A Model of Intramedullary Hematopoietic Microenvironments
Based on Stereologic Study of the Distribution of Endocloned Marrow Colonies

By Richard H. Lambertsen and Leon Weiss

Hematopoietic colonies were studied in the marrow of alternate fraction-irradiated mice by light microscopic stereology to investigate the microenvironmental organization of marrow. Separate analyses of the relative colony cell density of undifferentiated, granulocytic, erythrocytic, and macrophage colonies in four marrow zones were carried out at 3, 4, and 5 days postirradiation (PI) for all colonies, all periarterial colonies, and all nonperiarterial colonies. The results demonstrate a differential colony cell distribution that does not appear to be due to a preferential distribution of certain colony types around arteries. Undifferentiated colony cells showed a consistent predilection for endosteal and periarterial regions, with the majority of colony cells occurring along bone. Erythrocytic colony cells proliferated initially in intermediate and central marrow zones and along arteries. Granulocytic colony cells occurred in all areas at 3 days PI, but increased in density along bone thereafter. Macrophage colony cells occurred in all zones at 4 days PI, but at 5 days were concentrated in subosteal and central regions. Macrophage colonies also occurred periarterially. To explain these findings and the organization of normal bone marrow, we present a detailed model of the microenvironmental organization of intramedullary hematopoiesis. This model portrays the stroma as engendering distinct microenvironments for stem cell replication, stem cell commitment, and early progenitor cell proliferation.

ELUCIDATING STROMAL EFFECTS on hematopoietic function remains a central problem in the physiology of hematopoiesis. Although in recent years there has been an accumulation of detailed information on possible control functions of the hematopoietic stroma, correlation of the results from different experimental systems is difficult. Even so, a number of related studies show that hematopoiesis, the most complex of all proliferating cell systems, is a spatially and functionally ordered process. Thus, the existence of microenvironmental controls, if not their mechanism of action, is beyond doubt.

In vivo hematopoietic colony formation provides insight into the nature and distribution of stromal effects. By lethally irradiating laboratory animals and administering, or shielding, viable hematopoietic stem cells, discrete clusters of hematopoietic cells can be formed in the spleen and marrow. During the first 10 days of their growth, these clusters tend to differentiate monotypically, forming distinct erythrocytic, granulocytic, megakaryocytic, and macrophage colony types. Nevertheless, karyologic and secondary transplant studies show that most spleen colonies are derived from, and contain, pluripotent cells. The pattern of colony formation thus suggests that the hematopoietic stroma is in some way capable of restricting stem cell differentiation. Furthermore, it has been found that the different types of hematopoietic colonies are differentially distributed in the spleen, that the ratio of erythrocytic to granulocytic colonies in the spleen and marrow is distinctly different, and that when marrow containing hematopoietic colonies is implanted into the spleen of irradiated animals, colonies that grow across the implant boundaries tend to change from granulocytic, in the implanted marrow, to erythrocytic, in the surrounding spleen. These findings suggest type-specific, organ-dependent modulations, and led Wolf and Trentin to hypothesize that the initiating event in pluripotent stem cell differentiation is stromally, not stochastically, determined. They proposed that the stroma of hematopoietic organs is compartmentalized into a series of hematopoietic inductive microenvironments (HIM), each of which commits its resident pluripotent hematopoietic cells to a particular type of differentiation. They suggested that regional differences in the expression of these microenvironments are responsible for the differential distribution of colonies in the spleen and the distinctive colony ratios of spleen and marrow.

In bone marrow, a spatially organized microenvironmental effect on stem cells is indicated by demonstrations that the proliferation of hematopoietic stem cells is under a local, or stromal, control, in conjunction with the observation that proliferating hematopoietic stem cells tend to concentrate in endosteal regions. Early histologic studies identified a proliferative zone along bone and one of increasing granulopoietic matu-
ration centrally,\textsuperscript{18,19} suggesting an intramedullary gradient of both proliferation and differentiation. This was confirmed and extended by Shackney et al.,\textsuperscript{20,21} who demonstrated a downward gradient of proliferation in the granulocytic and megakaryocytic series from bone to the diaphyseal marrow axis with autoradiographic and stathmokinetic techniques. In complementary studies, Lord et al.\textsuperscript{22} used a differential washout technique to obtain "axial" and "marginal" samples from normal diaphyseal marrow for in vivo and in vitro assays. They found a "continuous" concentration gradient of pluripotent cells (CFU-S) from bone to the marrow axis that corresponded to a similar gradient of stem cell proliferation; in vitro granulocyte/macrophage progenitors (CFU-C), in contrast, showed a peak in concentration approximately 110 μ from bone. Based on these findings, Lord et al.\textsuperscript{22} suggested that granulocytic differentiation is organized along a stem cell gradient originating from endosteal regions of proliferation, and that the peak in CFU-C arises from pluripotent cell differentiation near bone.

