days after 5FU, then increased to normal levels by day 12 without a compensatory overshoot.

When SCF mRNA species from normal or 5FU-treated mice were evaluated by polymerase chain reaction (PCR) techniques, two sizes of PCR products were observed corresponding in length to alternatively spliced SCF mRNAs, which either include or exclude exon 6. Increases in the amounts of both bands were observed from marrow harvested 5 or 7 days after 5FU treatment, compared with normal bone marrow, without a change in the ratio of the two bands (data not shown).

In vivo effect of SCF in normal mice. Figure 4 contains data from four independent experiments where normal Balb/c mice were injected subcutaneously for 6 or 7 days with SCF (100 μg/kg/d) or with vehicle. Blood cell analyses were performed throughout the injection period and for 3 weeks following the last SCF injection. This treatment had no statistically significant effect on either the platelet or RBC counts (Fig 4A and B, respectively). In contrast, the platelet counts in SCF-treated, but not vehicle-treated, mice increased to 140% to 200% of pretreatment levels by 11 to 12 days (Fig 4C). This initial increase in the platelet count was followed by a return to normal levels and then a second statistically significant increase by 21 to 24 days. In one of these four experiments, platelet counts were monitored for 42 days to detect a possible third cycle of thrombocytosis, but the counts remained at normal levels after the second cycle (data not shown). In initial experiments not shown here, Balb/c mice given SCF daily for 28 days exhibited the same pattern of cyclic thrombocytosis as mice given SCF for only 6 to 7 days. Because of this early observation, and to conserve on reagent, a treatment schedule of 6 to 7 days of SCF was adopted for our experiments.

In view of the effect of SCF on platelet counts, megakaryocytes from treated animals were examined. Table 2 pre-
SCF (100 μg/kg/d) was administered to Balb/c mice (n = 3 each group) for 3 or 6 days, after which bone marrow was harvested. Total cellularity is given as the number of bone marrow cells per mouse. The percentage of identifiable megakaryocytes (Megs) in the marrow was determined as described in the Methods. The total number of megakaryocytes per mouse was then calculated based on the known cellularity. As megakaryocytes were counted, they were identified as stage I, II, or III cells. For the control group, 250 megakaryocytes were counted and staged. For the 3-day group, 184 megakaryocytes were counted and staged. For the 6-day group, 309 megakaryocytes were counted and staged. The "percentage" of stage I, II, or III cells is relative to the total number of megakaryocytes. The number of each stage megakaryocyte per mouse was calculated based on the total number of megakaryocytes. Values given are the means of determinations from the three mice in each group. SEM was omitted for clarity, but in no case did it exceed 20% of the mean value.

Table 2. Effect of SCF In Vivo on Stage I, II, and III Megakaryocytes

<table>
<thead>
<tr>
<th>SCF</th>
<th>Cellularity</th>
<th>% of Total No.</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.04*</td>
<td>36 x 10^4</td>
<td>17</td>
<td>6 x 10^4</td>
<td>17*</td>
</tr>
<tr>
<td>3 d</td>
<td>0.06</td>
<td>42 x 10^4</td>
<td>21</td>
<td>9 x 10^4</td>
<td>28</td>
</tr>
<tr>
<td>6 d</td>
<td>0.10*</td>
<td>62 x 10^4</td>
<td>15</td>
<td>10 x 10^4</td>
<td>40*</td>
</tr>
</tbody>
</table>

SCF-treated animals was monitored throughout the two cycles of SCF-induced thrombocytosis. In the marrow, this value increased from 0.04% in untreated animals to 0.2% by day 6. This increase preceded the first thrombocytotic cycle by 3 to 5 days. By day 14, the percentage of marrow megakaryocytes had returned to normal levels, where they remained unchanged throughout the remainder of the experiment. A change in the percentage of splenic megakaryocytes was also observed in SCF-treated animals, increasing from 0.001% to 0.028% by day 9 and falling back to normal values by day 21. This transient increase in splenic megakaryocytes coincided with the first increase of thrombocytosis and preceded the second wave by 11 to 12 days. The cellularity of the bone marrow or of the spleen did not change at any time throughout this experiment (data not shown).

Localization of SCF-receptors on mouse megakaryocytes. The role(s) of SCF in influencing platelet levels could be either indirect through an action on early hematopoietic progenitor cells and/or direct through an interaction with megakaryocytes. A direct effect on megakaryocytes would require the presence of SCF receptors (c-kit) on those cells. To determine if identifiable megakaryocytes express SCF-receptors, low-density bone marrow cells from normal Balb/c mice were incubated with 125I-SCF then processed for autoradiography. Figure 6A shows two stage I megakaryocytes (black arrows) with high densities of radio-grains associated with them. An unidentified blast-like cell also binding 125I-SCF is pointed out with a white arrow. Figure 6B shows a parallel preparation of cells incubated with 125I-SCF and an excess of unlabeled SCF to demonstrate specificity of radiobinding. No grains are seen over megakaryocytes or over any cell type in this field. This control was repeated numerous times to eliminate the possibility of nonspecific binding of 125I-SCF to megakaryocytes due to their known propensity to endocytose proteins.37 In no case were radio-grains seen associated with megakaryocytes or any cell type if unlabeled SCF was included in the preparation. Since the labeling was performed in 1% FCS, it is unlikely that the competitive effect of unlabeled SCF on binding of radiolabeled SCF was due to the added protein concentration nonspecifically.

