Comparative Effects of Thrombopoietin and Interleukin-6 on Murine Megakaryocytopoiesis and Platelet Production

By T.P. McDonald, Marilyn B. Cottrell, Carol J. Swearingen, and Rose E. Clift

A thrombocytopoiesis-stimulating factor (TSF or thrombopoietin) derived from human embryonic kidney (HEK) cells is known to increase platelet production and to increase the number of morphologically unrecognizable early megakaryocytes, ie, small acetylcholinesterase-positive (SAChE+) cells in mice. Other recent studies have concluded that interleukin-6 (IL-6) also stimulates murine megakaryocytopoiesis both in vitro and in vivo. Some workers have suggested that IL-6 is thrombopoietin. Therefore, the purpose of this study was to compare the effects of TSF and IL-6 on percent $^{35}S$ incorporation into platelets, platelet sizes, and the percentages of SAChE+ cells in C3H mice, and to determine if they produce the same or different responses. The results showed that two or four injections of a partially purified TSF (total dose of 2 or 4 units [U] over a 1- or 2-day period) increased percent $^{35}S$ incorporation into platelets ($P < .005$) and platelet sizes ($P < .005$) of both normal and rebound-thrombocytotic mice when compared with values from other mice treated with human serum albumin, the carrier protein for both TSF and IL-6. In eight separate experiments, it was shown that IL-6 (40,000 U, 4 μg), when given to rebound-thrombocytotic mice in four injections over a 2-day period, produced a small but significant ($P < .005$) increase in percent $^{35}S$ incorporation into platelets. Additional studies showed that negative results were obtained when similar high doses of IL-6 were administered in two doses over a 1-day period. TSF, but not IL-6, stimulated an increase in platelet sizes of normal mice ($P < .005$); however, IL-6 increased platelet sizes of rebound-thrombocytotic mice when given in two or four injections ($P < .05$ to .0005). Also, IL-6, but not TSF, caused anemia in normal mice ($P < .0005$) that were given two injections and tested 3 days later. TSF stimulated an increase ($P < .005$) in the percentage of SAChE+ cells; whereas IL-6, even at high doses, did not. Because of the observed differences in biologic responses of these two cytokines, we conclude that TSF and IL-6 are separate entities.

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A THROMBOCYTOPOIESIS-stimulating factor (TSF or thrombopoietin) derived from human embryonic kidney (HEK) cells is a potent stimulator of thrombocytopoiesis and megakaryocytopoiesis. Previous work showed that, in vivo, TSF stimulates increases in megakaryocyte size and number, the number of precursor megakaryocytes (ie, small acetylcholinesterase-positive [SAChE+] cells), and maturation and endomitotic indices of megakaryocytes. More recently, it was shown that TSF increased megakaryocyte DNA content. In addition, thrombopoietin increases platelet numbers, platelet sizes, and percent $^{35}S$ incorporation into platelets of mice. One group of workers concluded that interleukin-6 (IL-6) functions as thrombopoietin. In recent work it was shown that high doses (4 to 16 μg to mice or 70 to 1120 μg/kg to monkeys) of IL-6 increased platelet counts, increases percent $^{35}$Se-selenometionine ($^{35}$SeM) incorporation into platelets in a dose-response relationship, increases megakaryocyte size, and increases the number of megakaryocyte and granulocyte-macrophage colony-forming cells in bone marrow (BM) and spleen. White blood cell (WBC) counts were also increased after 16 μg/mouse or after 70 μg/kg in monkeys by IL-6 treatment. However, the numbers of marrow megakaryocytes were not increased in mice treated with 20 to 100 μg of IL-6-7 and hematocrits were reported to be normal. A preliminary study by Hill et al reported that platelet sizes were not altered significantly by IL-6 administration. Moreover, several additional studies concluded that IL-6 causes an increase in megakaryocytopoiesis in vitro. For example, megakaryocyte sizes, acetylcholinesterase content, and DNA content were increased in vitro in the absence of IL-6. Bruno and Hoffman showed that IL-6 will increase the number of megakaryocytic colonies in the absence of IL-3, whereas these and other investigators concluded that pretreatment of BM cells with IL-3 before adding IL-6 will either improve or is required for increasing the number of megakaryocytic colonies. In all of these studies, it was concluded that IL-6 stimulated megakaryocytopoiesis and platelet production. However, measurement of platelet sizes after IL-6 treatment has only been reported in a preliminary study and the effects of IL-6 on SAChE+ cells have not been reported. Therefore, the purpose of the present study was to compare the effects of TSF and IL-6 on percent $^{35}S$ incorporation into platelets, platelet sizes, and the percent SAChE+ cells in the BM of C3H mice. Although both cytokines stimulated percent $^{35}S$ incorporation into platelets, IL-6 did not stimulate an increase in platelet sizes of normal mice nor elevate the proportion of SACH+ cells, indicating significant biologic differences in the two factors.

