Thrombocytotic Suppression of Megakaryocyte Production From Stem Cells

By Joan Goldberg, Elizabeth Phalen, Donald Howard, Shirley Ebbe, and Frederick Stohlman, Jr.

Megakaryocytopoiesis in the spleens of lethally irradiated mice transplanted with marrow cells was suppressed by platelet transfusions. In one group of experiments, animals were irradiated and transfused with bone marrow cells on day 0. They were then given either no treatment, platelets, platelet-poor plasma, or saline on days 0, 2, 4, 6, and 8, and then were sacrificed on day 10. Megakaryocytes per section in the spleens of mice receiving platelets were 24%-48% of the values in the groups given plasma, saline, or bone marrow only. The number of pure megakaryocyte colonies was also diminished by platelet hypertransfusion. Another experiment examined the effect of platelets or plasma administered on days 1 and 2 or days 6 and 7 after irradiation and bone marrow transfusion. Hypertransfusion on days 6 and 7 was as effective in suppressing megakaryocytopoiesis as hypertransfusion every other day for 10 days. Animals given platelets or plasma only on days 1 and 2 did not have any significant change in their megakaryocyte number. These results implied that committed megakaryocyte precursors were more sensitive to inhibition by increased platelet levels than pluripotential stem cells. Further experiments with plethoric animals indicated that different levels of erythropoietin did not account for the effects of platelet hypertransfusion. The findings could be explained by inhibition of cell proliferation or of differentiation of megakaryocyte precursors by increased platelet levels.

MEGAKARYOCYTOPOIESIS AND PLATELET PRODUCTION appear to be regulated, in part, by the numbers of circulating platelets. However, the cellular level at which such homeostatic mechanisms may exert their major effect is not clear. Changes in megakaryocyte ploidy and size in response to perturbation of platelet number suggest that polyploid immature megakaryocytes and megakaryocytic precursors may be influenced in their capacity to replicate DNA. Changes in number and the rate of turnover of megakaryocytes suggest that the rate of differentiation and cellular proliferation of more primitive precursors may also be affected. In addition, thrombocytopenia may lead to a change in the number of pluripotential stem cells.6

It is thought that the adjustment of platelet production, which occurs after acute changes in platelet numbers, may be mediated by adjustments in the level of a humoral thrombopoietin. The stimulatory effect of plasma from acutely thrombocytopenic animals on platelet production in assay animals has been shown. However, tests for a thrombopoietin in the blood of animals in which thrombocytopenia developed more slowly after irradiation have given conflicting results, even though other results indicate that the homeostatic mechanism is not destroyed by radiation.
An experimental system that has helped to clarify the differentiation of erythroid cells is the analysis of splenic colonies in irradiated mice transfused with bone marrow cells. Erythroid colonies are suppressed in plethoric mice and can be stimulated by erythropoietin. Erythropoietin is not effective in producing differentiated colonies early after transplantation; however, it will stimulate erythroid differentiation when given later.

It was of interest to adapt this experimental system to determine if early megakaryocyte precursors were responsive to changes in platelet levels and to verify the feedback control of megakaryocytopoiesis in animals in which the development of thrombocytopenia was a gradual process, rather than an abrupt one. Therefore, experiments were done to see if hypertransfusion of platelets at different times after irradiation and transplantation of bone marrow cells would suppress megakaryocyte production in splenic colonies of hematopoietic cells.

**MATERIALS AND METHODS**

Female mice, 12–14 wk old, of the CFI strain (Carworth Farms, New York, N.Y.) were used as donors and recipients of marrow cells. Female CF1 exbreeders, 6–7 mo old, were used as donors for platelet or red cell transfusions. Platelets were counted by phase microscopy.

**Transplantation of marrow cells.** Suspensions of femoral marrow cells were prepared in cold TC199 culture media, pH 7.3, according to the method of Kubanek et al. The cells were diluted to a concentration of 4 × 10^5 nucleated cells/ml, and 0.25 ml was injected into a tail vein of each recipient mouse within 6 hr of irradiation.

**Irradiation.** Recipient mice in all experiments and all platelet and red cell suspensions were irradiated with 950 R from a cesium source (Gamma Cell 40, Atomic Energy of Canada, Ltd. The LD_{50/30} for these mice is 800 R).