Although stage I and II megakaryocytes were consis-
DISCUSSION

This work proposes that the SCF/c-kis interaction is at least partially responsible for the compensatory thrombocytosis that follows 5FU treatment. This hypothesis follows from observations that both S/Sn and W/Wv mice are dysfunctional in this regard and that S/Sn, but not W/Wv, mice will undergo rebound thrombocytosis after being corrected by in vivo SCF treatment. The post-5FU thrombocytotic peak seen in SCF-treated S/Sn mice occurs several days later than that seen in wild-type mice, reminiscent of the delayed release of marrow cells reported by Arnold et al. in these mutant mice. The hypothesis that SCF is involved in 5FU-induced thrombocytosis is supported further by observations that in normal mice, 5FU treatment leads to increased expression of SCF mRNA during the platelet nadir at days 5 to 7. Expression levels of actin mRNA were monitored to test whether this was a general effect on mRNA levels. Actin mRNA levels were not elevated by 5FU treatment and, in fact, were depressed 2 to 5 days after 5FU, probably due to the effect of the drug on mitotically active cells. The cells within the marrow responding to 5FU treatment with an elevated expression of SCF mRNA have not yet been identified, although stromal elements have been implicated in other studies. In situ RNA hybridization studies in progress will address this question, as well as the issue of whether the increase in SCF mRNA levels reflects an enrichment of the cell types producing SCF or an increase in the mRNA expression levels per cell. Future work will also include measurement of serum SCF levels after 5FU treatment, an experiment that first requires the development of specific and sensitive immunological reagents.

It is not known what mechanism(s) induced by 5FU-treatment is responsible for the dramatic and prolonged rebound thrombocytosis. It has been hypothesized that the depletion of cycling hematopoietic cells by the cytotoxic drug is a signal for increased production of progenitor cells, including megakaryocyte precursors. SCF induced by 5FU treatment could contribute to this phenomenon, given its ability in vitro to support and direct human megakaryocyte-colony forming cells in combination with interleukin-3 (IL-3). As seen in this report, SCF administered for 6 days in mice increased the total number of marrow and splenic megakaryocytes. Since the percentage of acetylcholinesterase-positive cells (representing both cytoplasmically immature and mature cells) increased fivefold (Fig 5), and the percentage of cells identified by cytoplasmic and nuclear morphology (mature cells only) increased twofold (Table 2), it follows that the increase in the number of acetylcholinesterase cells must include an increase in the number of immature megakaryocytes. Of the mature cells, stage II cells appeared to be particularly increased in number. As these cells are not mitotically active, the increase must reflect a shift of stage I cells into the stage II compartment. Since the number of stage I cells did not concomitantly decrease, this compartment is probably replenished from the increased pool of immature cells. The SCF-mediated increase in marrow acetylcholinesterase-positive cells preceded the first cycle of thrombocytosis by 4 days, while the increase in splenic megakaryocytes was coincident with it. A second cycle of thrombocytosis was always observed in these experiments. This increase in platelet levels was not immediately preceded by a detectable increase in splenic or marrow megakaryocytes. Possibly, the increase in splenic megakaryocytes observed 11 to 12 days preceding the second cycle contributes to it, but in a delayed fashion due to a sequestering of splenic-derived platelets within that organ for a period of several days before release.
SCF may have a direct effect on megakaryocytes, as well as on precursor cells, as pointed out by the presence of SCF-receptors on stage I and II cells and by the shift of stage I cells into the stage II compartment. It is interesting that the most mature stage III megakaryocytes were never observed binding SCF, although, at least in the human system, they possess the capacity to bind interleukin-6 (IL-6)\(^4\) and granulocyte-macrophage colony-stimulating factor (GM-CSF).\(^4\)

The upregulation of platelet production in the 5FU model may result in part from the decrease in platelet counts occurring 5 to 7 days after treatment. Although the data from this report do not allow us to determine if thrombocytopenia is itself an inductive signal for SCF production, it is interesting that SCF mRNA expression is increased just as the platelet nadir is ending, the precise period when regulatory thrombopoietic factors are postulated to appear.\(^2\),\(^8\),\(^10\) Furthermore, \(SI/SI^4\) mice do not undergo rebound thrombocytosis when depleted of circulating platelets by APS, implying an involvement of SCF in that model as well.\(^27\) Paradoxically, \(W/W^v\) mice respond normally to APS,\(^\#\) pointing out the complexity of this model system.

A question that remains is the role of SCF in normal platelet homeostasis. Both \(SI/SI^4\) and \(W/W^v\) mice possess normal circulating platelet counts, despite other megakaryocyte abnormalities.\(^\#\),\(^\#\) Possibly, SCF influences platelet production primarily under situations of hematopoietic or thrombopoietic stress and other factors or combinations of factors such as thrombopoietin,\(^8\),\(^10\),\(^11\) IL-6,\(^\#\),\(^\#\) erythropoietin,\(^4\) IL-3, GM-CSF,\(^4\) leukemia inhibitory factor,\(^5\) or others are necessary for the physiological regulation of platelet mass. An alternative notion considers that \(SI/SI^4\) and \(W/W^v\) mice are both viable mutants and that the products of the mutated genes, while abnormal, are still sufficient for survival. The \(SI/SI^4\) version of SCF therefore may be adequate for platelet homeostasis in the absence of undue stress, whereas other aspects of hematological differentiation may require greater SCF levels or have specific requirements for membrane-bound presentation of the ligand. Nonetheless, from the data presented in this report, it can be concluded that SCF is thrombopoietic, i.e., SCF administered in vivo will significantly increase megakaryocyte numbers and platelet counts in treated animals.

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