Regulation of Bone Marrow Cell Growth in Diffusion Chambers: The Effect of Adding Normal and Leukemic (CML) Polymorphonuclear Granulocytes

By Arne Bøyum, Dagfinn Lövhaug, and Werner R. Boecker

Inhibition of granulopoiesis was studied using the diffusion chamber (DC) technique. When mature granulocytes from human blood or syngeneic mouse peritoneal fluid were added to mouse bone marrow cells cultured in DC, a significant depression of granulopoiesis took place, and a stimulation of macrophage formation was observed in 7-day cultures. Human granulocytes had a stronger inhibitory capacity than mouse granulocytes. The inhibition appeared to be tissue-specific and caused by a diffusible factor. A time study showed that the added granulocytes had no observable effect on the growth of proliferative granulocytes and CFU-C during the first days of culture. A rapid decrease of proliferative granulocytes after day 5 was preceded by a similar reduction of CFU-C 1 day earlier. The effect on CFU-S was more variable. In one strain of mice, there was a consistent increase in the granulocyte co-culture group, whereas in another strain a significant increase was observed only on day 2. Histologic examination showed that mature granulocytes changed the colony distribution, so that a significant relative decrease of granuloid colonies occurred. The nature of this delayed suppression of granulopoiesis is not evident from these data. A possible explanation is that factors released by mature granulocytes prevent recruitment of CFU-C and granulocyte precursors from the CFU-S compartment by blocking the granulopoietic pathway. Leukemic (CML) granulocytes isolated from blood were less able to inhibit granulopoiesis than normal granulocytes with mouse bone marrow as well as human bone marrow as target cells.

There is growing evidence that the stability of the granulopoietic system depends upon a balanced interplay of stimulatory and inhibitory factors. The stimulatory factors have been extensively studied (reviewed by Metcalf1), but recently considerable interest has also focused on inhibitors. These may be present in serum2-5 and urine,6 and are also produced by the functional end cells. A low-molecular-weight peptide,7-8 released by mature granulocytes, reduces DNA synthesis of granulocyte precursors in cultures of bone marrow cells.7-9 Furthermore, granulocytes10,11 or granulocyte extracts12,13 inhibit formation of granulocyte and macrophage colonies in agar. The inhibitor appears to be species independent but tissue specific.16,17

In the present experiments we have studied inhibition of granulopoiesis by adding mature granulocytes to bone marrow cells cultured in diffusion chambers (DC). The cells were harvested daily in an attempt to distinguish between an effect on the transit population and early progenitor cells. The effect on
multipotent stem cells was investigated by combining the DC technique and the spleen colony assay. A comparison was also made between the effects of mature granulocytes from normal and leukemic (CML) individuals.

MATERIALS AND METHODS

Male C3D2 mice and female and male NFMR/BOM mice were used as cell donors and as recipients in the spleen colony assay. Female and male NMRI/BOM mice were used as DC hosts.

Cell Separation

Following dextran sedimentation of human EDTA blood, the leukocytes were separated by the Isopaque Ficoll ("Lymphoprep," Nyegaard et Co., Oslo, Norway) technique. The cells, with an admixture of platelets and harvested from the interface region after centrifugation, were composed of approximately 85% lymphocytes and 15% monocytes; they were almost devoid of granulocytes and erythrocytes. In some experiments platelet contamination was avoided by using defibrinated blood. The granulocytes (99% purity) recovered from the bottom of the tube were contaminated with two to four erythrocytes per granulocyte. When blood from patients with CML was separated by the same technique, the bottom fraction comprised 0.5% lymphocytes, 1.7% myelocytes, 8.8% band forms and metamyelocytes, 85.3% neutrophils, and 3.7% eosinophils (eight separations).

The admixture of monocytes to lymphocytes was reduced from 15% to 1% by a procedure described elsewhere.

Human bone marrow cells were aspirated from the posterior iliac spine into syringes containing EDTA. Equal parts (4 ml) of the bone marrow specimen and 0.9% NaCl were mixed, layered over Isopaque Ficoll, and centrifuged (800 g, 20 min). The cells recovered from the interface region were used for DC cultures. They were composed of approximately 50% proliferative granulocytes, <1% mature granulocytes, 10% erythroid cells, 35% lymphocytes, and 3% of other cells (six separations).