MATERIALS AND METHODS

A total of 336 C3H/HENHSID (C3H; Harlan Sprague-Dawley, Indianapolis, IN) male mice weighing about 24 g each was used in these studies. Animals were injected with test materials using one of two different treatment schedules. The first schedule was as follows: mice were given one subcutaneous (SC) injection of test substance in the morning and another injection in the afternoon of the first day; these mice were killed 3 days after the first injection. The second schedule utilized a total of four injections: one injection was given in the morning and another in the afternoon of the first and second days. As before, mice were killed 3 days after

From the Department of Animal Science, The University of Tennessee, College of Veterinary Medicine, Knoxville.

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Address reprint request to T.P. McDonald, PhD, The University of Tennessee, College of Veterinary Medicine, PO Box 1071, Knoxville, TN 37901-1071.

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the first injection. Both normal and rebound-thrombocytotic mice were used in these studies and all mice were given the same total dose of test materials. Mice were made thrombocytotic by a single intraperitoneal (IP) injection of rabbit anticoagulant plasma expander (RAPMS) 5 days before injection of test substances, ie, human serum albumin (HSA), TSF, or IL-6.

The TSF used in this study was Step II material, a partially purified preparation from HEC culture media. A total dose of 2 or 4 units (U) of TSF was given per mouse. A unit of TSF is defined as the amount of material in milligrams of protein that is required to increase the percent $^{35}$S incorporation into platelets of immunothrombocytotic mice by 50% above baseline. The specific activity of the TSF used in this study was approximately 3.5 U/mg protein. The TSF preparation contained a small amount of IL-6, ie, $\approx 875$ U of IL-6/U of TSF as determined by the B9 cell proliferation assay. Because large amounts of HSA (approximately 1.8 mg for each TSF U) were added to stabilize the partially purified TSF, other mice were treated with 7.5 mg of HSA as a control. Previous studies showed that saline and HSA gave similar responses in mice, ie, platelet sizes, percent $^{35}$S incorporation into platelets, and average polyloid megakaryocyte DNA content were almost identical at 2 and 3 days after the beginning of the treatment. Therefore, in the present studies, HSA was used as the control substance.

Recombinant human IL-6 (rhIL-6) was obtained from Boehringer Mannheim (Indianapolis, IN). This preparation of rhIL-6 was produced utilizing Escherichia coli and purified by standard chromatographic techniques. The material was greater than 98% pure as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and endotoxin (LAL) was less than 10 EU/mg. Specific activity was greater than $1 \times 10^7$ U/mg as determined by the B9 cell proliferation assay. The material was diluted into HSA (7.5 mg/mouse) before injection.