**Platelet transfusions.** Platelet suspensions were prepared from blood obtained by cardiac puncture from mice anesthetized with 2 mg of Nembutal intraperitoneally. From 1/2 to 2 ml of blood were collected from each mouse into a syringe containing 0.2 ml acid-citrate-dextrose (ACD) solution. For each recipient mouse, seven to eight donors were bled. Approximately 10 ml of blood were placed in each 15-ml plastic centrifuge tube, which was then filled with sterile saline. This mixture was centrifuged at 110 g at 4°C for 20 min, and the platelet-rich supernatants were pooled. The remaining red cells were resuspended in saline to a total volume of 15 ml and centrifuged at 150 g, 4°C, for 20 min. The platelet-rich supernatants were again pooled, and total numbers of platelets were estimated from the platelet counts and volume. The platelet-rich suspensions were then spun at 1100 g, 4°C, for 30 min. The platelet-poor supernatant was removed and saved, and the platelet pellet was resuspended in a small volume of supernatant. Platelets or plasma were irradiated in 0.25-ml aliquots in plastic syringes prior to injection into a tail vein. Each platelet transfusion contained 5–12 × 10^9 platelets.

**Experimental Designs**

**The effect of transfusion of platelets for 10 days.** Duplicate experiments were done in which irradiated mice received platelets or plasma about 4 hr after irradiation and on days 2, 4, 6, and 8. Marrow cells were injected about 6 hr after irradiation. Coincident with one experiment, an additional group of mice was studied in which injections of saline were substituted for injections of platelets or plasma. Of the 14 mice in each injected group initially, 6–8 successfully completed the course of repeated injections. Two additional groups of irradiated mice received only platelet or plasma injections, and three mice in each group were killed 2 days after each infusion for determination of platelet counts and hematocrits from cardiac blood samples; these counts were taken as representative of all transfused mice.

**The effect of early and late platelet transfusions.** About 2 hr after irradiation, mice were transfused with bone marrow cells on day 0. Platelet or plasma transfusions were given to different groups either on days 1 and 2 or days 6 and 7. About 1 hr after each platelet transfusion, two or three mice were killed for determination of cardiac blood platelet counts and hematocrits.
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The effect of red cell transfusions. Heparinized blood was collected by cardiac puncture from mice anesthetized with Nembutal. The red cells were washed three times with saline, then adjusted to a packed cell volume of 70% and irradiated in plastic syringes. Mice were irradiated and infused with 10^7 marrow cells on day zero, then injected intraperitoneally with 0.8 ml of red cell suspensions or 0.8 ml of saline on days 1 and 2. Three irradiated mice, which received just red cells or saline, were killed every other day for blood counts.

Spleen Colony and Megakaryocyte Quantification

Ten days after irradiation and transplantation, recipient animals were killed, and their spleens were removed and fixed overnight in Bouin's solution. Gross colonies were counted; then the spleens were embedded and sectioned at 10-μm thickness. Five consecutive sections were mounted on each slide, and then 20 sections were discarded; this was continued until the entire spleen was cut, and 8-12 slides were prepared from each spleen. The sections were stained with hematoxylin and eosin; the best section on each slide, as judged by morphology and staining, was examined microscopically; the total number of megakaryocytes was counted, and the result was expressed as average number of megakaryocytes per section. By this technique, about 4% of the spleen was examined microscopically. Approximately every 25th section was viewed, and these sections were about 250 μm apart. The numbers of megakaryocytes found in pure megakaryocytic colonies, in mixed colonies, and in isolation were also noted. The total number of pure megakaryocytic colonies seen in all the slides examined in each spleen was recorded, with care taken not to count the same colony more than once if it appeared on consecutive slides. To be classified as a colony, rather than as isolated megakaryocytes, at least six megakaryocytes had to be found in close proximity. The location of colonies within sections was used to determine if colonies on adjacent slides were in fact, duplicate sections of the same colony. Microscopic colonies were counted and classified on a central slide according to the definitions of Curry and Trentin.24