Mouse granulocytes were obtained by peritoneal lavage 4 or 24 hr after injecting 1 ml of a 10% solution of Bacto tryptone.

Mouse lymphocytes were obtained by dissecting out mesenteric lymph nodes. After mincing the nodes with a pair of scissors, the cells were suspended using a Pasteur pipette.

Diffusion Chamber Cultures

Harvesting of mouse bone marrow, intraperitoneal implantation of chambers, and cell retrieval were done as described previously. In the cell-mixing experiments, equal parts of the bone marrow cell suspension and the suspension of either granulocytes or lymphocytes were mixed immediately before chamber filling. Each chamber contained 100 μl of the cell suspension.

Double diffusion chambers were made from two plastic rings and three Millipore filters, glued together so that the chamber halves were separated by one filter. The cell numbers harvested from these chambers after 7 days of culturing amounted to 70%, 80% of those obtained with single chambers.

Control chambers (six to seven) and experimental chambers (six to seven) were always run at the same time. The cultured bone marrow cells were identified as (1) proliferative granulocytes (myeloblasts, promyelocytes, and myelocytes), (2) nonproliferative granulocytes (metamyelocytes, band forms, and segmented cells), (3) macrophages (including monocytoid cells), and (4) other cells.

Phytohemagglutinin (PHA, Wellcome Reagents Ltd.) stimulation of lymphocytes was done as follows. The chambers were filled with the cell suspension, and before implantation they were incubated for 45 min at 4°C in Medium 199 containing 10% (by volume) of the reconstituted PHA solution. The harvested cells were identified as lymphocytes, blast cells, and macrophages.

Mitomycin (Kyowa Hakko Kogyo Co., Tokyo) treatment of granulocytes and lymphocytes was done by incubating the cells at 37°C for 30 min in medium containing mitomycin (0.04 mg/ml). The cells were thereafter washed twice.
CFU-S Assay

The recipient mice were irradiated with 950 rads. The spleens were removed 7 days after the cell injection. For histologic examination, every sixth section (5-μm thickness) was mounted and stained with hematoxylin and eosin.

CFU-C Assay

The concentration of colony-forming cells in vitro was determined using the methylcellulose technique,23 with medium conditioned by mouse embryo cells as source of colony-stimulating activity. After 7 days of culturing, cell aggregates of more than 50 cells were scored as colonies.

Statistics

In experiments with DC (except Table 5), the mean value of six to seven chambers was taken as one observation. In the CFU-S and CFU-C assays, the number of colonies per spleen per plate was used as one observation. The Student’s t test was used for statistical analysis, and significant differences were confirmed with the two-sample rank test.

RESULTS

Human, Mouse, and Pig Granulocytes Added to Mouse Bone Marrow Cells in DC: Dose-Response Studies

When $2 \times 10^6$ mouse bone marrow cells were inoculated, $9.2 \pm 0.4 \times 10^6$ cells were harvested after 7 days (13 experiments). The differential counts were as follows: proliferative granulocytes, $36\% \pm 3\%$; nonproliferative granulocytes, $43\% \pm 4\%$; macrophages, $21\% \pm 2\%$. Adding $5 \times 10^4$ human granulocytes per chamber had no effect upon the cell growth (Fig. 1), whereas with $10^5$ or more granulocytes added, there was a significant ($p < 0.05$) decrease in proliferative granulocytes and a concomitant increase in macrophage formation. With $10^6$ granulocytes added, the number of proliferative granulocytes...
was depressed to 7% of control. The total granulocyte number was reduced less (to 35%) because of a relative increase of nonproliferative granulocytes.

Syngeneic mouse granulocytes (Fig. 2) had a less pronounced, but the same effect in principle as human granulocytes. With $10^6$ cells added, the number of proliferative granulocytes ($p < 0.001$) as well as total granulocytes ($p < 0.05$) were depressed, whereas macrophage formation was enhanced ($p < 0.01$). The half-disappearance time of mouse neutrophils, as determined in separate chambers, was somewhat shorter (14 hr) than that of human neutrophils (22 hr). The inhibitory effect of pig granulocytes ($10^6$) was similar to that of mouse granulocytes (3 experiments).