Recently, however, Frassoni, Testa, and Lord\textsuperscript{23} presented data that appear to preclude the assumption that all hematopoietic cell differentiation in marrow is organized along a continuous, adaxial gradient of stem cell proliferation. Using the same sampling procedure as Lord et al.,\textsuperscript{22} they assayed BFU-E, a class of primitive erythropoietic cells,\textsuperscript{24,25} and CFU-E, relatively well differentiated erythropoietic cells thought to be derived from BFU-E.\textsuperscript{25} Their results show a bimodal BFU-E distribution from bone to the marrow axis, with sharp peaks in concentration about 50 and 250 μ from bone. CFU-E, on the other hand, were infrequent along bone but otherwise appeared to occur nearly uniformly across the marrow space.

We have sought to provide information on the organization of hematopoiesis by studying the details of hematopoietic colony formation in the bone marrow. Using light and electron microscopic techniques, we described the early formation and long-term growth of "endocloned" hematopoietic colonies in alternate fraction x-irradiated mice.\textsuperscript{2,22} In addition to observations made on the associations and interactions between colony stromal and hematopoietic elements, we found that undifferentiated, granulocytic, erythrocytic, and macrophage colonies could routinely be detected in sections of methacrylate-embedded marrow as early as 3–5 days postirradiation (PI), that megakaryocytic colonies first appear at 5 days PI, and that these colonies grow nonrandomly and dissimilarly in the marrow space. This suggested that proliferative and differentiative stimuli for different hematopoietic cell types are nonuniformly expressed in x-irradiated and possibly normal marrow. We also demonstrated the multicentric or burst-like configuration of early erythrocytic colonies in vivo, and thereby inferred the motility of early erythrocytic colony-forming cells (in vivo BFU-E). Furthermore, we concluded that two distinct subtypes of macrophage colonies may occur during early marrow regeneration.

Our present study examines undifferentiated, neutrophilic granulocyte, erythrocytic, and macrophage colonies during the earliest detectable phase of their development using a light microscopic stereologic technique. We have focused on the very early stage of marrow regeneration, reasoning that the distribution of colonies during this period might accurately reflect both the pattern and the nature of stromal effects related to the initiation of hematopoiesis. Moreover, since colonies derived from radioresistant cells are not evident until 7–8 days PI in animals receiving an equal but single-fraction dose of total body x-irradiation,\textsuperscript{27} it can be assumed that essentially all colonies in our endocloned marrow system prior to 6 days PI (as studied here) arise from radioprotected colony-forming units that circulate from the initially shielded marrow compartment. Our stereologic results demonstrate a differential distribution of these early colonies in x-irradiated bone marrow. To explain our findings and the organization of normal marrow, we propose a model of intramedullary hematopoiesis that defines distinct microenvironments for stem cell replication, stem cell commitment, and progenitor cell proliferation.