Twenty-three mice that were only irradiated had 1.6 ± 0.7 gross colonies per spleen. Fourteen of these spleens were sectioned and had 1.8 ± 0.5 microscopic colonies in a central section and 0.7 megakaryocytes per section. In experiment I, 16 irradiated mice were not transplanted with marrow cells, but 8 received platelets and 8 received plasma every other day; they had 0.8 ± 0.3 and 1.0 ± 0.5 gross colonies per spleen, 1.1 ± 0.4 and 2.1 ± 0.8 microscopic colonies, and 0 and 2.9 ± 2.0 megakaryocytes/section (insignificant difference), respectively. In experiments II and III, three or four irradiated mice received platelets, plasma, or red cells on days 1 and 2; in none of these were spleen colonies visible grossly, and sections were not examined.

RESULTS

Effect of Transfusion of Platelets for 10 Days

The infusion of platelets every other day resulted in platelet counts that were at least twice the control values through the 10 postirradiation days (Fig. 1). Since endogenous platelet production continues for about 4 days after

![Fig. 1. Platelet counts (average ± SEM) of mice irradiated on day 0 and then given infusions of platelets or plasma on days 0, 2, 4, 6, and 8. Values for day 10 represent 15 mice; other values represent four or five mice.](image-url)
irradiation, the highest platelet counts were seen during that period. The values shown were determined 2 days after infusion of platelets and, thus, represented minimal levels. For the first 6 days, these values were higher than normal. There were no differences in numbers of gross colonies or of microscopic splenic colonies on the central slide among the several groups (Table I). However, differentiation into megakaryocytes, as judged from the average total number of megakaryocytes per section, appeared to be reduced in those mice in which platelet counts were maintained at normal or increased levels (Fig. 2). When compared with those mice that were only irradiated and transplanted with marrow cells, mice that received platelet transfusions appeared to have a reduction in splenic megakaryocytes to 48% of control levels ($p < 0.10$). However, a more striking difference was observed when the number of splenic megakaryocytes was compared with controls that were subjected to a comparable amount of handling. Megakaryocytes per section in the platelet injected group were 30% of that seen in the saline injected animals ($p < 0.05$) and 24% of that seen in the plasma injected group ($p < 0.01$). Megakaryocytes in mixed, undifferentiated, or pure megakaryocyte colonies were comparably reduced. In addition, the platelet-infused animals had significantly fewer pure megakaryocyte colonies when compared with the saline- and plasma-injected groups (Table 2). Differential counts of colonies from central sections are
SUPPRESSION OF MEGAKARYOCYTES

Table 2. Numbers of Pure Megakaryocyte Colonies*

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment</th>
<th>No.</th>
<th>Pure Megakaryocyte Colonies</th>
<th>Difference From Platelet-treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Platelets</td>
<td>15</td>
<td>2.26 ± 0.33</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>16</td>
<td>3.00 ± 0.47</td>
<td>0.74 (not significant)</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>15</td>
<td>4.53 ± 0.69</td>
<td>2.27 (p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>8</td>
<td>4.00 ± 0.78</td>
<td>1.74 (p &lt; 0.05)</td>
</tr>
<tr>
<td>II</td>
<td>A. Platelets (days 1 and 2)</td>
<td>8</td>
<td>3.5 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
<td>4.1 ± 0.6</td>
<td>0.6 (not significant)</td>
</tr>
<tr>
<td></td>
<td>Plasma (days 1 and 2)</td>
<td>8</td>
<td>3.4 ± 0.8</td>
<td>0.1 (not significant)</td>
</tr>
<tr>
<td></td>
<td>B. Platelets (days 6 and 7)</td>
<td>8</td>
<td>1.1 ± 0.4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8</td>
<td>4.1 ± 0.6</td>
<td>3.0 (p &lt; 0.005)</td>
</tr>
<tr>
<td></td>
<td>Plasma (days 6 and 7)</td>
<td>8</td>
<td>3.8 ± 0.9</td>
<td>2.7 (p &lt; 0.02)</td>
</tr>
</tbody>
</table>

*Values represent the average (± SEM) of the total number of pure megakaryocyte colonies seen in each spleen. The entire spleen was cut at 10-μm thickness, and every 25th section was examined. The number of pure megakaryocyte colonies was recorded with care taken not to count the same colony more than once if it appeared on consecutive slides. In experiment I, mice were irradiated and transfused with marrow cells on day 0 and given platelet, plasma, or no infusions on days 0, 2, 4, 6, and 8. In experiment II, mice were irradiated and transfused with marrow cells on day 0 and given platelets or plasma on days 1 and 2, on days 6 and 7, or not at all.

shown in Table 3; differences among the groups were not striking, but megakaryocyte colonies in mice transfused with platelets were significantly fewer (p < 0.01) than in those transfused with plasma.