Percent $^{35}$S incorporation into platelets and platelet sizes were measured using previously described techniques. Thirty micromoles of Na$_2$SO$_4$ were injected intravenously (IV) 24 hours before killing. Blood for determination of percent $^{35}$S incorporation into platelets and platelet sizes was taken by cardiac puncture after injection of a heparin-sodium pentobarbital solution. Approximately 0.5 mL of blood was collected into syringes containing 1 mL of 3.8% sodium citrate solution and expressed into plastic tubes. Platelet-rich plasma (PRP) was obtained by centrifugation at 160g for 4.5 minutes at 22°C. The tube containing the PRP was capped to minimize pH changes, which can alter platelet sizes. The platelets were washed and the percent $^{35}$S incorporation measured as previously described.

Platelet size measurements were made using a Particle Data Instrument (Particle Data Inc, Elmhurst, IL) with a logarithmic scale as previously described. Platelet counts and hematocrits were taken by cardiac puncture after injection of a heparin-sodium pentobarbital solution. Approximately 0.5 mL of blood was collected into syringes containing 1 mL of 3.8% sodium citrate solution and expressed into plastic tubes. Platelet-rich plasma (PRP) was obtained by centrifugation at 160g for 4.5 minutes at 22°C. The tube containing the PRP was capped to minimize pH changes, which can alter platelet sizes. The platelets were washed and the percent $^{35}$S incorporation measured as previously described.

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Figure 1 shows the results of dose-response studies of TSF and IL-6 on $^{35}$S incorporation into platelets of rebound-thrombocytotic mice. As shown, TSF gave a highly significant linear relationship between the dose of TSF and the percent $^{35}$S incorporation into platelets ($r^2 = .99$). IL-6 also showed a dose-response relationship ($r^2 = .90$), but re-
The numbers on the bars represent the number of mice per treatment and the vertical bars represent the standard errors. HSA (7.5 mg/mouse), TSF (4 U/mouse), and IL-6 (40,000 U/mouse, 4 μg) were administered in either two or four injections. In all cases the mice were assayed 3 days after the first injection. The HSA control values for (A) were 3.99 ± 0.04 μl and the control values for (B) were 4.32 ± 0.16 x 10⁻³. The values were significantly greater compared with appropriate HSA controls: *P < .05, **P < .005.

Figure 2 shows the results of measuring percent ³⁵S incorporation into platelets of mice after two or four injections of either HSA, 4 U of TSF, or 40,000 U of IL-6 in both normal mice and mice in rebound-thrombocytosis (RAMPS-treated mice). The results showed that in both normal mice and RAMPS-treated mice, TSF significantly (P < .005) increased percent ³⁵S incorporation into platelets of mice after two or four injections. In agreement with previous studies, TSF had greater effects when administered to mice in rebound-thrombocytosis than when given to normal mice. IL-6, on the other hand, did not increase percent ³⁵S incorporation into platelets of mice after two injections, but after four injections IL-6 significantly increased platelet production (P < .05) in both normal and rebound-thrombocytotic mice. Although not shown, platelet counts of mice treated with either IL-6 or TSF were not increased when compared with counts of mice injected with HSA.

Platelet size measurements on blood of the same mice as presented in Fig 2 are shown in Fig 3. TSF caused significant (P < .005 to P < .0005) increases in platelet sizes at all injection schedules in both normal mice and rebound-thrombocytotic mice when compared with other mice treated with HSA. IL-6 did not increase platelet sizes of normal mice, but there were significant (P < .05 to P < .0005) increases in sizes of platelets in RAMPS-treated mice after IL-6 treatment. In the present study, TSF and IL-6 did not increase platelet counts of mice when measured 3 days after the initial injection.

Table 1 shows the results of measuring hematocrits of these mice. Normal mice treated with two injections of IL-6 had significantly (P < .0005) lower hematocrits on day 3 than mice treated with HSA. Otherwise, there were no changes in packed cell volumes of treated mice.