**MATERIALS AND METHODS**

**Animals, Microscopy, and Endocloning Procedure**

Twelve C576J/BL female mice, 4–8 wk old, were used for this study. Hematopoietic marrow colonies were produced by an alternate-fraction x-irradiation procedure, with a total body exposure of 850 R (250 Kvp, 1.5 mm Cu half-value layer, 97.0 R/min). Animals were restrained, two at a time, within plastic tubes. The left hind leg of each was pulled through a small hole in the wall of the tube and shielded with a 4-mm-thick lead collar. An x-ray dose of 850 rads was then administered to both animals, with the primary beam collimated to exclude the shielded legs. Ten hours later, the initially shielded legs were irradiated with the same dose, with lead shielding molded around the tubes to prevent scatter irradiation to the animals’ bodies. Collimation at this time was adjusted so that a small portion (2–3 mm) of the proximal left femur received double irradiation, assuring that no marrow region escaped exposure to the primary x-ray beam. Additional details on this procedure are available elsewhere.\textsuperscript{2,26} Three animals were sacrificed at 3 days, 6 at 4 days, and 3 at 5 days PI, with time measured from the beginning of the initial x-ray exposure. Their sternums and right (initially unshielded) femurs were processed for methacrylate sectioning\textsuperscript{1} and subserially sectioned in 100-μm steps. Six to twelve 1.5–2.0-μm thick sections were taken at each step, mounted on slides, and stained using May-Grunwald-Giemsa. All observations were carried out using a Zeiss
Ultraphot II microscope. Measurements were made with an American Optical eyepiece micrometer calibrated, for each objective, to an American Optical stage micrometer.

**Classification of Colonies and Colony Cell Types**

Hematopoietic colonies were morphologically identified as undifferentiated, neutrophilic granulocyte (granulocytic), erythrocytic, or macrophage, as described in detail previously. The relatively rare eosinophilic granulocyte and late-appearing megakaryocytic colonies were not studied here. Colonies were further classified for analysis as being either periarterial or nonperiarterial: periarterial colonies occurred as discrete clusters of cells around or along marrow arteries or arterioles; nonperiarterial colonies did not.

To provide additional information on the composition of macrophage colonies, the cells of these colonies were counted as either “differentiated” macrophages, identified by their elongate form and abundant lysosomes, or “monocyte-like” cells. Monocyte-like cells were spheroidal cells with a round or slightly indented, but not lobed, nucleus. These additional data, however, were not considered in the main colony distribution analysis.

**Stereology**

Data on colony distribution were obtained by defining four marrow zones relative to endosteal surfaces and the wall of the central venous sinus, as set forth below. Each nucleated cell of a given hematopoietic colony was then counted and scored according to which zone it occupied. This permitted a quantitative expression of a colony's spatial distribution within the marrow as the relative frequency of its cells in each zone, with the total colony cell count (in section) equaling the sum of the number of cells from all zones. This procedure was carried out for all colonies identified at each 100-μm step of subserially sectioned femurs and sternums, taking great care between sections to count a particular colony only once.

The marrow zones for stereology of femoral colonies were defined as follows.

**Endosteal zone.** This zone included the marrow space bordered on one side by cortical or trabecular bone and extending 0.02 mm from the bone surface.

**Subendosteal zone.** This zone encompassed the marrow space beginning 0.02 mm from the surface of cortical or trabecular bone and extending away from bone an additional 0.08 mm.

**Central zone.** This was a band of marrow surrounding the central venous sinus and extending perpendicularly outward from the wall of that sinus no greater than 0.10 mm. The abaxial boundary of the central zone was further limited by a line drawn 0.20 mm from, and parallel to, the bone surface. Hence, the central zone was no wider than 0.10 mm, and in areas where the central sinus wall is closer than 0.20 mm from bone, it narrowed accordingly.

**Intermediate zone.** This zone included the marrow space between the subendosteal and the central zones. In paraaxial sections not including the central sinus (and therefore not having a central zone) the intermediate zone was bordered only by the axial margins of the subendosteal zone.

**Relative Areas of Marrow Zones and Normalization of Femoral and Sternal Data**

Normalization of data from femoral and sternal colonies (see below) required information on the relative areas of the above-defined marrow zones. To provide this, the relative areas of the endosteal, subendosteal, intermediate and central zones of the femoral and sternal marrow were measured using a point-counting method. An eyepiece micrometer was used in one ocular to measure marrow zones, and a reticle grid in the other to establish points. Zonal relative areas were determined as the ratio of the total number of points over a particular zone to the total number of points over all zones in one complete set of sections of a subserially sectioned femur or sternum. All normalization calculations employed the mean zonal relative areas of five subserially sectioned femurs and five subserially sectioned sternums taken at 4 days PI; these data are given in Table 1.

