Effects of Actinomycin D In Vivo on Murine Erythroid Stem Cells

By Kenneth S. Zuckerman, Richard Sullivan, and Peter J. Quesenberry

Low-dose actinomycin D (Acto) selectively suppresses murine erythropoiesis without decreasing erythropoietin (Ep) production. We used the plasma clot system to determine the stage of erythroid differentiation at which this inhibition occurs. Late erythroid precursors, CFU-E, and less differentiated committed erythroid stem cells, BFU-E, were assayed in CF1 mice given Acto 75–82 μg/kg/day or saline subcutaneously for 5 days. We also assayed pluripotent (CFU-S) and committed granulocyte-monocyte (CFU-C) stem cells. Reticulocytes and marrow and spleen nucleated erythroid precursors were decreased by 99% in the Acto-treated mice; tibial marrow CFU-E were decreased by 97% and splenic CFU-E by 99%. Tibial BFU-E were not decreased by Acto, although there was a 66% diminution in splenic BFU-E. Acto increased tibial CFU-S, but splenic CFU-S and tibial and splenic BFU-E were unchanged. Thus Acto inhibits erythropoiesis by suppressing the ability of immediate committed erythroid precursors of CFU-E or CFU-E themselves to differentiate further in response to Ep. Acto does not affect survival or proliferation of the less differentiated cells—CFU-C, CFU-S, and marrow BFU-E. The suppression of splenic BFU-E in Acto-treated mice may indicate that marrow and splenic BFU-E are basically different stem cells. Alternatively, Acto treatment may impair migration of BFU-E from marrow to spleen.

INJECTION OF LOW DOSES of actinomycin D (Acto) in mice suppresses erythropoiesis, as judged by reticulocyte counts, bone marrow differential cell counts, and 59Fe incorporation into circulating red blood cells.1-4 Daily administration of Acto 60 μg/kg results in almost complete inhibition of erythropoiesis within 3–4 days, which is completely reversible within 5 days of discontinuation of the drug.1

Erythropoietin (Ep), which is required for erythroid differentiation,5-7 induces RNA synthesis in erythroid precursor cells within 10–15 min of their exposure to Ep.8 This change is the earliest detectable biochemical event preceding differentiation into recognizable erythroid cells. This burst of RNA synthesis is prevented completely by prior exposure of the cells to Acto.8 Thus it seems likely that Acto blocks erythropoiesis by impairing Ep-mediated differentiation of erythroid precursor cells.

In doses of less than 400 μg/kg Acto does not prevent maturation of erythroid cells more mature than pronormoblasts,1 does not interfere with pluripotent stem cell (CFU-S) proliferation or differentiation,1,9 does not inhibit proliferation of granulocytic progenitors (CFU-C),4,9,11 and does not inhibit...
transplantable erythroid repopulating cells, which are presumably pluripotent or committed erythroid stem cells.\textsuperscript{3,10,11}

Recently a plasma clot clonal assay system in vitro was introduced that permitted the quantification of relatively mature erythroid precursors (CFU-E) and of less differentiated committed erythroid stem cells (BFU-E). We used the plasma clot assay along with the CFU-S assay in order to assess at what stage of stem cell differentiation Acto exerts its inhibitory effect. We also studied the effect of Acto on the CFU-C, a putative granulocytic stem cell, in order to assess the specificity of any observed inhibition.

\section*{MATERIALS AND METHODS}

Virgin female CF\textsubscript{1} (Carworth Farm) mice 12-16 wk old were used in most experiments. In two separate experiments 20-24-wk-old virgin female BDF\textsubscript{1} (C57B1/6J x DBA-2 F\textsubscript{1} hybrid) mice (Jackson Laboratories) were used. Five mice were included in each experimental group. Actino-
mycin D (Cosmegen, Merck Sharp & Dohme, West Point, Pa.) was diluted in sterile triple-distilled water to a concentration of 7.5-8.2 \textmu g/ml. Each mouse receiving Acto was injected subcutaneously (SC) with 0.01 ml of this solution/g of body weight (75-82 \textmu g/kg) daily for 5 days. Control mice received 0.9 NaCl (saline) 0.01 ml/g body weight subcutaneously daily for 5 days.

