Characteristics of colony growth from normal human bone marrow

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SYNOPSIS The number of granulocyte/macrophage colonies grown in vitro from bone marrow cells obtained from 90 rib segments and 30 'normal' bone marrow aspirates was found to be highly variable. Considerable variation was also noted in the relationship between colony number and the number of cells cultured in both groups. The aspirate group was found to have a significantly greater ability to form colonies without the addition of colony-stimulating factor to the culture medium at low cell concentrations.

The technique whereby colonies of granulocyte and monocyte/macrophage cells can be grown from their progenitor cells (colony-forming cells) in soft agar culture has been very extensively studied in the experimental animal. In this situation colony growth is dependent on the presence of a colony-stimulating factor in the culture system. The potential application of this technique to human myelopoiesis, both normal and disordered, is twofold, in that the granulopoietic potential of a marrow sample may be assessed and biological fluids can be assayed for their content of stimulating and inhibiting substances (Metcalf, 1973).

While the growth characteristics of marrow cells in small rodents has been extensively studied, much less work has been done to establish the pattern of colony growth from normal marrow cells in the human subject. A major problem in this regard is the difficulty of obtaining marrow samples from normal donors and most work has been done on material aspirated from patients subsequently shown not to have any haematological abnormality. Another source of marrow cells would be from the portions of rib removed routinely at thoracotomy and in this paper we report on the characteristics of growth from 90 such samples taken from patients not suffering from any haematological disorder. For purposes of comparison, a study of 30 samples obtained by aspiration was also made.

Materials and Methods

CELL COLLECTION
Bone marrow cells from surgical patients (rib group) were obtained from small segments of fifth rib removed routinely at thoracotomy and also from some portions of 12th rib removed during major renal surgery; all patients had no primary haematological disorder. The rib segment was placed in collecting medium (BHK Eagle's, Wellcome Reagents Ltd) supplemented with 10% fetal calf serum (Flow Laboratories Ltd) and 10% trypticase soy broth (Difco) and the cells were suspended after washing medium through the medullary cavity with a Sahli marrow biopsy needle attached to a syringe. Smears for differential counting were prepared by re-suspending a centrifuged pellet in a drop of serum and staining by May-Grunwald Giemsa.

Samples of marrow aspirated from the anterior iliac crest were placed in bottles containing 5 ml collecting medium (with 100 μ preservative-free heparin: Weddel Pharmaceuticals Ltd, London). The marrow donors in this group were patients who had undergone marrow biopsy for investigative purposes which subsequently revealed no significant haematological disorder. Excess erythrocytes were removed by layering the diluted sample over methylcellulose/triosil (Hullinger and Blaztiovec, 1967) and allowing them to sediment at room temperature for 30 to 50 minutes. The leucocyte-rich supernatant was removed and nucleated cell counts performed.
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Samples in which the total cell count was less than $1 \times 10^7$ were discarded.

**CULTURE OF COLONY-FORMING CELLS**

All cultures were performed by the double-layer technique in Nunclon 30 mm plastic dishes using the modified Eagle’s medium previously described (McNeill, 1971). In brief, a 2 ml underlayer of Eagle’s 1.2% agar was placed in each dish. Colony-stimulating factor was provided by the inclusion of 5% (v/v) human embryo or human spleen cell conditioned medium in the underlayer. Eagle’s 0.3% agar was held at 37°C, cells were added to the appropriate concentration, and 1 ml aliquots placed on the gelled underlayers. Cultures were routinely performed in quadruplicate at cell concentrations of $1 \times 10^5$, $2.5 \times 10^5$ and $1 \times 10^6$ per ml with intermediate concentrations in some instances. Cultures were incubated for seven days at 37°C in sealed boxes containing 10% CO$_2$ in humidified air and colonies counted by a single observer at x 40 magnification using an Olympus dissecting microscope. Aggregates of greater than 20 to 30 cells were scored as colonies.

**Results**

The contents of both rib and aspirate marrow samples with particular regard to cells of the granulocytic series, are shown in table I. The mature polymorph content of the aspirated group was higher than the rib group but the proportions were otherwise fairly

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**Fig 1** Histogram of numbers of colonies per culture from rib samples cultured at (a) $1 \times 10^5$ marrow cells per culture; (b) $1 \times 10^6$ marrow cells per culture.
similar; rib marrow would seem to provide only a marginally more constant sample.

**RIB GROUP**

Figure 1 shows the number of colonies per culture when marrow cells were plated at $1 \times 10^5$ (fig 1a, 79 samples) or $1 \times 10^6$ (fig 1b, 90 samples). Colony-stimulating factor was added to all cultures. The mean colony number was 39 at $1 \times 10^5$ (range 0-102, SD ± 27) and 179 at $1 \times 10^6$ (range 1-423, SD ± 100). Analysis of clinical data from these patients in terms of diagnosis, peripheral blood leucocyte count, and body temperature revealed no correlation between these parameters and the capacity for colony growth by marrow cells. No single cell type shown in table I was found to have a direct relationship with the colony count.