**Human Granulocytes and Lymphocytes Added to Mouse Bone Marrow Cells in DC: Time Studies**

The added granulocytes ($10^6$ per culture) had no effect upon the growth of proliferative granulocytes during the first 5 days, but thereafter a significant decrease to $7.5\% \pm 1.3\%$ of control on day 7 occurred (Fig. 3). Conversely, macrophage formation was significantly enhanced from day 3 ($p < 0.05$). The results were essentially the same when contaminating erythrocytes had been removed by NH$_4$Cl lysis prior to culturing. Lymphocytes ($10^6$ per culture) apparently had a similar effect as granulocytes (Fig. 3), and the number of proliferative granulocytes was depressed to 50% of control on day 7. This effect on day 7 persisted when monocytes (15%) were removed. The large number of macrophages during the first days was probably due to admixture of monocytes among the added lymphocytes.

**Specificity of the Inhibition**

Morphological examination indicated inhibition by an immune reaction of xenogeneic lymphocytes (Fig. 3), since in single chambers with severe depression of granulopoiesis, there was mostly a large number of lymphoblasts. Irradiation (2000 rads) of lymphocytes from EDTA–blood (Table 1) reduced their inhibitory effect ($p < 0.05$). However, some inhibition persisted, possibly due to an effect of contaminating platelets, as suggested by the observation that the inhibitory effect of lymphocytes from defibrinated blood was com-
Fig. 3. Time study of the effect of adding human blood granulocytes and lymphocytes to mouse bone marrow cells. Three groups were compared, with chambers containing: (1) \(2 \times 10^5\) mouse bone marrow cells; (2) \(2 \times 10^5\) mouse bone marrow cells and \(10^6\) human blood granulocytes; or (3) \(2 \times 10^5\) mouse bone marrow cells and \(10^6\) mononuclear cells (85% lymphocytes, 15% monocytes) from human EDTA-blood. The number of experiments (six to seven chambers each) per point are shown. The cells were harvested from days 1 to 7, and the figure shows mean cell numbers (+ SE) per \(10^6\) bone marrow cells cultured.

Completely eliminated by irradiation (Table 1). Irradiation did not significantly affect the inhibitory effect of granulocytes (Table 1). Furthermore, mitomycin treatment, which did not affect inhibition by granulocytes (4 experiments), eliminated the inhibitory effect of lymphocytes from defibrinated blood (4 experiments).

Syngeneic lymph node lymphocytes (\(10^6\) per chamber) did not affect the proliferation of bone marrow cells in 7-day cultures (3 experiments). Larger cell numbers (2, 3, or \(18 \times 10^6\)) increased the macrophage yield to approximately 200\% of control, whereas granulocyte formation was unaffected.

With mouse bone marrow cells on one side and human lymphocytes on the other side, the mononuclear cell yield was increased to approximately 200\% of control (Table 1).

Table 1. The Effect of Irradiating the Granulocytes and Lymphocytes Added to Mouse Marrow Cultures

<table>
<thead>
<tr>
<th>Cells Added</th>
<th>Irradiation Dose</th>
<th>No. of Exp</th>
<th>No. of Cells (Per Cent of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10^6) Granulocytes</td>
<td>0</td>
<td>5</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>from EDTA-blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^6) Lymphocytes</td>
<td>2000</td>
<td>5</td>
<td>24 ± 9</td>
</tr>
<tr>
<td>from EDTA-blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^6) Lymphocytes</td>
<td>0</td>
<td>3</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>from EDTA-blood</td>
<td>2000</td>
<td>3</td>
<td>72 ± 10</td>
</tr>
<tr>
<td>(10^6) Lymphocytes</td>
<td>0</td>
<td>3</td>
<td>64 ± 9</td>
</tr>
<tr>
<td>from defibrinated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>blood</td>
<td>2000</td>
<td>3</td>
<td>91 ± 5</td>
</tr>
</tbody>
</table>

Human granulocytes were isolated from EDTA-blood, and human lymphocytes from EDTA-blood or defibrinated blood. Each chamber contained \(2 \times 10^5\) mouse bone marrow cells and \(10^6\) unirradiated or irradiated granulocytes or lymphocytes. Culture period was 7 days, six to seven chambers per experiment.
Table 2. Effect of Diffusible Factors Released From Human and Mouse Leukocytes on Growth of Mouse Bone Marrow Cells in Double DC