Approximately 4 hr after the last saline or Acto injection on day 5, mice were anesthetized with ether and blood was collected by cardiac puncture. A small sample of blood was anticoagulated with sodium EDTA (ethylenediaminetetraacetate) and used for hematocrit and reticulocyte determinations. In those studies in which blood values were not determined, mice were killed by cervical dislocation without ether anesthesia. Results were similar with either method of killing. The spleen was removed under sterile conditions. Single-cell spleen suspensions were prepared in single-strength Eagle medium (E\textsubscript{1010}) or Eagle minimum essential medium -Hank's balanced salt solution with 2\% heat-inactivated fetal calf serum (MEM-HBSS). The spleens were minced with scissors, and the suspension was aspirated 20 times through a syringe with no needle and then twice through a 25-gauge needle. Cells were flushed from individual tibias with E\textsubscript{1010} or MEM-HBSS. Nucleated cell counts were performed using a hemocytometer, and total tibial and spleen cellularity was calculated.

Paint brush smears were obtained from the femurs of the experimental animals. Five hundred cells per femur were counted from Wright-Giemsa stained smears for calculating the marrow differential counts. Paint brush smears were also made of the cell buttons from centrifuged spleen cell suspensions. One thousand cell differentials were performed on Wright-Giemsa stained smears of each cell suspension.

Hematocrits were done by the microhematocrit method. Reticulocyte counts were determined by counting 1000 erythrocytes per slide on smears stained with new methylene blue.

\textbf{CFU-C Assay}

Assays of the number of CFU-C per tibia and spleen were performed using a previously described modification\textsuperscript{16} of the double-layer soft agar technique of Bradley and Sumner\textsuperscript{17} or the single-layer technique of Metcalf and Foster.\textsuperscript{18}

\textbf{CFU-S Assay}

The number of CFU-S per tibia and spleen was determined by a modification\textsuperscript{19} of the method of Till and McCulloch.\textsuperscript{20} In the most recent experiments assay mice received 900 R whole-body gamma irradiation from a \textsuperscript{137}Cs radiation source delivered at a dose rate of 118 R/min.

\textbf{CFU-E and BFU-E Assays}

The number of CFU-E in tibial marrow and spleen was assessed using a modification of the plasma clot technique described by McLeod et al.\textsuperscript{13} A mixture was prepared consisting of the following components: (1) 2 parts NCTC-109; (2) 2 parts heat-inactivated fetal bovine serum; (3) 1 part citrated bovine plasma; (4) 1 part 10\% deionized fraction V bovine serum albumin:
Means ± 1 SEM of the total number of nucleated cells per tibia (x 10^6). Five hundred cell differential counts were done on marrows from 19 saline-treated and 20 Acto-treated mice.

ACTINOMYCIN AND ERYTHROID PRECURSORS 959

(5) 1 part l-asparagine (0.5 ml l-asparagine 2 mg/ml; 0.25 ml 7% NaHCO₃; 4.25 ml NCTC-109); (6) 1 part Ep 2.5 U/ml in NCTC-109 (final concentration in the clot was 0.25 U/ml). In the initial studies sheep plasma Ep was used (4.7 U/mg protein) (Connaught Laboratories, Willowdale, Ont.). In later experiments human urinary Ep was used (54.5 U/mg protein) (supplied by the National Heart, Lung and Blood Institute).

Tibial marrow cells were suspended in MEM-HBSS in the proper concentration for plating, 5-10 x 10^6 nucleated cells per 0.1 ml plasma clot. Spleen cells were also suspended in MEM-HBSS and plated at a concentration of 2.5-5 x 10^5 nucleated cells per 0.1 ml plasma clot. After addition of the cell suspension to the above mixture, 1 part beef embryo extract (diluted 1:5; beef embryo extract to NCTC-109) was added, the solution was mixed well, and 0.1 ml was pipetted into each of four microtiter wells (Linbro Scientific, New Haven, Conn.). The cultures were then incubated and numbers of CFU-E were determined as described by McLeod et al. 13

Tibial and splenic BFU-E were assayed by a modification of the technique of Axelrad et al. 14

The culture medium and cell suspensions were prepared as described above for the CFU-E assay, except that the Ep dilution used was 30 U/ml for a final concentration in the plasma clot of 3 U/ml clot. No Ep was added to cultures on subsequent days. Then 0.5 ml of culture solution was pipetted into each of four culture plate wells of 2 ml capacity each (Linbro). The number of nucleated tibial cells per 0.5 ml clot was 2.5-5 x 10^5, and the number of spleen cells was 1.25-2.5 x 10^6 per clot. Cells were cultured and BFU-E derived colonies counted as described by Axelrad et al. 14

Ep Sensitivity Curves

Ep sensitivity of tibial and splenic CFU-E from control and Acto-treated mice was assessed using varying concentrations of Ep (0.1-2.0 U/ml clot). Four clots were used for each experimental group.