Femoral and sternal colony distribution data were initially treated separately. To facilitate their comparison, the relative density (weighted relative colony cell frequency) of each colony's cells within each marrow zone of the femur or sternum was computed by dividing the relative colony cell frequency in a given zone by the mean relative area of that zone. This normalized for the differences in relative areas of marrow zones between femur and sternum and...
permitted a direct graphic comparison of colony cell distribution with that which would occur if colonies were uniformly distributed throughout the marrow (i.e., weighted relative colony cell frequency equals 0.25 in each of four zones). A comparison of sternal and femoral colony data based on this approach showed a similar colony distribution pattern. Sternal and femoral colony data were therefore combined. These data represent a total of 85 differentiated colonies, 255 neutrophilic granulocyte colonies, 69 erythrocytic colonies, and 69 macrophage colonies.

RESULTS
Colony Distribution Analysis Without Regard to Colony Type

Figure 2A depicts the mean weighted relative colony cell frequency data for all colonies without regard to colony type (i.e., undifferentiated, granulocytic, etc.) or periarterial status. Data from 3, 4, and 5 days are represented separately, as indicated. The same data, sorted into those derived from “nonperiarterial” or “periarterial” colonies, are depicted in Fig. 2B and Fig. 2C, respectively, with the plots for 3, 4, and 5 days P1 in Fig. 2A representing the algebraic sum of those in 2B and 2C. For convenience in the description of these and the following graphs, the term “mean weighted relative colony cell frequency” is replaced with “frequency.”

From Fig. 2 it can be seen that hematopoietic colony cells are not uniformly distributed throughout the marrow space, but occurred with greater frequency in the endosteal zone. A slightly bimodal colony cell distribution is apparent (Fig. 2A) and is essentially abolished when periarterial colonies are removed from consideration (Fig. 2B). Thus, colony cell proliferation overall was focused in periarterial and subosteal regions. The distribution of periarterial colonies (Fig. 2C), on the other hand, appears merely to reflect the histologic distribution of arterial vessels in the marrow: in marrow sections, arterial vessels most commonly occurred in the intermediate zone.

Colony Distribution

To determine whether 3–5-day colonies of different types were similarly or differentially distributed in the marrow, the data in Fig. 2 were sorted according to colony type (Fig. 3).

From Fig. 3 it can be seen that hematopoietic marrow colonies of different types are, in fact, differentially distributed, and that this pattern is not abolished when periarterial colony data are omitted (Fig. 3, B, E, H, K). Thus, the observed distribution of colony cells cannot be readily explained on the basis of a preferential distribution of certain colony types in periarterial regions.

In contrast, periarterial colony cells, with the possible exception of those of periarterial macrophage colonies (see below), do not appear differentially distributed (Fig. 3, C, F, I, L). The prevalence of their cells within the intermediate zone correlates, as noted above, with the histologic distribution of arterial vessels.

Undifferentiated colony data indicate a marked predilection of undifferentiated colony cells for the endosteal zone (Fig. 3, A–C). This persists throughout the 3-day P1 period and is almost absolute (i.e., mean weighted relative colony cell frequency approached 1.00) when periarterial colonies are not considered (Fig. 3B). It is therefore apparent that a minor population of periarterial undifferentiated colonies accounts for most of the undifferentiated colony cells that occurred outside of endosteal or subendosteal zones.

Granulocytic colony cells, like those of undifferentiated colonies, were preferentially distributed in the

---

**Table 1. Relative Area of Marrow Zones**

<table>
<thead>
<tr>
<th>Bone</th>
<th>Endosteal</th>
<th>Subendosteal</th>
<th>Intermediate</th>
<th>Central</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femur</td>
<td>0.13 ± 0.01</td>
<td>0.45 ± 0.03</td>
<td>0.30 ± 0.02</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>Sternum</td>
<td>0.22 ± 0.01</td>
<td>0.57 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0 ± 0.01</td>
</tr>
</tbody>
</table>

Mean of 5 femurs and 5 sternums at 4 days P1, ± standard error.

---

**Fig. 2.** Relative frequency of hematopoietic colony cells in endosteal, subendosteal, intermediate, and central marrow zones at 3 (○), 4 (△), and 5 (□) days postirradiation. Relative frequencies are weighted according to the relative area of marrow zones (see Table 1 and text). Open, offset symbols (○, △, □) indicate ± standard error of means at 3, 4, and 5 days postirradiation, respectively.
Fig. 3. Relative frequency of undifferentiated, granulocytic, erythrocytic, and macrophage colony cells in the endosteal, subendosteal, intermediate, and central marrow zones. The data represented in Fig. 2 are expanded here vertically according to colony type.

