Assessment of Erythrocytic and Granulocytic Colony Formation in an In Vivo Plasma Clot Diffusion Chamber Culture System

By H. N. Steinberg, E. S. Handler, and E. E. Handler

Normal rat bone marrow cells seeded into a plasma clot diffusion chamber culture developed into erythrocytic and granulocytic colonies in vivo. Chambers implanted into the peritoneal cavity of normal hosts showed erythrocytic colony numbers reaching an initial peak on day 2, declining on days 3–5, and increasing in a secondary growth phase on day 7. Day 2 colonies were evenly dispersed; day 7 colonies were grouped into discrete areas of bursts. Granulocytic colony numbers reached a peak on day 4 and gradually declined through day 7. Cells in various stages of differentiation could be detected in both colony types. Colony numbers were proportional to the number of marrow cells seeded into the chamber. Host animals treated with phenylhydrazine induced a marked increase in erythrocytic colony numbers and size and a decrease in granulocytic colony formation. Host animals treated with endotoxin suppressed erythrocytic colonies while increasing granulocytic colony size. This method may prove advantageous for the study of hematopoietic colony formation in a physiologic environment.

METHODS are presently available for the quantitative assessment of hematopoietic stem cells. These procedures depend upon the formation of observable discrete colonies of progeny cells whose number and morphological characteristics can be readily determined. Colonies derived from pluripotential stem cells (CFU-S) have been assayed in vivo using the spleen colony method. Committed precursor cells of the granulocytic (CFU-C) and erythrocytic (CFU-E) lines have been assayed in vitro in agar colony and plasma clot culture systems. The proliferation and subsequent differentiation of progenitor cells require a proper hematopoietic inductive microenvironment and/or the addition of specific regulatory serum factors. The recent use of diffusion chambers (DC) to compartmentalize hematopoietic elements has allowed progenitor cells to grow in a more physiologic environment. At the same time, this isolation has eliminated problems of cell migration which may complicate interpretation of data from in vivo systems. However, colony formation, not normally seen in DC cell suspensions, may require an environmental architectural support. Recently, Gordon has used an agar diffusion chamber technique to support the growth of granulocytic and macrophage colonies. We have successfully placed plasma clots into DC, thereby providing a support system to make possible the study of colony formation by erythroid and myeloid progenitors in vivo. The method permits the development of erythrocytic and granulocytic colonies in the same chamber.

The present study was designed to (1) establish a reliable system for the direct...
assessment of CFU-E and CFU-C using a combined in vivo plasma clot-diffusion chamber technique, and (2) examine alterations in the functional capacity of these committed stem cells when cultured in host animals exposed to hematopoietic perturbation.

**MATERIALS AND METHODS**

All animals used were male Long-Evans rats weighing 280–300 g maintained on a diet of Purina Lab Chow and tap water ad libitum. Untreated rats were the source of normal bone marrow cells for culture in diffusion chambers implanted into recipient hosts. Chambers were implanted into normal untreated rats and rats pretreated with endotoxin or phenylhydrazine. Experiments were performed so that the same marrow sample was implanted into different host animals. Rats receiving endotoxin were injected intraperitoneally with 20 µg Salmonella typhosa lipopolysaccharide 4–5 hr prior to chamber implant. Rats rendered anemic received two intraperitoneal injections of 50 mg/kg phenylhydrazine; one 24 hr and the second 3–4 hr prior to chamber implantation. Hematocrits and total and differential peripheral leukocyte determinations were made on free-flowing tail blood of chamber recipients.

**Cell Suspensions and Medium**

All surgical equipment and glassware were dry-heat sterilized. Medium consisting of 20% heat-inactivated fetal calf serum in McCoy’s 5A was passed through a 0.2 µ Nalge Filter Unit to prevent bacterial contamination. Preparations of cell suspensions and surgical procedures were performed under a vertical laminar air-flow unit. Both femurs of normal donor rats were aseptically removed and the marrow flushed out with media using a hypodermic syringe. A cell suspension was prepared by passage through a 44.0-µ nylon mesh. An aliquot was removed for total and differential cell analysis. Cells were diluted to the appropriate concentration and stored on ice.