Cell Mixture Experiments

In order to assess if enough Acto could have been carried over to the culture plates in the marrow or spleen cell suspensions to account for the observed inhibition of CFU-E from the Acto-treated mice, cells from Acto-treated mice were mixed with cells from saline treated mice; 5 x 10^6 control tibial cells were mixed with 5 x 10^6 or 1 x 10^7 Acto tibial cells, and the ensuing number of CFU-E derived colonies was compared to that seen with 5 x 10^6 or 1 x 10^7 control cells alone or 5 x 10^4 Acto cells alone. Similarly, in three experiments 2.5 x 10^5 control spleen cells were mixed with 2.5 x 10^5 or 5 x 10^5 Acto spleen cells, and the number of CFU-E derived colonies was compared with that seen with 2.5 x 10^5 or 5 x 10^5 control cells or with 2.5 x 10^5 Acto cells alone. In some experiments, Acto cells were washed with 15-50 ml MEM-HBSS five times prior to plating Acto cells either alone or in combination with control cells. Similar studies were performed with tibial and splenic BFU-E. Since the plasma clots used in the BFU-E assay contained five times the volume used in the CFU-E assay, all BFU-E clots contained five times the number of cells used in the equivalent groups in the CFU-E assay.

Kinetic Studies

In two experiments, we varied the Acto dosage schedule to evaluate progressive changes in peripheral blood reticulocyte counts and marrow differentials, CFU-E, BFU-E, CFU-S, and, in one experiment, CFU-C. Groups of four CF1 female mice 14-16 wk old received Acto 75 μg/kg/
Table 2. Effect of Low-Dose Actinomycin D on Spleen Nucleated Cell Differential Counts

<table>
<thead>
<tr>
<th></th>
<th>Proliferative</th>
<th>Nonproliferative</th>
<th>Lymphoid Cells</th>
<th>Erythroid Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline</strong></td>
<td>1.17 ± 0.30</td>
<td>6.56 ± 0.99</td>
<td>222 ± 11</td>
<td>25.5 ± 1.0</td>
</tr>
<tr>
<td><strong>Acto</strong></td>
<td>1.72 ± 0.61</td>
<td>19.6 ± 4.90</td>
<td>209 ± 33</td>
<td>0.26 ± 0.16</td>
</tr>
</tbody>
</table>

Means ± 1 SEM of the total number of nucleated cells per spleen (× 10^-6). One thousand cell differential counts were done on each of four spleen cell suspensions from both saline-treated and Acto-treated mice.

day or saline subcutaneously for, 1, 2, or 3 days and were killed approximately 24 hr after the last injections.

**Statistical Methods**

Statistical significance was assessed using Student’s t test.21

**RESULTS**

**Hematocrits, Reticulocytes, Marrow and Spleen Differential Cell Counts**

The mean hematocrit ± 1 SEM for the saline-treated mice was 46.0\(\pm\)1.2\(\%\), and for the Acto treated mice was 45.2\(\pm\)1.0\(\%\). Acto treatment, however, resulted in a 99\(\%\) reduction in peripheral blood reticulocytes, from 1.47\(\pm\)0.07\(\%\), in saline-treated mice to 0.02\(\pm\)0.00\(\%\), in the Acto-treated mice (\(p < 0.001\)). Marrow cell differentials, expressed as number of cells per tibia, are shown in Table 1. The Acto-treated group had significant increases in tibial marrow proliferative granulocytes (\(p < 0.001\)) and nonproliferative granulo-

![Fig. 1. Effect of actinomycin D on CFU-E. Mean ± SEM. Number of experiments shown in parentheses.](image)
cytes \( p < 0.001 \), a moderate decrease in lymphoid cells \( p < 0.001 \), and a marked diminution \( 99.6\% \) in nucleated erythroid cells \( p < 0.001 \). Splenic nucleated cell differentials are presented as number of cells per spleen in Table 2. The Acto-treated group had no significant increase in proliferative granulocytes, a threefold increase in nonproliferative granulocytes, no change in lymphoid cells, and a \( 99\% \) decrease in nucleated erythroid cells in the spleen \( p < 0.001 \).