Figure 4 shows the results of measuring the percent SAE<sup>+</sup> cells in marrow of mice treated with HSA, 2 U of TSF, or 40,000 U of IL-6. In agreement with previous studies, TSF in small doses increased the percentage of SAE<sup>+</sup> cells (P < .005) compared with the results of other mice treated with HSA. High doses of IL-6 did not increase the percentages of SAE<sup>+</sup> cells of mice. Furthermore, preliminary experiments showed that 2, 4, or 10 x 10⁸ U of IL-6 did not stimulate an increase in percent SAE<sup>+</sup> cells, ie, after treatment with 7.5 mg of HSA, 6.7 ± 0.7 (4) percent SAE<sup>+</sup> cells were found in the BM of normal mice. After 2 x 10¹⁰ U of IL-6/mouse gave 7.1 ± 1.5 (6) percent SAE<sup>+</sup> cells; and 10 x 10¹⁰ U IL-6/mouse, resulted in 6.9 ± 0.8 (5) percent SAE<sup>+</sup> cells.

Table 2 shows the results of measuring the percent ³⁵S incorporation into platelets of TSF assay mice after injec-

<table>
<thead>
<tr>
<th>Material</th>
<th>Normal Mice</th>
<th>RAMPS-Treated Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Injections</td>
<td>4 Injections</td>
</tr>
<tr>
<td></td>
<td>2 Injections</td>
<td>4 Injections</td>
</tr>
<tr>
<td>HSA</td>
<td>46.2 ± 0.2 (20)</td>
<td>46.1 ± 0.2 (14)</td>
</tr>
<tr>
<td>TSF</td>
<td>45.5 ± 0.8 (5)</td>
<td>46.2 ± 0.3 (9)</td>
</tr>
<tr>
<td>IL-6</td>
<td>44.6 ± 0.3 (15)</td>
<td>46.3 ± 0.4 (10)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (number of mice/treatment). HSA was injected at 7.5 mg/mouse. TSF was injected for a total dose of 4 U/mouse. IL-6 was injected for a total dose of 40,000 U/mouse.

*Significantly lower than hematocrits of HSA-treated control mice, P < .0005.

Fig 2. A comparison of the percent ³⁵S incorporation into platelets of both normal mice (A, pool of three separate experiments) and rebound-thrombocytotic mice (B, pool of four separate experiments). The numbers on the bars represent the number of mice per treatment and the vertical bars represent the standard errors. HSA (7.5 mg/mouse), TSF (4 U/mouse), and IL-6 (40,000 U/mouse, 4 μg) were administered in either two or four injections. The HSA control values for mice in (A) were 6.27 ± 0.23 x 10⁻³ and the control values for (B) were 3.48 ± 0.16 x 10⁻³. The values were significantly greater compared with appropriate HSA controls: *P < .05, **P < .005.

Fig 3. A comparison of average platelet size of mice after various treatments in both normal mice (A) and rebound-thrombocytotic mice (B). The numbers on the bars represent the number of mice per treatment and the vertical lines represent the standard errors. HSA (7.5 mg/mouse); TSF (4 U/mouse), and IL-6 (40,000 U/mouse or 4 μg/mouse) were administered in either two or four injections. In all cases the mice were assayed 3 days after the first injection. The HSA control values for (A) were 3.99 ± 0.04 μl and the control values for (B) were 4.32 ± 0.05 μl. The values were significantly larger compared with appropriate HSA controls: *P < .05, **P < .005, ***P < .0005.
the vertical lines represent the standard errors. Mice treated with TSF administered as a single IP injection 8 hours before the mice were killed had significantly more SAChE+ cells than HSA-treated mice or IL-6.