**Diffusion Chambers**

Diffusion chambers were prepared by adhering, with MF cement No. 1, 0.22-µ GS type Millipore filters to both sides of a plastic ring. The constructed chamber was tested for leaks and placed under UV light for 2 hr. Each chamber was wetted with media and seeded with $5 \times 10^5$ cells in 120 µl of medium. The cell suspension was injected and 20 µl of citrated bovine plasma was added to the chamber, which was immediately stoppered with a plastic plug, quickly heat sealed with a hot metal rod, and shaken vigorously to insure a uniform distribution of cells within the chamber. Each completed chamber was set aside for 3–5 min until the clot was formed. The DC were transferred into ice-cold medium where they remained until implantation. We found that seeded chambers may be stored in cold medium for up to 1 hr without altering the numbers of colonies formed during subsequent incubation. Four chambers were implanted into the peritoneal cavity of each host animal.

**Harvest and Fixation of Plasma Clot DC Culture**

Diffusion chambers were removed from host animals at intervals over a period of 7 days. The chambers were cleaned of adhering tissue and placed in ice-cold medium. Using a thin, sharp, disposable microtome knife, one Millipore filter was carefully removed without disturbing the clot and examined under the microscope; it was routinely found to be free of cells. The remaining filters and their adhering clots were then removed from the plastic ring by careful trimming with a blood styllet. The clot, still attached to the filter, was placed face down on a precleaned slide and allowed to settle for 2–3 min. We found that leaving the clot attached to one of the Millipore filters insured ease of handling, maintained the integrity of the clot and did not interfere with the final examination of colonies on a permanent slide. Excess fluid was absorbed from the clot by the repeated application of Whatman No. 1 filter paper to the filter; the clot was pressed lightly to insure flattening. The clot preparation was then fixed with 5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.0–7.2). Using a pasteur pipet, the fixative was added slowly to the filter paper overlying the preparation and allowed to stand for 6 min. The clot was then removed from the slide and placed in distilled water for 8 min. Excess water was removed by gentle blotting with filter paper, and the clot was stained with benzidine-hematoxylin. The clots were
placed face up on a slide (the Millipore filter adhered to the slide), and the stains were added
directly with a pasteur pipet. Clots were thoroughly dried with a cold-air hair dryer. In order
to reduce shrinkage and cracking, clots were sandwiched between fine wire gauze during the
drying period. The dried preparation was then placed in immersion oil until the attached Milli-
pore filter appeared transparent. A permanent slide was then prepared, the cover slip was
weighted to insure a flat preparation and allowed to set for 2 days.

**Scoring of Colonies**

Clot preparations were evaluated at × 400 magnification. Colonies were examined according
to type, numbers of cells per colony, and the degree of cell maturation within each colony. My-
eloid colonies were scored when consisting of at least 20 cells. Erythroid colonies were scored as
benzidine-positive units of four to seven cells and eight cells or greater. Bursts were counted
depending upon the spatial orientation of erythroid colonies.

**RESULTS**

Rats injected with endotoxin within 1 hr manifested an initial leukopenia
followed by a gradual leukocytosis which was maintained for approximately
10 hr. The total WBC counts during the remainder of the 7-day study were
normal; differential counts displayed a slight neutrophilia. In rats rendered
anemic with phenylhydrazine, hematocrit values dropped from an average of
45% to a mean of 26% during the first 3 days of study. Thereafter, hematocrits
rose slowly and returned to normal by day 6.

The proliferation of normal bone marrow cells seeded into diffusion cham-
ber plasma clot cultures implanted into the peritoneal cavity of rats was char-
acterized by the formation of discrete erythrocytic and granulocytic colonies.
The pattern of colony formation over the time course of 7 days in normal hosts
is given in Fig. 1. Pretreating recipient rats with endotoxin (Fig. 2) and phenyl-
hydrazine (Fig. 3) markedly altered both erythroid and granulocytic colonies.

**Granulocytic Colonies**

Granulocytic colony growth in normal hosts was characterized by an increase
in colony numbers from days 1–4. Linear regression analysis indicated a coeffi-
Fig. 2. Mean numbers of colonies counted per clot in chambers removed daily from rats receiving 20 μg endotoxin 4–5 hr prior to chamber implant. Each point represents the mean of three to five diffusion chambers obtained in two experiments; vertical lines, SEM.

cient of correlation of $r = 0.97$ with a slope of $m = 39.2$ colonies/day. The peak number of colonies was observed on day 4, declined on day 5 ($p < 0.05$), and tended to level off through day 7. Small colonies containing approximately 20–100 cells consisting of myeloblast-promyelocyte cells and myelocyte–metamyelocyte cells could be detected on days 2 and 3. By day 4, a wide range of colonies in terms of size and cell maturation could be observed. Most of the colonies were of the myelocyte–metamyelocyte type containing several hundred cells. Having reached the metamyelocyte-mature neutrophil stage of differentia-
tion, many colonies by day 5 were in the process of breaking up. Individual cells appeared to migrate away from the colony which at this time appeared as a diffuse aggregate of cells. This finding was in sharp contrast to the day 4 colony which was observed as a tight group of cells. The period between days 5 and 7 was characterized by the migration of cells from colonies, the development of new colonies and the maintenance and continued growth of some old colonies.