**CFU-E**

The numbers of tibial marrow and splenic CFU-E in the saline and Acto-treated mice are compared in Fig. 1. Acto treatment resulted in a \( 97.3\% \), decrease in tibial CFU-E \( p < 0.001 \) and a \( 98.7\% \), decrease in splenic CFU-E \( p < 0.02 \). These changes were comparable to those seen with peripheral blood reticulocytes and with marrow and splenic nucleated erythroid cells.

In order to evaluate the possibility that the decreased CFU-E in Acto-treated mice could be due to altered sensitivity of the CFU-E to Ep, tibial marrow and spleen cells were cultured for 2 days with concentrations of Ep ranging from

---

**Fig. 2.** Erythropoietin sensitivity of CF1 marrow and splenic CFU-E. Mean ± SEM from quadruplicate plasma clots in one experiment.

---

**Fig. 3.** Mixture of cells from actinomycin D-treated and control CF1 mice. Mean ± SEM. There were four experiments with marrow cells and three with spleen cells. Value for the group in which cells from Acto-treated and control mice were mixed expressed as number of control CFU-E/10⁷ control cells. This number was derived by subtracting the number of CFU-E in Acto-treated group from total CFU-E in the mixture group, then calculating the results as CFU-E/10⁷ control cells in the culture.
0.1 to 2.0 U/ml clot. The results of one experiment using human urinary Ep are shown in Fig. 2. In one other experiment, using sheep plasma Ep, tibial CFU-E were assayed; the results were similar to those seen with the human urinary Ep. The number of assayable tibial CFU-E from Acto-treated mice increased progressively from 35 per $10^6$ nucleated cells with 0.1 U Ep/ml to 193 per $10^6$ nucleated cells with 2.0 U Ep/ml. However, with as much as four to eight times the usual optimal Ep concentration the maximum number of tibial CFU-E from the Acto-treated mice was only 12%, of the maximum number from the saline-treated mice. There was no increase seen in the number of splenic CFU-E from Acto-treated mice over the range of Ep concentrations from 0.1 to 2.0 U/ml. At the highest concentration of Ep used, the maximum number of splenic CFU-E in the Acto-treated group was 2%, of the maximum number from the spleens of saline-treated mice.

Table 3. Effect of Low-Dose Actinomycin D on Tibial Marrow and Spleen BFU-E in BDF$_1$ Mice

<table>
<thead>
<tr>
<th></th>
<th>Tibia</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2174 ± 16</td>
<td>338 ± 68</td>
</tr>
<tr>
<td>Acto</td>
<td>2531 ± 31</td>
<td>98 ± 98</td>
</tr>
</tbody>
</table>

Means ± 1 SEM of the total number of BFU-E per tibia or spleen. Results are based on quadruplicate plasma clots in each of two experiments.
In order to rule out a carryover effect of Acto from the cell suspensions on culture growth, Acto cells and control cells were mixed and numbers of CFU-E were compared with the number seen with each cell population cultured separately. The results of these experiments with both tibial and spleen cell suspensions are shown in Fig. 3. Mixture of Acto cells with control cells using either tibial marrow or spleen resulted in no significant decrease from the number of CFU-E expected if there was neither inhibition nor potentiation of growth in these mixtures. Extensive washing of both tibial and splenic cell suspensions from Acto-treated mice resulted in no increase in the number of erythroid colonies as compared with that seen with unwashed cells. In addition, as expected, mixture of these washed Acto cells with control cells resulted in no inhibition of colony growth by the cells from the control mice.

BFU-E

Tibial and splenic BFU-E from saline- and Acto-treated mice are shown in Fig. 4. In marked contrast to CFU-E, tibial BFU-E were not diminished in the Acto-treated group. In contrast, splenic BFU-E in Acto-treated mice were decreased by $66\%$ ($p < 0.05$). These results, obtained in CF1 mice, were confirmed in two additional experiments with BDF1 mice, the results of which are shown in Table 3. Tibial BFU-E were increased by $18.4\%$ in the Acto-treated group; as in the experiments with CF1 mice, splenic BFU-E in Acto-treated BDF1 mice were decreased (by $71\%$) from control values.