Endosteal zone; they occurred, however, with greater frequency in subendosteal and intermediate zones (Fig. 3, D–F). From 3–5 days, a trend of increasing granulocytic colony cell frequency in the endosteal zone with a decreasing frequency in the intermediate zone is evident. Histologically, this correlated with (A) the apparent transformation (differentiation) of undifferentiated colonies to granulocytic colonies in the endosteal zone, (B) a tendency for granulocytic colonies to grow along, rather than away from, the bone, and (C) the disappearance of some granulocytic colonies from intermediate and central zones.

Erythrocytic colony cells at 4 days PI (the earliest these colonies were observed), peaked in frequency in the intermediate zone (Fig. 3, G–I). Because of the scarcity of erythrocytic colonies at this time, however, the frequency distribution shown represents a small number of colonies (n = 18).
At 5 days PI ($n = 51$), the frequency of erythrocytic colony cells is greatest in the subendosteal zone, approximately equal in the endosteal and intermediate zones, and least in the central zone (Fig. 3, G–I). The combined frequency of 5-day erythrocytic colony cells in the intermediate and central zones, however, was greater than that of any other colony type at 5 days (Table 2). The apparent lack of predilection of erythrocytic colony cells for bone surfaces could not be explained by a preferential occurrence of erythrocytic colonies in periarterial regions, for a very similar distribution pattern occurs when only nonperiarterial colonies in periarterial regions, for a very similar distribution pattern occurs when only nonperiarterial colonies are considered (Fig. 3H).

Macrophase colony data are shown in Fig. 3, J–L. At 3 days PI, macrophase colonies occurred in such small numbers that analysis of their spatial distribution in the marrow was not done. At 4 days PI, macrophase colony cell frequency was essentially equal in all four zones. Macrophage colony cells, however, had a greater frequency in the central zone than other 4-day colonies.

At 5 days PI, a bimodal distribution of macrophase colony cells is evident (Fig. 3, J–K). Colony cells appeared to concentrate somewhat in the central zone and also in the endosteal and subendosteal zones.

The frequency of macrophase colony cells in the central zone was dampened, but not abolished, when periarterial colony data were removed from consideration (Fig. 3K). Also, there is a tendency of periarterial macrophase colony cells to distribute in the central zone in greater frequency than those of other colony types is also evident (Fig. 3L).

The cells of macrophase colonies were also somewhat distinct in their differentiation status. "Central" macrophase colonies consisted predominantly of well differentiated macrophages, whereas "subosteal" colonies appeared to be discrete foci of normal monocytopenous cells. To evaluate this further, the original macrophase colony cell counts (which were scored separately for differentiated macrophages and "monocyte-like" cells) were used to calculate the percentage of mature macrophages in "central" and "subosteal" macrophase colonies in the femur. "Central" macrophase colonies were defined as those having more than 50% of their cells in the central zone. "Subosteal" macrophage colonies were defined as those having more than 50% of their cells within the combined endosteal and subendosteal zones. Data from 3-, 4-, and 5-day colonies were not considered separately. It was found that the percentage of differentiated macrophages in central macrophase colonies ranged from 56 to 100, with an average of 78 ($n = 13$). The percentage of differentiated macrophages in subosteal macrophase colonies ($n = 8$) ranged from 13 to 64, with an average of 41.

### DISCUSSION

In order to interpret our observations, one must consider whether marrow colony-forming units (CFU-M) include both pluripotent and committed cells, whether populations of these cells seed differentially into the marrow space, and whether the immediate progeny of pluripotent CFU-M are motile and move out of the area in which CFU-M initially lodge. If any of these possibilities is true, one cannot presume, despite the findings of Wolf and Trentin, that the distribution of a particular type of colony necessarily reflects an ability of the stroma to induce or determine that type of hematopoietic differentiation. Without a detailed knowledge of CFU seeding into marrow, moreover, the subosteal/periarterial "focusing" of hematopoietic proliferation without regard to colony type (Fig. 2) provides unsatisfactory evidence for a subosteal/periarterial stimulus to proliferation.