Cultures grown in endotoxin treated hosts (Fig. 2) also displayed a linear increase in the number of granulocytic colonies up to day 4. This linear increase, however, occurred after a lag period of 1 day. Despite this delay, the numbers of colonies observed on day 4 were similar to those seen in normal hosts. The rate of colony formation was increased as shown by the slope of the growth curve of $m = 57.9$. The size of many of the colonies observed on day 4 was approximately twice that which developed in normal hosts. There also appeared to be an increase in the rate of maturation within the colonies with many more myelocyte-metamyelocyte colonies appearing on day 3 than were observed in normal hosts. In endotoxin treated hosts there was no leveling off of colony numbers between days 5 and 7, but, rather, a sharp decline to levels half that scored in cultures grown in normal hosts on day 7. This finding was due to greater numbers of colonies breaking up and fewer numbers of new early colonies developing. Similar results were observed when 100 μg of endotoxin was administered to host rats.

In phenylhydrazine-treated hosts (Fig. 3) the number of granulocytic colonies assessed on day 4 was significantly ($p < 0.001$) less than seen in normal hosts. The rate of growth and the size of the colonies observed appeared normal up to day 3. Thereafter, colony numbers declined and leveled off through day 7.

Between days 5 and 7 in normal, endotoxin, and phenylhydrazine host animals, the presence of colonies composed of eosinophilic leukocytes was observed. Eosinophilic colonies showed cells in different stages of maturation and could be distinguished from the predominant granulocyte colonies containing neutrophils. Although the appearance of such colonies has been noted, quantitative assessment awaits improved staining techniques.

**Erythrocytic Colonies**

The formation of erythrocytic colonies in chambers implanted into different host animals followed patterns influenced by the pretreatment. An initial increase in the numbers of colonies was seen reaching a peak on day 2. This increase was followed by a significant decline and a secondary wave of erythroid colony development on days 6–7. In normal hosts, 188 erythrocytic colonies were scored on day 2; this number was significantly ($p < 0.05$) increased in anemic hosts to 275 colonies and reduced to 36 colonies in hosts treated with 20 μg of endotoxin. Colonies could be grouped according to two separate criteria, number of cells, or maturation of cellular elements. Colonies were scored as benzidine-positive aggregates consisting of four to seven cells and greater than eight cells. The number of cells per colony up to day 2 rarely exceeded 20 cells. Early erythroid colonies, consisting of basophilic normoblasts lightly stained with benzidine, were scored separately. Table 1 shows the size and maturation distribution of colonies counted daily after chamber implantation.

It was evident that anemia in host animals elicited an increase in colony
Table 1. Maturation and Size Distribution of Individual Erythrocytic Colonies Counted per Plasma Clot in Different Host Animals

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Phenylhydrazine</th>
<th>Endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Mature</td>
<td>Early</td>
</tr>
<tr>
<td></td>
<td>&lt;8</td>
<td>4-7</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.0 ± 2.7</td>
<td>46.5 ± 13.8</td>
<td>38.8 ± 10.6</td>
</tr>
<tr>
<td>Day 2</td>
<td>1.7 ± 1.2</td>
<td>111.7 ± 9.0</td>
<td>75.0 ± 9.7</td>
</tr>
<tr>
<td>Day 3</td>
<td>3.6 ± 1.4</td>
<td>6.1 ± 3.0</td>
<td>8.4 ± 3.0</td>
</tr>
<tr>
<td>Day 4</td>
<td>4.4 ± 1.7</td>
<td>6.3 ± 2.0</td>
<td>8.1 ± 2.0</td>
</tr>
<tr>
<td>Day 5</td>
<td>5.3 ± 1.5</td>
<td>3.6 ± 2.2</td>
<td>5.7 ± 2.2</td>
</tr>
<tr>
<td>Day 6</td>
<td>12.1 ± 2.6</td>
<td>0.7 ± 1.2</td>
<td>73.0 ± 16.2</td>
</tr>
<tr>
<td>Day 7</td>
<td>28.5 ± 6.5</td>
<td>6.0 ± 0.2</td>
<td>172.5 ± 5.9</td>
</tr>
</tbody>
</table>