Tibial and splenic BFU-E assays were also performed with washed Acto cells alone or with mixtures of control cells and Acto cells or washed Acto cells in two experiments. The results, similar to those found with CFU-E, demonstrated neither potentiation nor suppression by Acto or washed Acto cells of BFU-E from tibias or spleens of saline-treated animals.
Means ± 1 SEM of 6-8 mice per group; 500 cell differential counts were done from the marrow of each of eight mice per group.

1. **CFU-S**

   The numbers of CFU-S per tibia and spleen of both saline- and Acto-treated mice are shown in Fig. 5. There was a significant increase in tibial marrow CFU-S in the Acto-treated mice ($p < 0.02$) but no change in splenic CFU-S.

2. **CFU-C**

   The numbers of CFU-C per tibia and spleen in saline- and Acto-treated mice are shown in Fig. 6. Acto treatment of the mice resulted in no significant change in the numbers of CFU-C in either organ, although there was great experimental variability.

### Table 4. Progressive Effect of Low-Dose Actinomycin D Treatment on Marrow Nucleated Cell Differential Counts and Peripheral Blood Hematocrit and Reticulocytes

<table>
<thead>
<tr>
<th>Acto (Days)</th>
<th>Hct (%)</th>
<th>Rets (%)</th>
<th>Granulocytes</th>
<th>Lymphoid Cells</th>
<th>Erythroid Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proliferative</td>
<td>Nonproliferative</td>
<td>Cells</td>
</tr>
<tr>
<td>0</td>
<td>42.3 ± 1.3</td>
<td>1.2 ± 0.1</td>
<td>1.40 ± 0.11</td>
<td>5.47 ± 0.34</td>
<td>2.48 ± 0.12</td>
</tr>
<tr>
<td>1</td>
<td>42.2 ± 1.4</td>
<td>1.6 ± 0.2</td>
<td>1.08 ± 0.03</td>
<td>5.73 ± 0.15</td>
<td>2.75 ± 0.19</td>
</tr>
<tr>
<td>2</td>
<td>42.3 ± 1.9</td>
<td>1.4 ± 0.2</td>
<td>1.45 ± 0.06</td>
<td>5.97 ± 0.16</td>
<td>2.65 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>40.1 ± 1.3</td>
<td>0.6 ± 0.1</td>
<td>2.20 ± 0.11</td>
<td>7.44 ± 0.30</td>
<td>2.32 ± 0.16</td>
</tr>
</tbody>
</table>

Means ± 1 SEM of 6-8 mice per group; 500 cell differential counts were done from the marrow of each of eight mice per group.
Fig. 7. Effect of actinomycin D on CF₁ tibial stem cell levels. Mean ± SEM. Values for BFU-E and CFU-E are means of seven or eight cultures in two separate experiments with pooled cells of four mice per group. CFU-S values are means of 14 or 15 total assay mice in two experiments using pooled cells from four mice per group. CFU-C values are means of five cultures in one experiment.

Kinetic Studies

Peripheral blood and marrow changes over 3 days of Acto treatment are shown in Table 4. Reticulocyte numbers remained at or above control levels after 1 and 2 days of Acto but decreased to 46% of control after 3 days. This decrease in peripheral blood reticulocytes was preceded by a decrease in marrow nucleated erythroid cells to 15% of control after 2 days of Acto treatment and less than 1% of control after 3 days. The progressive changes in numbers of marrow stem cells after 0–3 days of Acto treatment are shown in Fig. 7 as percentages of control values. Absolute numbers of stem cells (± I SEM) per tibia in control mice in these experiments were 30,401 ± 4,929 CFU-E, 984 ± 38 BFU-E, 52,276 ± 2,654 CFU-C, and 3,333 ± 517 CFU-S per tibia.

DISCUSSION

The present studies show that concomitant with the inhibition of differentiated erythropoiesis, both tibial marrow and splenic CFU-E are virtually abolished by low-dose Acto treatment (Fig. 1). This depression is a true effect in vivo of Acto and is not due to carryover in vitro of Acto into the cultures (Fig. 3). The abolition of CFU-E is most likely due to the permanent inability of the CFU-E or their immediate precursors to differentiate further in response to Ep, although this finding may be due, at least in part, to a markedly impaired sensitivity of CFU-E to Ep (Fig. 2).