Given these limitations we interpret our results by making two major assumptions: (1) that the presence of a particular type of colony in a marrow zone indicates a coincident stromal capacity to support proliferation of the cells of that colony type, and (2) that the colonies typed as "undifferentiated" contain a large proportion of pluripotent hematopoietic cells. Support for the latter assumption has been reviewed. The colonies we type as "undifferentiated" are composed of mononuclear cells morphologically similar to the cells described by others as likely candidates for the pluripotent marrow stem cell. They do not include cells that can be identified as erythropoietic, monocytopenous, megakaryopoietic, or granulopoietic by conventional cytology or by the presence of distinctive morphological features unique to a particular type of well differentiated colony. Moreover, they occur only during the first few days PI, a time when pluripotent cells, as assayed by the spleen colony technique, are increasing in colonized marrow at maximal rates. Last, they decrease in frequency as differentiated colonies develop.

In accordance with these assumptions, the distribution of undifferentiated colony cells (Fig. 3, A–C) strongly suggests, as concluded by others, that the stroma immediately adjacent to bone provides a spe-

### Table 2. Combined* Mean Relative Colony Cell Frequency

<table>
<thead>
<tr>
<th>Colony Type</th>
<th>Intermediate and Central Zones</th>
<th>5 Days Postirradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undifferentiated</td>
<td>0.00 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Granulocytic</td>
<td>0.07 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Erythrocytic</td>
<td>0.38 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>Macrophage</td>
<td>0.09 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

*Summed means ± SEM.
cialized region for pluripotent stem cell proliferation. The frequency of undifferentiated cells in the endosteal zone was consistently high during the 3–5-day PI period, despite a zonal width of only 20 μ. This reflects the previously reported tendency of undifferentiated colonies in this system to grow along bone rather than in all available directions, which in turn can be simply explained only by a proliferative stimulus, chemotactic attraction, or migration inhibition of undifferentiated cells arising from endosteal regions.\(^7\) However, the mechanistic correlates of this “stem cell microenvironment” might additionally include a selective seeding of pluripotent CFU to the endosteal regions, as the small caliber and finely divided character of subosteal vessels should favor the lodgment of CFU-M along bone.\(^26\)

Similarly, the finding of a subset of undifferentiated cells localized around arterial vessels suggests that a microenvironment for stem cell proliferation also exists in periarterial marrow. Corroborating our observation that periarterial undifferentiated colonies tend to grow along the vessel rather than equally in all directions,\(^7\) this suggests a local influence on undifferentiated cell proliferation and/or migration.

If one accepts the conventional view that hematopoietic differentiation involves an irreversible commitment of morphologically undifferentiated pluripotent stem cells to distinctly recognizable hematopoietic cell lines, our results allow certain conclusions regarding where the process of commitment occurs in endoceloidized marrow. Assuming that pluripotent cells do not leave an area of early undifferentiated cell proliferation to become committed elsewhere and then return to the initial area to form differentiated colonies, it follows that a particular type of commitment (e.g., granulocytic) probably occurs in areas which supported undifferentiated colonies and that particular colony type. Accordingly, the finding that undifferentiated granulocytic and macrophage colony formation occurred in endosteal and periarterial regions suggests that commitment to granulocytic and macrophage differentiation occurs in endosteal and periarterial marrow, while the finding that both undifferentiated and erythrocytic colonies grew in periarterial regions suggests that erythrocytic commitment occurs at least periarterially. In addition, our previous finding that megakaryocytic marrow colonies develop almost exclusively in subosteal locations\(^7\) suggests that commitment to thrombopoiesis occurs along bone.

The present results therefore support the hypothesis of Lambertsen\(^26\) that microenvironments sustaining stem cell proliferation and commitment in marrow occur as a mosaic along endosteal surfaces and arterial vessels. While commitment might occur elsewhere, such a possibility seems less likely. This is so because it is difficult to argue that areas generally devoid of undifferentiated colonies (i.e., nonendosteal/nonperiarterial marrow) support a significant level of commitment necessarily involves undifferentiated cells. Moreover, an apparent transience and uniformly well-differentiated cell composition of many granulocytic and macrophage colonies in nonendosteal/nonperiarterial regions suggests that they are derived from committed, not pluripotent, CFU,\(^7\) and the relatively high frequency of erythrocytic colony cells in nonendosteal/nonperiarterial regions (Fig. 3, G–I) need not indicate that erythroid commitment occurred there. From the multicentric configuration of erythrocytic colonies in this system, it can be inferred that early erythrocytic colony cells in vivo, as in vitro, are actively motile.\(^7\) Hence, erythroid cells might move out of endosteal and periarterial regions, once committed, and form detectable colonies elsewhere.