*Early denotes colonies composed of basophilic normoblasts that are randomly dispersed within the clot matrix and stain lightly with benzidine.*

*Mature colonies are composed of cells in later stages of erythroid development which appear to fall into two size classes.*

formation with augmented numbers of both early basophilic normoblasts and orthochromatic and polychromatic normoblasts in larger colony units. Endotoxin treatment depressed erythroid development in all categories. The decline in colony numbers in cultures from day 3 to day 6 was due primarily to the maturation of erythroid elements into anucleate red blood cells. Such mature red blood cells could be observed in aggregates, but they were not scored as colonies in this study. Erythrocytic colonies persisted longer in anemic hosts as shown by both the number and size of the colonies observed on days 3, 4, and 5. On occasion, colonies were seen with greater than 100 cells in anemic rats, whereas normal hosts never induced colonies of that size.

A second wave of colony growth was observed on day 7 in normal hosts. However, these colonies differed from those on day 2 in their spatial orientation within the clot matrix. Similar to the bursts described by Axelrad et al., a second wave of colony growth was observed on day 7 in normal hosts. These colonies were grouped into discrete aggregates consisting of six to eight identifiable erythroid colonies, whereas day 2 colonies were randomly dispersed throughout the clot matrix. In addition, these larger entities (bursts) could be counted effectively without scoring the individual colonies within them. Thus, the secondary increase in the total number of colonies was concomitant with the linear increase in the number of bursts quantitated starting on day 5 and reaching 28 bursts on day 7. In anemic hosts, this secondary wave of differentiation started on day 4, a day earlier than seen in normal hosts. The peak number of colonies was reached on day 6 and was maintained on day 7. Approximately twice the number of colonies, as well as bursts, were observed in this secondary wave compared with normal hosts. The numbers of colonies...
per burst remained from six to eight; however, the number of cells per colony was markedly increased. In endotoxin-treated hosts, the secondary wave of colony formation was minimal as was the corresponding number of bursts. Within all host animals studied, each colony comprising the burst displayed predominantly orthochromatic and polychromatic normoblasts (Table I, day 7).

In order to determine the relationship between the numbers of marrow cells cultured and the numbers of colonies observed, 0.5, 1.0, and $5.0 \times 10^3$ normal bone marrow cells were implanted into normal hosts. Figure 4 shows a linear relationship for granulocytic colonies scored on day 4 with a coefficient of correlation of $r = 0.99$. Figure 4 also shows a linear relationship for erythrocytic colonies scored on day 2 with a coefficient of correlation of $r = 0.99$. These studies suggest that both granulocytic and erythrocytic colonies are of clonal origin.

The numbers of committed stem cells per $10^5$ cells cultured calculated from peak day colonies is given in Table 2. Comparable numbers of CFU-E on day 2

### Table 2. Number of Committed Stem Cells per $10^5$ Cells Seeded Into Plasma Clot Diffusion Chamber Cultures.

<table>
<thead>
<tr>
<th>Hosts</th>
<th>Day 2 CFU-E</th>
<th>Day 7 CFU-E</th>
<th>Day 7 BFU-E</th>
<th>Day 4 CFU-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>38.1 ± 6.9*</td>
<td>41.1 ± 11.6</td>
<td>5.5 ± 1.9</td>
<td>27.3 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>(6:2)†</td>
<td>(6:2)</td>
<td>(6:2)</td>
<td>(10:4)</td>
</tr>
<tr>
<td>Phenylhydrazine</td>
<td>54.9 ± 4.9</td>
<td>80.7 ± 16.3</td>
<td>10.5 ± 2.3</td>
<td>17.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>(6:2)</td>
<td>(6:2)</td>
<td>(6:2)</td>
<td>(12:4)</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>8.2 ± 5.6</td>
<td>7.1 ± 1.2</td>
<td>0.8 ± 0.5</td>
<td>34.6 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>(5:2)</td>
<td>(5:2)</td>
<td>(5:2)</td>
<td>(5:2)</td>
</tr>
</tbody>
</table>

*Mean ± SEM.
†Numbers in parentheses represent number of chambers scored: number of experiments.
and day 7 are noted in normal hosts. Anemic hosts induced an increase in CFU-E with elevated values on day 7 and a twofold increase in BFU-E. Endotoxin treated hosts effectively suppressed the growth of CFU-E and BFU-E. Numbers of CFU-C were slightly suppressed in anemic hosts and mildly elevated in endotoxin-treated hosts. The numbers of cells per colony in these endotoxin-treated hosts were markedly increased.