Effects of Early and Late Platelet Transfusions

To investigate whether early or late megakaryocyte precursors were affected by the platelet transfusions, platelet or plasma infusions were given either early (day 1 and 2) or late (day 6 and 7) in the period after irradiation and marrow transplantation. As shown in Fig. 3, substantial levels of thrombocytosis were achieved, but the group that was transfused early was thrombocytopenic by day 6. The platelet counts shown in Fig. 3 were determined about 1 hr after transfusion, and, thus, represented maximum values, in contrast to the minimum values shown in Fig. 1. Numbers of spleen colonies, counted grossly or microscopically, were the same in all groups of mice (Table 1). Megakaryocytes

Table 3. Number of Colonies of Each Type in Central Spleen Section

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment*</th>
<th>N Erythroid</th>
<th>Myeloid</th>
<th>Megakaryocytic</th>
<th>Mixed</th>
<th>Undifferentiated</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>0</td>
<td>16</td>
<td>4.8 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Platelets</td>
<td>15</td>
<td>4.9 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>15</td>
<td>4.7 ± 0.6</td>
<td>1.7 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>1.9 ± 0.4</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>8</td>
<td>6.1 ± 0.6</td>
<td>1.0 ± 0.4</td>
<td>0.6 ± 0.3</td>
<td>1.0 ± 0.4</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>8</td>
<td>6.1 ± 0.7</td>
<td>1.2 ± 0.4</td>
<td>0.5 ± 0.2</td>
<td>1.1 ± 0.5</td>
<td>0.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Platelets</td>
<td>(days 1 and 2)</td>
<td>8</td>
<td>5.9 ± 0.8</td>
<td>2.6 ± 0.6</td>
<td>0.5 ± 0.2</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>(days 1 and 2)</td>
<td>8</td>
<td>5.5 ± 1.0</td>
<td>1.6 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>1.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Platelets</td>
<td>(days 6 and 7)</td>
<td>8</td>
<td>7.0 ± 0.7</td>
<td>2.3 ± 0.6</td>
<td>0.4 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>(days 6 and 7)</td>
<td>8</td>
<td>4.9 ± 0.9</td>
<td>2.1 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>1.9 ± 0.4</td>
</tr>
</tbody>
</table>

*All mice irradiated with 950 R and injected with 10⁶ marrow cells on day 0.
†Treatments given on days 0, 2, 4, 6, and 8.
‡Average ± SEM.
per splenic section were reduced to 25% of control values, compared to groups receiving plasma or bone marrow only ($p < 0.001$ and $p < 0.02$) when platelets were given on days 6 and 7, but were not significantly reduced by platelet infusions on days 1 and 2 (Fig. 4). This reduction was comparable to that seen with transfusion of platelets every other day throughout the postirradiation period. The total number of pure megakaryocyte colonies seen in the platelet-treated animals was similarly reduced when platelet infusions were given on days 6 and 7 (Table 2). Differential counts of colonies on central splenic section (Table 3) did not show this reduction in pure megakaryocytic colonies, probably due to the small sample size.

**Effect of Transfusion of Red Cells**

Of note in the preceding experiments was that both groups in which megakaryocyte numbers were reduced also showed higher hematocrit values than their respective controls (Table 1). This finding was presumably due to reduction of the amount of blood loss by amelioration of the radiation-induced thrombocytopenia. This finding raised the possibility that erythropoietin might have influenced megakaryocyte differentiation. To investigate this question, the
production of splenic megakaryocytes was evaluated in mice in which erythropoietin production was inhibited by transfusion-induced polycythemia. Figure 5 shows that hematocrit values were increased by transfusions and that platelet counts began to fall about 2 days earlier in the transfused mice than the controls. Both grossly visible and microscopic spleen colonies were reduced in number by plethora, and the reduction appeared to be totally accounted for by a reduction in erythroid colonies (Table 4). The numbers of megakaryocytes in the spleen were the same in the plethoric and the nonplethoric controls.