The present study shows that both IL-6 and TSF will increase percent 35S incorporation into platelets of mice if given in four injections (Figs 1 and 2), but only TSF is effective when the material is given in two doses (Fig 2). Unlike TSF, IL-6 did not increase the numbers of SACH+E cells (Fig 4) or platelet sizes (Fig 3) of normal mice. This study agrees with previous studies showing that TSF increased both platelet production and megakaryocytopoiesis in rodents, i.e., percent 35S incorporation into platelets, percent SACH+E cells, and platelet sizes. Previous experiments showed that TSF will elevate platelet counts, megakaryocyte sizes and numbers, megakaryocyte endomitosis, megakaryocyte maturation, and megakaryocyte DNA content. The stimulatory effects of IL-6 on megakaryocyte endomitosis and maturation have apparently not yet been tested, but in the present study IL-6 did not stimulate percent SACH+E cell numbers and platelet sizes in normal mice. We present below additional evidence that the two cytokines are probably separate factors.

Previous studies have shown that administration of IL-6 in vivo will increase platelet counts, megakaryocyte size, and 35S incorporation into platelets of both mice and monkeys. Also platelet sizes were reported not to be elevated in mice by IL-6 treatment in a preliminary study. However, 16 μg (160,000 U) of IL-6 were reported to increase WBC counts, without significant changes in hematocrits or megakaryocyte numbers of recipient animals. Hill et al showed that 8 μg of IL-6 given to mice produced an increase in megakaryocyte and granulocyte-macrophage colony-forming cells in both the BM and spleen of mice. However, Asano et al showed in primates that injection of 80 μg/kg per day of IL-6 produced a loss of body weight, anemia, and other adverse side effects. It should be mentioned that in all these studies, large doses of rIL-6 (microgram quantities) were required to increase either megakaryocytopoiesis or thrombocytopoiesis.

In vitro studies, Ishibashi et al and Koike et al showed that IL-6 increased megakaryocyte sizes. Megakaryocyte ploidy and acetylcholinesterase content of megakaryocytes in culture were also reported to be increased by IL-6 treatment, and if IL-3 was added to cultures containing IL-6, an increase in megakaryocyte colonies was observed. However, Bruno and Hoffman showed that the addition of TSF or erythropoietin (Epo) to IL-6 decreased megakaryocytic colony formation. It was concluded that the effects of IL-6 are probably on accessory cells for the release or production of other cytokines. Williams et al showed that IL-6 would stimulate growth of immature mouse megakaryocytes. A similar conclusion was reached by other investigators who showed that IL-6 would increase the acetylcholinesterase content of cells without an increase in colony formation. Two recent reviews summarize this work. All these in vitro studies showed that IL-6 would enhance megakaryocytopoiesis, but the possibility exists that the effects may not be direct.

In the present study we used a partially purified TSF preparation that contained a small amount of IL-6 (~875 U of IL-6/U of TSF). Sufficient amounts of the more highly purified preparation for these studies were not available. Preliminary experiments and work presented herein showed no effects of IL-6 on percent 35S incorporation into platelets (Fig 1) or on the percentage of SACH+E cells when IL-6 was given in doses that were present in the TSF. Moreover, one experiment (Table 2) using a more highly purified preparation of TSF that was found to be free of IL-6 showed that this TSF preparation would still stimulate platelet production in rebound-thrombocytotic mice. Therefore, we do not believe that the small amount of IL-6 that was present in the TSF was responsible for the thrombocytopoietic stimulating effects seen in this study. It does not seem possible that a combination of IL-6 and other un-

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**DISCUSSION**

The table below shows the effects of Step III TSF on percent 35S incorporation into platelets of immunothrombocytotic mice:

<table>
<thead>
<tr>
<th>Material</th>
<th>Number of Mice</th>
<th>U/Mouse</th>
<th>Percent 35S Incorporation Into Platelets x 10^3 ± SE</th>
<th>% of Control*</th>
<th>P &lt; t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5</td>
<td>—</td>
<td>3.00 ± 0.16</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Step III TSF</td>
<td>5</td>
<td>0.3</td>
<td>3.73 ± 0.35</td>
<td>124</td>
<td>NS</td>
</tr>
<tr>
<td>Step III TSF</td>
<td>5</td>
<td>0.6</td>
<td>3.94 ± 0.37</td>
<td>131</td>
<td>.05</td>
</tr>
<tr>
<td>Step III TSF</td>
<td>5</td>
<td>0.9</td>
<td>4.40 ± 0.12</td>
<td>146</td>
<td>.0005</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. Step III TSF was found to be free of IL-6 by both Western blotting techniques and by the B9 cell proliferation assay.