CFU-E are believed by most investigators to be either pronormoblasts or immediate precursors of pronormoblasts, rather than less mature committed erythroid stem cells. Reissman and Ito showed convincingly that the major effect of Acto in suppressing erythropoiesis is the prevention of Ep-induced differentiation of erythroid precursor cells into recognizable pronormoblasts. Exposure of erythroid precursor cells to Ep results in a rapid induction
of RNA synthesis, which is the first detectable change prior to recognizable differentiation into more mature erythroid cells. Both Ep-mediated RNA synthesis and erythroid differentiation are blocked by prior exposure of the cells to Acto.

Two other experimental models in which detectable erythropoiesis is abolished are the exhypoxic polycythemic mouse and the hypertransfused polycythemic mouse. In both cases erythropoiesis is almost completely eradicated, as judged by the criteria of peripheral blood reticulocyte numbers, 59Fe incorporation into peripheral blood erythrocytes, and marrow and splenic erythroid nucleated cell numbers. These two models differ from the Acto-treated mouse model in that in the former cases erythropoiesis is abolished by suppression of Ep production and is readily reestablished by Ep administration, whereas in Acto-treated mice Ep production remains normal but its effect in inducing differentiation of committed erythroid precursor cells into recognizable pronormoblasts is blocked by Acto. As shown by the current study of Acto treatment and prior reports utilizing the polycythemic mouse, both of these types of erythropoietic suppression are characterized by a diminution in assayable CFU-E that parallels the other measured parameters of erythropoiesis.

In contrast to the marked suppression of differentiated erythropoiesis and assayable CFU-E in Acto-treated mice, CFU-S and marrow BFU-E were not suppressed. Since Acto blocks the effect of Ep on erythroid precursor cells, the lack of effect of Acto on BFU-E suggests that CFU-E are not dependent on Ep activity for their existence. This hypothesis is supported by other studies utilizing the polycythemic mouse system in which Ep has been suppressed. These experiments demonstrated that BFU-E survival is not Ep dependent. An alternative explanation for these results is that CFU-E but not BFU-E are sensitive to a direct effect of Acto separate from its effect on Ep-induced differentiation. Similarly, as shown in a number of studies, CFU-S survival and proliferation does not appear to be Ep dependent. Acto therefore apparently blocks erythroid differentiation at some stage between the BFU-E and the pronormoblast.

The fact that there are virtually no CFU-E-derived colonies in cultures of cells from Acto-treated animals suggests three possibilities: (1) CFU-E are present but were permanently prevented by Acto from differentiating in response to Ep and thus cannot be detected in this system in vitro; (2) CFU-E were selectively killed because of selective diffusion into these cells of high concentrations of Acto; (3) there is a block in the erythroid differentiation pathway at or beyond the BFU-E stage so that there are no CFU-E present (in vivo or in vitro) on which Ep can act. The last possibility is supported by observations in the polycythemic mouse models, in which there is a transient block in differentiation of BFU-E to CFU-E because of absence of Ep rather than damage to erythroid precursor cells. Because the Acto-treatment model of suppressed erythropoiesis depends on a different mechanism to block erythroid differentiation, i.e., rendering erythroid precursor cells insensitive to Ep, the presence or absence of CFU-E is not necessarily analogous in the two different situations. If there is a block at the BFU-E stage in Acto-treated mice, it is pre-
sumably only a transient block rather than permanent damage to the BFU-E, since tibial BFU-E proliferate and differentiate normally in vitro in the presence of Ep. Additional evidence that committed erythroid stem cells are not permanently prevented from differentiating is the rapid recovery of erythropoiesis that occurs once Acto is discontinued. It remains to be shown which of the above mechanisms is operative in the case of Acto suppression of erythroid differentiation.