**DISCUSSION**

These studies have demonstrated that platelet transfusions suppress megakaryocyte production from transplanted marrow stem cells in the spleens of irradiated mice. There are several possible explanations for this result. By correcting or preventing the radiation-induced thrombocytopenia, the development of a stimulus (i.e., thrombopoietin) for megakaryocyte differentiation may have been abrogated. The transient periods of thrombocytosis may also have effectively interfered with the generation of the normal amount of thrombo-

<table>
<thead>
<tr>
<th>Red Cells (n = 7)</th>
<th>Saline (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spleen Colonies</strong></td>
<td></td>
</tr>
<tr>
<td>Gross</td>
<td>5.4 ± 1.2*, †</td>
</tr>
<tr>
<td>Microscopic</td>
<td>3.4 ± 0.9†</td>
</tr>
<tr>
<td>Erythroid</td>
<td>0.8 ± 0.8†</td>
</tr>
<tr>
<td>Megakaryocytes/section</td>
<td>5.5 ± 1.2</td>
</tr>
</tbody>
</table>

* Average ± SEM.
† Difference from saline-injected controls significant with p < 0.01.
‡ Difference from saline-injected controls significant with p < 0.05.
poietin that has been reported to be present in the circulation of animals with normal levels of circulating platelets. Alternatively, it could be suggested that the platelets themselves may have directly influenced the splenic microenvironment, so that megakaryocyte differentiation was not encouraged, or directly inhibited megakaryocytic precursors.

When platelets were transfused only on days 1 and 2, no significant suppression of megakaryocyte production was seen. During the first few days in this experimental model, pluripotential stem cells are thought to settle in the spleen and proliferate. Depending upon the microenvironment, some of the cells may become committed to megakaryocytosis, erythropoiesis, or granulocytosis. These early events for the megakaryocytic system were not demonstrably affected by high platelet levels, as similar numbers of megakaryocytes were seen in the early transfused and control groups. Induction of thrombocytosis on days 6 and 7, however, suppressed the production of recognizable megakaryocytes. The maturation time of recognizable mouse megakaryocytes has been estimated to be about 2-3 days, and that of an unrecognizable immediate precursor compartment to be about 1 day. Thus on day 6, when thrombocytosis was first induced, those cells that would have been mature megakaryocytes on day 10 would have been in the compartment of proliferating committed stem cells. This cell population may be the most sensitive to feedback regulatory mechanisms, but the data do not indicate whether their proliferation or their differentiation was inhibited. Since thrombocytosis persisted after day 6, later events such as development of polyploidy and cytoplasmic differentiation could likewise have been inhibited. Unfortunately, in our histologic sections only large mature megakaryocytes were identifiable, and very small or immature cells would not be identified as megakaryocytes.

Continuous hypertransfusion of platelets and infusions on days 6 and 7 also appeared to suppress the number of pure megakaryocyte colonies seen. Pure megakaryocyte colonies were relatively small, averaging in size from 15 to 40 cells on each section in the various control groups. Since undifferentiated megakaryocyte precursors cannot be distinguished by our methods, we cannot tell if the decrease in pure megakaryocyte colony number was accompanied by an increase in smaller undifferentiated colonies that presumably would consist of only several cells.

The failure of thrombocytosis to alter the numbers of gross splenic colonies could be explained by the small number and size of megakaryocytic colonies and the fact that grossly visible colonies were, for the most part, erythroid. In the chronically transfused mice, stem cells were infused about 2 hr after platelets, plasma, or saline, and it could be suggested that the pretreatment may have altered the quantity (f fraction) or quality of stem cells to lodge in the spleen. The finding that there was no difference in numbers of gross colonies or microscopic colonies on the central slide implied that the f fraction was similar in all groups. In the other experiments, treatments were not given until at least a day after stem cell transplantation, and, in the group with the most impressive reduction in megakaryocytes, 6 days after transplantation.