DISCUSSION

The in vivo plasma clot-diffusion chamber technique described above combines the advantages of the in vitro plasma clot culture and the in vivo diffusion chamber system. Bone marrow cells seeded into this culture are immobilized in a supportive semisolid matrix so that differentiating progeny cells remain localized and develop into discrete quantifiable colonies of erythroid and granuloid cells. The clonal nature of the observed colonies allows for the quantitative assessment of the numbers of progenitor cells seeded into the chamber. The number and morphological characteristics of the cells comprising each colony provide a means of evaluating the proliferative and developmental potential of the progenitor cells. The system circumvents problems of stem cell migration noted in spleen colony assays as well as the need for exogenous humoral factors (e.g., EPO, CSF). The host animal, within an experimental design, can provide a physiologic environment to supply the nutritive as well as the humoral regulators requisite for cell growth and differentiation.

The clonal origin of granulocytic cells grown in cell suspensions in diffusion chambers has not as yet been established. Boyum and Borgström, however, have quantitated the number of granulocytic progenitor cells per chamber using a limiting dilution technique. These investigators reported one diffusion chamber progenitor cell in 2000 bone marrow cells in C₃H × DBA mice, and one in 2700–4900 marrow cells in white outbred mice. Gordon reported one CFU-C in 1000 mouse marrow cells in an agar diffusion chamber system. The present study on in vivo granulocyte colony formation revealed one CFU-C in approximately 3700 rat bone marrow cells. This incidence was in the reported range and represents expected variations in growth potential between rat and mouse marrow in diffusion chambers. Paran et al. have noted one CFU-C in 1500 mouse bone marrow cells assayed in vitro; Metcalf and Moore placed a figure closer to one CFU-C in 500 marrow cells. Our previous findings using the adult Long–Evans rat reported one CFU-C in approximately 2200 marrow cells plated. It should be noted, however, that in vitro studies have demonstrated the growth of both granulocyte and macrophage colonies. The present plasma clot-diffusion chamber method of culture up to 7 days allowed for only the growth of granulocytic colonies; macrophages, although seen as isolated cells in the background matrix, never appeared as colonies. We have not as yet determined if macrophage colonies may form over longer periods of time.

Erythroid progenitor cells do not grow well in cell suspensions in diffusion chambers. Erythropoiesis tends to be minimal, with few normoblasts observed beyond day 3. The addition of the plasma clot supportive structure allows for the growth of erythroid colonies in a manner closely resembling the pattern observed in in vitro studies. The in vitro culture introduced by Stephenson et al. and improved by McLeod et al. has demonstrated the growth of benzidine-
positive erythroid colonies derived from a committed stem cell (CFU-E). Colony growth is erythropoietin dependent and shows a peak number on day 2. Axelrad et al.,6 using repeated additions of erythropoietin, have reported on a secondary increase in erythroid colony numbers on day 8. These colonies are dispersed in a burst formation, giving evidence for the concept of a committed stem cell preceding the CFU-E. The burst-forming unit (BFU-E) presumably serves as the progenitor cell for the CFU-E. Iscove and Sieber6 have recently reported on erythroid burst formation in a methylcellulose-supported in vitro system having the characteristics of a giant erythroid colony. The supporting matrix (plasma clot versus methylcellulose) may play an important role in the morphology of the burst. Iscove and Sieber6 report 25 BFU-E/10⁵ adult mouse bone marrow cells. Axelrad et al.6 have found five BFU-E/10⁵ marrow cells in plasma clot cultures. The present study shows approximately five BFU-E/10⁵ normal adult rat bone marrow cells with a twofold increase in anemic hosts. The fewer numbers of BFU-E compared with CFU-E are consistent with a precursor–progeny relationship. We observe in the present study 38 CFU-E/10⁵ normal bone marrow cells compared with in vitro studies7 in the same rat strain showing 200 CFU-E/10⁵ marrow cells. The in vitro assays are closer to reported values in mice of approximately 300 CFU-E/10⁵ bone marrow cells.5,6 The fewer numbers of CFU-E seen in the diffusion chamber may reflect the still less favorable growth conditions for erythropoiesis in the chamber and/or the important differences in the actual amount of erythropoietin reaching the cells. In vitro studies clearly delineate colony dependence on erythropoietin with the BFU-E, relying on even larger doses of the hormone. The increased number of CFU-E and BFU-E observed in anemic hosts suggests the stimulatory influence of the increased titers of the hormone. The presence of BFU-E in normal hosts indicates that the added “push” required in vitro may not be necessary in a more physiologic environment.