*Correlation coefficient of data: r^2 = .95.

†Significantly greater than saline-injected control mice.

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**Table 2. Effects of Step III TSF on Percent 35S Incorporation Into Platelets of Immunothrombocytotic Mice**

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The stimulatory effects of IL-6 on megakaryocyte endomitosis and maturation have apparently not yet been tested, but in the present study IL-6 did not stimulate percent SACH+E cell numbers and platelet sizes in normal mice. We present below additional evidence that the two cytokines are probably separate factors.

Previous studies have shown that administration of IL-6 in vivo will increase platelet counts, megakaryocyte size, and 35S incorporation into platelets of both mice and monkeys. Also platelet sizes were reported not to be elevated in mice by IL-6 treatment in a preliminary study. However, 16 μg (160,000 U) of IL-6 were reported to increase WBC counts, without significant changes in hematocrits or megakaryocyte numbers of recipient animals. Hill et al showed that 8 μg of IL-6 given to mice produced an increase in megakaryocyte and granulocyte-macrophage colony-forming cells in both the BM and spleen of mice. However, Asano et al showed in primates that injection of 80 μg/kg per day of IL-6 produced a loss of body weight, anemia, and other adverse side effects. It should be mentioned that in all these studies, large doses of rIL-6 (microgram quantities) were required to increase either megakaryocytopoiesis or thrombocytopoiesis.

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known factors present in the TSF preparation could have caused the stimulation of thrombocytopoiesis observed herein, based upon our previous findings using purified TSF and the data presented in Table 2. A more likely explanation is that a specific thrombopoietic factor (TSF) within our preparation caused the increases in thrombocytopoiesis that we have observed. Experiments with rTSF will be necessary to resolve this issue.

The present work agrees with the work of Hill et al showing that high doses of IL-6 will increase platelet production by increasing incorporation of isotopes into platelets. Moreover, the two studies agree that there is a dose-response relationship between iso peptide incorporation and the amounts of IL-6 injected. However, it should be noted that in both studies positive responses required multiple injections of large doses of IL-6 (in the order of 40,000 to 80,000 U/mouse, 4 to 8 μg) for increase of iso peptide incorporation into platelets. In our study, two injections of IL-6 administered over a 1-day period did not elevate percent 35S incorporation into platelets of either normal or rebound-thrombocytotic mice. All other studies14 showing increase of thrombocytopoiesis by IL-6 required large doses over an extended period of time. It should also be mentioned that high levels of another cytokine (rEpo) will also increase 35S incorporation into platelets of assay mice.26

Our study agrees also with a preliminary report of Hill et al showing that mean platelet volumes were unaffected by IL-6 administration into normal mice.7 Although we showed that TSF treatment resulted in significant increases in platelet sizes of both normal and rebound-thrombocytotic mice, IL-6 did not increase platelet sizes of normal mice. However, increased platelet sizes of rebound-thrombocytotic mice were found after IL-6 injections. The reason for altered platelet sizes in rebound-thrombocytotic mice but not in normal mice after IL-6 is unknown. However, the fact that the rebound-thrombocytotic mice are more sensitive to exogenous preparations of platelet stimulating factors than are normal mice may play a role. Unlike IL-6, thrombopoietin from plasma of thrombocytopenic rabbits27 and TSF from HEK cell culture media7 were shown previously to stimulate increases in platelet sizes of normal mice. This apparent discrepancy in activities of the two cytokines on platelet sizes illustrates another reason for suspecting that TSF and IL-6 are separate entities.