The distribution of erythrocytic colony cells manifests a tendency of early erythroblasts to concentrate in circumaxial marrow regions, which becomes increasingly evident as such colonies mature. In erythrocytic colonies at 10 days PI, large deeply basophilic erythroblasts typically concentrate in central regions, whereas polychromatophilic blasts and reticulocytes increase in concentration toward bone.\(^7\) Comparing this with the tendency of granulocytic colonies to grow along bone and, in some cases, to be transient in areas away from bone, we conclude that circumaxial regions in this system preferentially support the proliferation of early erythroid progenitor cells, while subosteal regions preferentially support progenitor cell proliferation of the granulocytic type. It is clear that none of the marrow zones exclude any line of differentiation, however, as all zones contained some cells of each colony type (Fig. 3).

By comparison, the results on macrophage colonies suggest that two spatially distinct stimuli of macrophage-monocyte differentiation exist in regenerating, x-irradiated marrow. On the one hand, the transformation of macrophage colony cell distribution from being nearly uniform at 4 days PI to somewhat bimodal at 5 days PI suggests, in conjunction with the relative abundance of “monocyte-like” cells in subosteal colonies, that the subosteal stroma is preferentially supportive of monocytopoiesis. This might be due in part to a selective seeding of CFU. On the other hand, the prominence of well differentiated macrophage colonies in central marrow regions suggests a centralized stimulus for macrophage maturation. Since irradiation preferentially disrupts the stroma and sinuses of central marrow,\(^7\) this maturation stimulus appears to be abnormal, i.e., promoting the formation of central macrophage colonies for the purposes of repair.
sections through the center of the murine femur. Rather, it rebates to our previous finding directly based on overlapping distributions of the core-apparently motile and therefore may move from endosteal, as periarterial, regions. Unlike the microenvironments for commitment to other cell types, this is not expected to originate from endosteal bone and arterial vessels. Noting that Lord et al. have found in intermediate and central regions of marrow, 100 μ or more from bone. Noting that Lord et al. concluded that a "continuous" gradient of CFU-S exists from bone to the marrow axis, however, we point out that our model instead predicts a decreasing concentration of stem cells away from bone on the basis of multiple, overlapping stem cell gradients. Given the distribution of early undifferentiated colony cells, such gradients would be expected to originate from endosteal bone and arterial vessels. We find this hypothesis attractive because it can explain the occurrence of pluripotent stem cell replication in nonbony areas (e.g., the spleen) as due to the periartrial component of an individual's microenvironmental complex for hematopoiesis.

The peak in CFU-C that Lord et al. found at about 110 μ from bone can be explained if one assumes, from the ability of CFU-C to form macrophage and granulocytic colonies in vitro, that they are monocytic and granulocytic progenitors in vivo. The increasing intensity of the microenvironment for nonerythroid progenitor cell proliferation toward bone (Fig. 4) implies that the probability of a committed cell proliferating increases with decreasing distance to bone. Thus,
progenitors produced by de novo commitment of pluripotent cells to monocytopenesis and granulopoiesis in endosteal microenvironments would tend to be amplified immediately by their own proliferation. This would produce a subosteal accumulation of monocytic and granulocytic progenitors (e.g., CFU-C). Competition for endosteal space caused by ongoing proliferation of stem cells and the primitive cells of other cell lines, on the other hand, would continually crowd these progenitors away from bone, contributing to the formation of a peak. Progenitors arising from low-level commitment in periarterial regions, by comparison, would be preferentially amplified abaxially (i.e., toward bone) and contribute to the axial slope and body of the same peak.