<table>
<thead>
<tr>
<th>Cell Type Added</th>
<th>No. of Cells Added</th>
<th>No. of Exp.</th>
<th>No. of Cells (± SE) Harvested (Per Cent of Control)</th>
<th>No. of Cells (± SE) Harvested (Per Cent of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>10^6</td>
<td>2</td>
<td>75 ± 9 38 ± 3 57 ± 14 157 ± 14</td>
<td>55 ± 4 8 ± 2 32 ± 3 163 ± 22</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>4 x 10^6</td>
<td>3</td>
<td>83 ± 12 90 ± 15 80 ± 10 100 ± 17</td>
<td>84 ± 12 85 ± 13 144 ± 21</td>
</tr>
<tr>
<td>Human lymphocytes</td>
<td>4 x 10^6</td>
<td>5</td>
<td>61 ± 11 55 ± 10 56 ± 9 74 ± 12</td>
<td>94 ± 12 84 ± 12 144 ± 21</td>
</tr>
<tr>
<td>Syngeneic</td>
<td>4 x 10^6</td>
<td>2</td>
<td>114 ± 14 107 ± 14 109 ± 9 129 ± 14</td>
<td>83 ± 12 90 ± 15 80 ± 10 100 ± 17</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>4 x 10^6</td>
<td>4</td>
<td>114 ± 14 107 ± 14 109 ± 9 129 ± 14</td>
<td>83 ± 12 90 ± 15 80 ± 10 100 ± 17</td>
</tr>
<tr>
<td>Syngeneic lymphocytes</td>
<td>40 x 10^6</td>
<td>3</td>
<td>114 ± 14 107 ± 14 109 ± 9 129 ± 14</td>
<td>83 ± 12 90 ± 15 80 ± 10 100 ± 17</td>
</tr>
</tbody>
</table>

Normal mouse bone marrow cells (2 x 10^5) were cultured for 7 days in double DC. The other half of the chamber contained either: (1) medium (control); (2) human blood granulocytes; (3) human lymphocytes (15% monocytes); (4) syngeneic granulocytes (93% purity) obtained without further separation from peritoneal fluid 4 hr after injection of Bacto tryptone; and (5) syngeneic lymphocytes from mesenterial lymph nodes. All compartments contained medium with 10% mouse plasma.

other side of the Millipore filter of double DC, there was a slight but insignificant depression of granulopoiesis (Table 2), whereas the stimulation of macrophages was of borderline significance. With human granulocytes, the results were essentially the same as for co-cultures in single chambers. Large numbers (40 x 10^6) of syngeneic granulocytes inhibited granulocyte formation as well as macrophage formation (p < 0.025), whereas 40 x 10^6 syngeneic lymphocytes had no effect (Table 2).

Human granulocytes (10^6) did not inhibit PHA-induced blastoid transformation of autologous lymphocytes co-cultured in single DC for 3 or 7 days (3 experiments).

The Effect of Co-culture With Human Granulocytes and Lymphocytes on CFU-C and CFU-S

The number of CFU-C per chamber was not affected by the added granulocytes during the first 4 days of culturing (Fig. 4). Thereafter the number decreased rapidly to 32% of control on day 7. The number of CFU-S was significantly increased (p < 0.001) on days 1 and 2 and remained above control

![Fig. 4. The effect of human blood granulocytes on CFU-S and CFU-C. Group I (control): 2 x 10^5 mouse bone marrow cells per chamber. Group II: 2 x 10^5 mouse bone marrow cells + 10^6 human blood granulocytes devoid of erythrocytes (NH4 Cl lysis). The chambers were removed from days 1 to 7, and the contents of five chambers per group were pooled, washed twice, and then tested for CFU-S and CFU-C concentration. The figure shows the mean number (± SE) of CFU-S and CFU-C per chamber. The number of experiments, each comprised of seven to eight spleens and six plates, are shown. The cell loss by washing was somewhat larger in Group II (26%-37%) than in Group I (18%-24%). No corrections were made for this cell loss.](image-url)
Table 3. Distribution of Spleen Colony Types (Per Cent)