In addition to a discrepancy in the effects of TSF and IL-6 on platelet sizes, SACH+E cells also were not altered by IL-6 treatment, whereas low doses of TSF (2 U/mouse) significantly increased (P < .005) the percent SACH+E cells in marrow of TSF-treated mice (Fig 4). Original studies by Jackson38 showed that large increases in the proportions of SACH+E cells occurred when rats were made thrombocytopenic. This study was confirmed in thrombocytopenic mice and in additional studies using TSF from HEK cell culture media.19 Plasma from thrombocytopenic mice that contained thrombopoietin,29 but is apparently free of IL-6,30 stimulated significant increases in SACH+E cells of recipient mice. The fact that IL-6 did not increase the percentages of SACH+E cells is another reason to suspect that IL-6 and TSF are separate cytokines.

In contrast to the results of previous studies,16 platelet counts were not increased by IL-6 in the present work. The reason(s) for this is probably because of different IL-6 injection schedules. All previous investigators gave large doses of IL-6 over an extended period of time (2½ to 16 days), whereas in our study the effects of IL-6 were examined after administering two or four injections over a 1- to 2-day period. This schedule was chosen because our previous study3 showed maximum increased platelet production in mice given TSF at this time.

Hematocrits in the present study were slightly decreased in normal mice given two injections of IL-6; no other hematocrit changes were noted. Why four injections of IL-6 did not produce anemia is unknown. Anemia was noted by Asano et al32 in monkeys given 20 to 80 μg/kg/d of IL-6. TSF did not cause significant decreases in hematocrits of treated mice, but did increase megakaryocytopoiesis and thrombocytopoiesis.

In addition to the differences in biologic responses of the two cytokines, there are several other reasons to predict that thrombopoietin and IL-6 are separate growth factors: (1) The two compounds have different molecular weights, ie, IL-6 has a molecular weight of about 26 Kd and the molecular weight of TSF is ~15 Kd.14 (2) Partially purified preparations of TSF (Step III TSF)14 have been shown to be free of IL-6 but still increase percent 35S incorporation into platelets of immun thrombocytopenic assay mice (Table 2). IL-6 was not found in Step III TSF by Western blotting techniques and by the B9 cell proliferation assay for IL-6.15 (3) Previously, nanogram quantities of purified TSF stimulated significant increases in percent 35S incorporation into platelets of assay mice,14 whereas in the present study and elsewhere16 microgram quantities of pure IL-6 were required for similar stimulation of platelet production. (4) Some kidney cell lines will not produce IL-6, but produce large quantities of TSF (unpublished results). (5) IL-6 is produced by a variety of tissues,28,29 whereas TSF appears to be produced in vitro only by kidney cells.1 (6) Recently, Hill et al30 reported that plasma IL-6 levels were not increased by induction of thrombocytopenia in mice, indicating that IL-6 is probably not involved in stimulating platelet production. (7) Some kidney cell lines will not produce IL-6, but produce large quantities of TSF (unpublished results). (5) IL-6 is produced by a variety of tissues,28,29 whereas TSF appears to be produced in vitro only by kidney cells.1 (6) Recently, Hill et al30 reported that plasma IL-6 levels were not increased by induction of thrombocytopenia in mice, indicating that IL-6 is probably not involved in stimulating platelet production. (7) Another recent study2 found that IL-6 levels did not correlate with platelet counts of patients, but IL-6 was elevated in sera of patients in response to inflammation. (8) Many growth factors (including Epo) will stimulate platelet production30 in mice if given in high enough doses. (9) Unlike thrombopoietin, IL-6 has a broad range of effects in vivo,32 as well as in vitro. For these reasons, we believe that IL-6 is not thrombopoietin and that thrombopoietin, not IL-6, is the physiologic regulator of thrombocytopoiesis.

ACKNOWLEDGMENT

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