Following the same reasoning, our model predicts that megakaryocytic progenitors of an equivalent state of differentiation as CFU-C should also peak in close proximity to bone in normal, steady-state marrow. Given the hypothesized lack of a periarterial microenvironment for megakaryocytic commitment, however, such a peak is expected to occur closer to the endosteal surface than that of CFU-C. Furthermore, the decline in the concentration of megakaryocytic progenitors toward the marrow axis is expected to be less gradual than that of CFU-C, as megakaryopoiesis involves a relatively small number of cell divisions. While we know of no data on the in medullary distribution of assayable megakaryocytic progenitors, stereologic and autoradiographic studies support these predictions. Shackney et al.20 found a steep decline in the frequency of megakaryocytes from bone to the marrow axis. Moreover, they found, from the pattern of pulsed 3H-thymidine uptake by immature marrow cells and by mature megakaryocytes, that myeloid megakaryocytes originate from rapidly proliferating “subendosteal” cells.

In further support of our model we note its ability to explain, apparently for the first time, the twin peaks in BFU-E concentration found in normal marrow by Frassoni et al.23 Although these workers noted their results to be compatible with microenvironmental regulation, they advanced no hypothesis to account for the bi-modal distribution of BFU-E. Because their marginal (subosteal) BFU-E peak had a greater proportion of BFU-E of high proliferative capacity than the paraxial peak, however, they suggested that the former represents an earlier erythropoietic population. Our model accounts for these two peaks on the basis of the dual representation of microenvironments for erythroid commitment and the proposed microenvironment for erythroid progenitor cell proliferation. A paraxial peak would be expected because the intensity of the erythroid microenvironment for progenitor cell proliferation increases toward the marrow axis. Such a microenvironment would promote proliferation of the erythroid progenitors that arise from the commitment of pluripotent cells in periarterial regions, but provide little or no stimulus for proliferation in subosteal areas. Under steady-state conditions, a peak of erythroid progenitors some generations removed from pluripotency would thus be expected in periarterial regions, in what we term the intermediate zone. This zone extends from 100 to approximately 350 μm from bone in normal diaphyseal marrow (unpublished observations), thereby including the paraaxial peak of BFU-E (about 250 μm from bone) reported by Frassoni et al.23

To account for a second, subosteal peak of early erythroid progenitors (i.e., BFU-E), we assume that the rate of production of committed progenitors in a microenvironment that supports commitment is directly related, within limits, to the local availability of pluripotent cells. A subosteal accumulation of erythroid progenitors would then be expected from the...
high concentration of pluripotent cells established along bone by the endosteal microenvironment for pluripotent cell replication, coupled with the microenvironment for erythrocytic commitment in endosteal regions (Fig. 4). Moreover, these cells should represent an earlier population of progenitors than hypothesized in intermediate zones, as they would be derived largely from de novo commitment of pluripotent cells. As with other cell lines, a decline in the concentration of progenitors immediately adjacent to bone to form a concentration peak would be expected to result from competition for limited endosteal space by very primitive hematopoietic cells.

Factors intrinsic to the behavior of early erythroidic cells may play a role in maintaining segregation between subosteal and paraaxial peaks of erythroid progenitors. The motility of early erythroid cells in vivo certainly decreases with increasing differentiation, while their direction of movement appears to be toward the marrow axis. Thus, the distinctness of the two erythroid peaks accounted for by our model might be accentuated by an axial migration of recently committed erythrocytic progenitors prior to large scale proliferation. This would have the effect of maintaining an area of cellular traffic between the peaks where only few progenitors could be found (Fig. 5).

To conclude, stereologic study of the distribution of early marrow colonies supports a model of the organization of bone marrow that defines distinct microenvironments for stem cell replication, stem cell commitment, and progenitor cell proliferation. Although schematic and largely qualitative, this model is capable of explaining findings on the normal intramedullary distribution of stem cells and early progenitors for all nonlymphoid hematopoietic cell lines. Refinement of this model to reflect exact mechanisms of local influence is expected once the pattern of CFU-M seeding and the behavior of hematopoietic cells in marrow are better known.

ACKNOWLEDGMENT

We are grateful to Roger Sessions, of the Institute for Environmental Medicine, University of Pennsylvania Medical Center, who helped generate the computer programs used in our analysis of the stereologic data. We wish also to thank Sheri Padgett and Jeannie Starr for typing the manuscript.

REFERENCES

23. Frassoni F, Testa N, Lord B: The relative spatial distribution
of erythroid progenitor cells (BFUe and CFUe) in the normal mouse femur. Cell Tissue Kinet 15:447, 1982


A model of intramedullary hematopoietic microenvironments based on stereologic study of the distribution of endocloned marrow colonies

RH Lambertsen and L Weiss