Several workers have demonstrated that thrombocytopenia results in increased megakaryocyte diameter, and that thrombocytosis is accompanied
by smaller megakaryocytes. Possibly, megakaryocytes in the control groups were larger than normal because of the stimulation from thrombocytopenia. Likewise, the megakaryocytes in the platelet-hypertransfused groups may have been smaller than normal. Differences in megakaryocyte size between the two groups would affect the number of megakaryocytes counted by an amount proportional to the difference in diameter. Harker noted that megakaryocyte diameter after 10 days of suppression by increased platelets was 67% of that seen after 10 days of stimulation by antiplatelet serum in rats. Since we observed a reduction of megakaryocytes to about 25% of control values, differences in megakaryocyte size would explain only a part of the changes seen.

The mice in which megakaryocyte production was reduced also showed less severe reduction in hematocrit than control animals. It is, thus, conceivable that the higher erythropoietin levels in the thrombocytopenic animals in some way stimulated megakaryocyte production. However, erythropoietin must have been almost totally suppressed in the mice with transfusion-induced plethora, since the numbers of erythroid colonies were extremely small. The similar numbers of splenic megakaryocytes in the plethoric and control animals indicated that reduced levels of erythropoietin alone did not account for reduced megakaryocytopoiesis in the animals in which the severity of the radiation-induced anemia was reduced by platelet transfusions. Although platelet levels fell somewhat earlier in the plethoric group than in control animals, it is doubtful that this effect was sufficient to stimulate megakaryocytopoiesis and to counteract a possible effect of a lack of erythropoietin. The mechanism responsible for this reduction in platelet counts after hypertransfusion with red cells is not clear. It has been a reproducible finding in nonirradiated mice also and is associated with reduction of the total number of circulating platelets, indicating that it is not due simply to blood volume expansion. Recognized homeostatic mechanisms operate largely by regulating megakaryocytic precursors, thus there is a delay between activation of the mechanism and a change in effective platelet production. Therefore, the prompt fall in platelet counts after red cell transfusion suggests that it is probably not due to suppression of production as a result of inhibition of erythropoietin. Furthermore, platelet counts and total circulating platelets recover during a period of sustained erythrocytosis, reticulocytopenia, and hence, deficiency of erythropoietin. Additional studies are necessary to clarify the mechanism of this interesting phenomenon.

It is of interest that megakaryocytopoiesis was stimulated in the chronically transfused mice by the multiple tail vein injections (six altogether) of plasma or saline, and was suppressed by injections of viable platelets. Odell et al. felt that the “nonspecific” thrombocytosis resulting from injections of foreign substances was probably mediated by activation of the same mechanism, i.e., a thrombopoietin, that accounted for the “specific” thrombocytosis that follows a transient period of thrombocytopenia. The present findings suggest that the “nonspecific” stimulation from trauma to the tail could be at least partially inhibited by circulating platelets. However, megakaryocyte development in these studies was never completely suppressed by platelet hypertransfusion. Other workers have similarly not shown complete suppression of mega-
karyocytopoiesis and platelet production after platelet hypertransfusion.\textsuperscript{1,6,7,31,32} Possibly there is a basal level of megakaryocytopoiesis unresponsive to homeo-
static mechanisms, or, alternatively there may have been residual levels of a
thrombopoietin in our hypertransfused animals. It is conceivable that the in-
jected platelets would have totally inhibited megakaryocytopoiesis were it not
for the nonspecific stimulation from the injections or the plasma injected. This
hypothesis is supported by the observation that the amount of stimulation of
megakaryocytopoiesis seen in the plasma-infused group (Fig. 2), i.e., the in-
crease over uninjected controls, can more than account for the residual amount
of megakaryocytopoiesis seen in the hypertransfused group.

These findings substantiate the feedback regulatory control of megakaryo-
cytopoiesis by the number of circulating blood platelets. They further suggest
that the sensitive cell compartment is committed to megakaryocytopoiesis,
rather than being the pluripotential stem cell itself.

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J Goldberg, E Phalen, D Howard, S Ebbe and F Jr Stohlman

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