Clearly in terms of absolute counts these results show great variation between individuals. In fig 1 the two samples which failed to grow colonies at $1 \times 10^5$ also gave poor growth at $1 \times 10^6$ but in samples with more profuse growth (11 > 80 colonies at $1 \times 10^5$ and 12 > 280 colonies at $1 \times 10^6$) only four individuals were common to both. The data indicated that colony number was not directly proportioned to the number of cells plated. Figure 2 shows the relationship between colony number and

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**Table I Results of differential marrow counts performed on 16 rib and 16 aspirate specimens**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Mean Rib</th>
<th>SD Rib</th>
<th>Range Rib</th>
<th>Mean Aspirate</th>
<th>SD Aspirate</th>
<th>Range Aspirate</th>
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<tr>
<td>Blast</td>
<td>3-3</td>
<td>2-9</td>
<td></td>
<td>0-9</td>
<td>1-1</td>
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<td>Promyelocyte</td>
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<td></td>
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<td>1-0</td>
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<tr>
<td>Myelocyte</td>
<td>14-5</td>
<td>13-6</td>
<td>2-3</td>
<td>4-3</td>
<td>11-2-18-4</td>
<td>4-8-19-2</td>
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<tr>
<td>Metamyelocyte</td>
<td>36-1</td>
<td>23-5</td>
<td>5-6</td>
<td>6-0</td>
<td>23-8-42-2</td>
<td>14-9-30-2</td>
</tr>
<tr>
<td>Polymorph</td>
<td>7-2</td>
<td>19-9</td>
<td>2-7</td>
<td>5-1</td>
<td>3-8-13-1</td>
<td>10-4-29-2</td>
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<tr>
<td>Lymphocyte</td>
<td>4-7</td>
<td>2-8</td>
<td>1-9</td>
<td>1-9</td>
<td>2-1-9-3</td>
<td>0-8-7-2</td>
</tr>
<tr>
<td>Monocyte</td>
<td>3-1</td>
<td>2-2</td>
<td>1-6</td>
<td>1-6</td>
<td>1-2-7-0</td>
<td>0-6-4-8</td>
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<tr>
<td>Erythroblast</td>
<td>30-3</td>
<td>33-5</td>
<td>5-8</td>
<td>6-7</td>
<td>16-8-42-0</td>
<td>23-8-47-0</td>
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<tr>
<td>Plasma cell</td>
<td>0-9</td>
<td>0-8</td>
<td>0-6</td>
<td>0-5</td>
<td>0-2-2-6</td>
<td>0 - 1-2</td>
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<tr>
<td>Reticulum cell</td>
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<td>0-5</td>
<td>0-3</td>
<td>0-4</td>
<td>0 - 1-2</td>
<td>0 - 1-0</td>
</tr>
</tbody>
</table>

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**Fig 2 Relationship between colony number and marrow cells per culture, standardizing data from each individual rib sample by taking colony number at $1 \times 10^5$ as 100. All other counts expressed as mean and standard deviation relative to this.**

**Fig 3 Graphs showing the relationship between number of bone marrow cells per culture and colony number for individual rib samples. Mean ± SD of colony count from groups of four cultures at each cell concentration. Results of two separate marrows are shown on each graph.**
the number of cells per culture, standardizing the data from each individual sample by taking the colony number at $1 \times 10^5$ as 100 and expressing counts at other cell concentrations relative to this. Each marrow was not cultured at every cell concentration and the number of estimations for each point is shown. It can be seen that lack of proportionality between colony number and number of cells plated was a feature of normal marrow cultures. This analysis, however, fails to demonstrate the different patterns of individual marrows in which four separate groups could be identified by plotting individual graphs; fig 3 demonstrates two examples for each of these variations. The majority (57 out of 77 suitable for analysis) gave the type of non-linear relationship shown by the averaged data of fig 2, eg, fig 3c. A further four samples gave a count at $1 \times 10^6$ cells per culture, which was lower than at either $2.5 \times 10^5$ or $5 \times 10^5$ cells per culture (fig 3d). Thirteen of the samples showed a direct proportionality up to and including $1 \times 10^6$ cells per culture (fig 3b), while three individuals showed disproportionately high colony counts at $1 \times 10^6$ cells per culture in relation to lower cell counts (fig 3a).