Preliminary studies in our laboratory in which normal bone marrow is incubated with erythropoietin prior to chamber implantation reveals significant increases in day 1 colony formation. Such studies may prove to be an important link in interpreting data from different culture systems. Increased erythropoiesis has been shown to occur in cell suspensions in diffusion chambers implanted into hypoxic, hemorrhaged, or erythropoietin-injected host animals.7,8 These investigators have found that the increase in the number of erythroid cells represents less than 10% of the total number of cells harvested. Small increases in ⁵⁹Fe incorporation in mouse bone marrow cultures in diffusion chambers in the presence of erythropoietin have also been reported.9 We suspect that, whether in cell suspensions or plasma clots, relatively low levels of erythropoietin penetrate the diffusion chamber—levels of the hormone that may well be more within the physiologic range than are used in in vitro culture systems that tend to induce a maximal response from the marrow cells.

Committed stem cells of the erythroid and granuloid series are presumably derived from the common pluripotent stem cell (CFU-S) as assayed in spleen colonies in irradiated mice. Shifts in populations of progeny cells reflect competitive proliferative demands on the subsequent differentiation of the CFU-S. Decreases in diffusion chamber progenitor cells are noted in hypoxic animals.7 Injections of erythropoietin during the regenerative phase of transplanted bone
marrow into lethally irradiated mice results in a decrease in granulopoiesis. Reductions in CFU-C in bone marrow under conditions which augment erythropoiesis have been used as further evidence for competing pathways from a common stem cell origin. The earlier studies of Fruhman, indicating reduced erythropoiesis following endotoxin treatment, support a similar conclusion. This same pattern of erythroid–granuloid directional competition is seen in the present study in perturbed hosts. In this instance, the normal marrow is isolated within the chamber so that directional hematopoiesis cannot be ascribed to shifts in cell populations due to stem cell migration. This in vivo assay shows the erythroid line as being the more labile of the two.

Attempts have been made to correlate the numbers of granulocytes harvested from diffusion chambers with CFU-S and CFU-C by methods of recloning in spleen colonies and agar cultures. Breivik using the limiting dilution technique, although finding similarities between the chamber progenitor cell and the CFU-S, has demonstrated a clear distinction of these two cell types in response to vinblastine. The question of whether the diffusion chamber progenitor cell is a multi- or unipotential stem cell has not as yet been resolved. The growth and differentiation of the CFU-C in vitro is dependent on the addition of colony-stimulating factor (CSF). In diffusion chambers, implanted into hosts rendered neutropenic by cyclophosphamide or irradiation, the numbers of differentiating myeloid elements as well as the proliferation of myeloid precursors have been shown to be increased. This finding has been attributed to the presence of circulating humoral factors that possibly are related to CSF. Serum levels of CSF are indeed augmented in animals made neutropenic by irradiation and endotoxin. However, have shown that CSF is different from a diffusible granulocytic stimulator (DGS) affecting granulopoiesis in diffusion chambers in endotoxin-treated hosts. Serum CSF levels peak at 24 hr, whereas DGS levels are elevated 72 hr after endotoxin treatment.

In the present study, granulocyte colony size but not number was increased in hosts experiencing a mild transient neutropenia. Whether this was due to CSF and/or DGS has not as yet been determined. The approximate doubling in colony size suggested an extra division at some time after the colony was formed. The in vivo plasma clot–diffusion chamber technique may prove useful in determining the associations which exist between progenitor cells, humoral control agents, and the development of progeny within the competitive complexities of the hematopoietic system.

REFERENCES

6. Axelrad AA, McLeod DL, Shreeve MM, Heath DS: Properties of cells that produce erythrocytic colonies in plasma culture, in Robinson WA (ed): Hematopoiesis in Culture,
PLASMA CLOT DC CULTURE SYSTEM


29. Breivik H: Response of multipotent (CFU) and granulocyte (diffusion chamber assay) progenitor cells and differentiating cells of murine haemopoietic tissue to a perturbation of the steady state. J Cell Physiol 79:171-180, 1972


Assessment of erythrocytic and granulocytic colony formation in an in vivo plasma clot diffusion chamber culture system

HN Steinberg, ES Handler and EE Handler