The studies of Ep sensitivity of CFU-E from control and Acto-treated mice indicate that there may be a small population of Acto-resistant marrow CFU-E (Fig. 2). Increasing concentrations of Ep resulted in a definite, although small, increase in numbers of detectable marrow CFU-E from Acto-treated mice. This finding suggests that there may be a subset of CFU-E requiring high levels of Ep and resistant to the action of Acto. Interestingly, there did not appear to be any increase in the numbers of splenic CFU-E from Acto-treated mice with increasing Ep concentrations. Further studies will be required to determine the significance of these findings.

An unexpected finding of this study was the decreased number of assayable splenic BFU-E in the Acto-treated mice, despite normal to elevated marrow BFU-E. Our experiments demonstrated that these results were not simply due to a carryover of Acto from the murine spleen suspensions into the culture plates. One possible explanation would be that splenic “BFU-E” and marrow “BFU-E” are actually different cells. If this is the case the difference is not based on differing proliferative status, since Hara and Ogawa and Iscove showed that marrow and splenic BFU-E have virtually identical proliferative rates that do not change with either stimulation or suppression of erythropoiesis. Splenic BFU-E may represent a more differentiated progeny of marrow BFU-E and thus be more responsive to Ep and more sensitive to inhibition by Acto.

An alternative possible cause of this decrease in splenic BFU-E would be an impairment of migration of BFU-E from marrow to spleen. Studying hypertransfused mice, Hara and Ogawa obtained similar results to those reported here, i.e., slightly increased marrow BFU-E and greatly decreased splenic BFU-E 5 days after the beginning of hypertransfusion. In sequential studies they found a partial recovery in splenic BFU-E from a nadir of about 25% of normal on day 5 to about 60% of normal on days 7–12. In separate studies they showed that after erythropoietic stimulation there were decreased marrow and increased splenic BFU-E, implying that there is normally a continual marrow to spleen migration of BFU-E and that this rate of migration diminishes with suppression of erythropoiesis. Thus the decrease in splenic BFU-E in Acto-treated mice may be attributable to a decrease in this proposed migratory stream of erythroid stem cells from the bone marrow to the spleen.

We found a modest but not significant increase in marrow granulocyte-monocyte precursors (CFU-C) in mice treated with Acto. We also found significant increases in marrow and spleen differentiated granulocyte precursors in Acto-treated mice. In addition, Reissman and Ito showed that daily low-dose Acto administration resulted in a sustained granulocytosis at least through 4 wk of Acto treatment and that repeated endotoxin challenges always resulted
in increased blood granulocyte counts, presumably from mobilization of marrow granulocytes. Thus low-dose Acto did not cause a depletion of CFU-C or more differentiated marrow granulocytes.

In these studies there was no suppression of pluripotent stem cells by Acto. In fact, marrow CFU-S were significantly increased. The reasons for the increase in CFU-S and possible increase in CFU-C after Acto treatment need to be elucidated further.

We conclude that low-dose Acto inhibits erythropoiesis by selectively suppressing the ability of a committed erythroid precursor cell to differentiate further in response to Ep. The number of assayable CFU-E is suppressed to a degree similar to that of more mature erythroid cells. The CFU-E, a relatively differentiated precursor of the pronormoblast, most likely represents a stage of development at which Ep is required for further maturation into recognizable nucleated erythroid cells. Our studies indicate that Acto selectively blocks the ability of an immediate committed erythroid precursor of the CFU-E or the CFU-E itself to differentiate in response to Ep. Acto does not impair the ability of CFU-C, CFU-S, or marrow BFU-E to proliferate, and at least does not prevent them permanently from differentiating. Furthermore, we showed that marrow BFU-E do not require Ep for their maintenance and proliferation but do require Ep for further differentiation into CFU-E and more mature erythroid cells. Finally, these results indicate that splenic BFU-E numbers are depressed in Acto-treated mice at a time when marrow BFU-E numbers are unchanged. This differential effect may indicate that marrow and splenic BFU-E are basically different stem cells, possibly having a parent-progeny relationship. A reasonable alternate explanation of these results is that Acto treatment may impair migration of BFU-E from marrow to spleen.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Marie Ryan for her excellent technical assistance, to Michael Austin and Ronna Goodman for their secretarial assistance in preparation of this manuscript, and to Dr. H. F. Bunn for his helpful review of the manuscript.

REFERENCES

9. Preisler HD, Henderson ES: Effect of


Effects of actinomycin D in vivo on murine erythroid stem cells

KS Zuckerman, R Sullivan and PJ Quesenberry