Parallel cultures were made in which extraneous colony-stimulating factor was omitted and colony counts in these cultures showed that below a critical cell concentration the count fell rapidly to zero; a single example is shown in figure 4. This cell concentration at which spontaneous colony formation occurred varied from marrow to marrow (usually within the range 1 to $5 \times 10^5$ cells per culture) and was not related to the actual number of colonies growing at that cell concentration.

Aspirated marrow

Results from cultures of 30 marrow samples taken by aspiration from the iliac crest are shown in fig 5 for comparison with the results for rib marrow (fig 1). The distribution of colony counts at $1 \times 10^6$ and $1 \times 10^5$ cells per culture was very similar to that shown for rib marrow. For aspirated marrow the mean colony counts were 45 at $1 \times 10^5$ (range 0-175, SD ± 41) and 174 at $1 \times 10^6$ (range 1-470, SD ± 121).
Figure 5  Histogram of numbers of colonies per culture from aspirated samples cultured at (a) $1 \times 10^5$ cells per culture; (b) $1 \times 10^6$ marrow cells per culture.

Figure 6  Relationship between colony number and marrow cells per culture, standardizing data from each individual aspirate sample by taking colony number at $1 \times 10^5$ as 100. All other counts expressed as mean and standard deviation relative to this.
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<table>
<thead>
<tr>
<th></th>
<th>1 × 10⁴</th>
<th>2.5 × 10⁴</th>
<th>5 × 10⁴</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rib</td>
<td>Aspirate</td>
<td>Rib</td>
<td>Aspirate</td>
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<tr>
<td>Number of samples</td>
<td>66</td>
<td>24</td>
<td>68</td>
<td>25</td>
</tr>
<tr>
<td>Growth equal to stimulated plates</td>
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<td>2</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Growth less than stimulated plates</td>
<td>7</td>
<td>7</td>
<td>35</td>
<td>9</td>
</tr>
<tr>
<td>No growth</td>
<td>58</td>
<td>15</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>P</td>
<td>0.0185</td>
<td>0.0636</td>
<td>0.4567</td>
<td>1.000</td>
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</tbody>
</table>

Table II Comparison of spontaneous colony formation in rib and aspirate samples

These characteristics of human colony growth can obviously lead to difficulties in the interpretation of results from patients with haematological disease. For example, we have found (unpublished data) that colony counts from cultures of marrow from patients with acute lymphoblastic leukaemia in remission were significantly lower than normal when cultured at 10⁶ cells per culture but there was no significant difference between the groups when cultures containing 10⁵ cells were used.

Taken together these results show that a simple estimation of colony-forming cells in a single marrow sample is unlikely to be of value in assessing the granulopoietic capacity of an individual. Further investigations are necessary to establish whether or not sequential samples from the same individual over a period of time would yield more informative results.

Discussion

Our results from cultures of rib marrow taken from non-haematological patients showed a wide variation in colony-forming capacity between individuals. This was not reflected in differing content of granulocytic precursors in the samples as judged by examination of Giemsa-stained preparations, since these lay within relatively narrow ranges (table I).

Similar variations in colony number have been reported by others in smaller series of marrow aspirates (table III).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Date</th>
<th>No. of Samples</th>
<th>Cell Concentration</th>
<th>Range</th>
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<tr>
<td>Senn et al</td>
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<td>15</td>
<td>1 × 10⁴</td>
<td>15-50</td>
</tr>
<tr>
<td>Harris and Freireich</td>
<td>1970</td>
<td>6</td>
<td>1 × 10⁴</td>
<td>26-410</td>
</tr>
<tr>
<td>Brown and Carbone</td>
<td>1971</td>
<td>17</td>
<td>2 × 10⁴</td>
<td>8-48</td>
</tr>
<tr>
<td>Greenberg et al</td>
<td>1971</td>
<td>37</td>
<td>1 × 10⁴</td>
<td>14-126</td>
</tr>
<tr>
<td>Greenberg and Schrier</td>
<td>1973</td>
<td>22</td>
<td>1 × 10⁴</td>
<td>14-36</td>
</tr>
<tr>
<td>Moore et al</td>
<td>1973(a)</td>
<td>6</td>
<td>2 × 10⁴</td>
<td>13-90</td>
</tr>
</tbody>
</table>

Table III Culture of marrow aspirates from non-haematological patients

Colony growth from marrow samples obtained by aspiration gave essentially the same mean values and showed the same degree of variation as that from rib samples. It is clear, therefore, that variation introduced by the method of sampling is a minor factor in the observed variation of results for colony-forming capacity. The fact that colony number was often not proportional to the number of marrow cells plated (figs 2 and 3) also limits the value of any figure for colony-forming potential in a given marrow, particularly since the degree of disproportionality at higher cell concentrations varied from marrow to marrow (fig 3). Rib and aspirate marrows had similar characteristics with regard to this disproportionality.

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References


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