<table>
<thead>
<tr>
<th>Day</th>
<th>DC Contents</th>
<th>Erythroid</th>
<th>Granuloid</th>
<th>Megakaryoid</th>
<th>Mixed + Unidentified</th>
<th>No. of Colonies Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Mouse BMC</td>
<td>35 ± 4</td>
<td>51 ± 2</td>
<td>12 ± 4</td>
<td>2 ± 1</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>2 Mouse BMC</td>
<td>37 ± 2</td>
<td>51 ± 2</td>
<td>9 ± 1</td>
<td>3 ± 1</td>
<td>154</td>
<td></td>
</tr>
<tr>
<td>BMC + granulocytes</td>
<td>41 ± 3</td>
<td>46 ± 4</td>
<td>8 ± 1</td>
<td>5 ± 2</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>5 Mouse BMC</td>
<td>36 ± 5</td>
<td>48 ± 5</td>
<td>7 ± 1</td>
<td>9 ± 1</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>BMC + granulocytes</td>
<td>54 ± 2</td>
<td>35 ± 2</td>
<td>7 ± 2</td>
<td>4 ± 1</td>
<td>176</td>
<td></td>
</tr>
<tr>
<td>BMC + lymphocytes</td>
<td>43 ± 4</td>
<td>40 ± 3</td>
<td>9 ± 1</td>
<td>8 ± 4</td>
<td>113</td>
<td></td>
</tr>
</tbody>
</table>

Chambers containing bone marrow cells only (control), and chambers where in addition \(10^6\) human granulocytes or lymphocytes had been added, were implanted. The cells were retrieved after 2 and 5 days of culturing to be assayed with the spleen-colony technique. The spleens were examined histologically. Mean values (± SE) of five spleens per group are given.

values throughout the culture period (Fig. 4). The effect on CFU-S number was less pronounced with another strain (NMFR/BOM) of mice, although some increase (35\(\%\), \(p < 0.05\)) was observed on day 2, but not on days 3 and 5. However, the effect on proliferative granulocytes and CFU-C was the same in both strains.

Histologic examination showed no change of colony distribution on day 2 (Table 3), but on day 5 there was a significant decrease of granuloid colonies (\(p < 0.02\)) and a corresponding increase of erythroid colonies, when granulocytes had been added. Lymphocytes \((10^6)\) had no effect on colony number or distribution (Table 3).

Comparisons Between Normal and Leukemic (CML) Granulocytes

Mature blood granulocytes were obtained from healthy and leukemic individuals (see Materials and Methods). With \(2 \times 10^5\) granulocytes added per chamber, the leukemic cells depressed granulopoiesis in 7-day mouse bone marrow cultures less (\(p < 0.05\)) than normal cells did (Table 4), and the difference was even more pronounced with \(10^6\) granulocytes added (\(p < 0.01\)). Macrophage formation was enhanced to a similar degree in both groups.

Experiments With Human Bone Marrow Cells

When \(2 \times 10^5\) Isopaque Ficoll separated human bone marrow cells were cultured for 13 days in irradiated mice, the numbers \((\times 10^5)\) of the predominant

Table 4. The Effect of Normal and Leukemic Mature Granulocytes on Mouse Bone Marrow Cells Cultured in Diffusion Chambers

<table>
<thead>
<tr>
<th>Granulocytes Added Type</th>
<th>No.</th>
<th>No. of Experiments</th>
<th>No. of Cells (Per Cent of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prolif. Gran.</td>
</tr>
<tr>
<td>Normal (2 \times 10^5)</td>
<td>4</td>
<td>33 ± 7</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>Leukemic (2 \times 10^5)</td>
<td>4</td>
<td>65 ± 10</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>Normal (10^6)</td>
<td>5</td>
<td>12 ± 3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>Leukemic (10^6)</td>
<td>5</td>
<td>44 ± 6</td>
<td>51 ± 6</td>
</tr>
</tbody>
</table>

Normal and leukemic blood granulocytes were added to mouse (NMRI) bone marrow cells \((2 \times 10^5)\) in DC. The cells were harvested after 7 days of culturing. The cell numbers are given in per cent of controls without added granulocytes, seven to eight chambers per group in each experiment. Mean ± SE.
Table 5. The Effect of Autologous, Allogeneic, and Leukemic Granulocytes on Growth of Human Bone Marrow Cells in DC

<table>
<thead>
<tr>
<th></th>
<th>Cell No. (Per Cent of Control)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. A</td>
<td>Exp. B</td>
<td>Exp. C</td>
</tr>
<tr>
<td>Autologous</td>
<td>22 ± 5</td>
<td>361 ± 222</td>
<td>39 ± 11</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>17 ± 10</td>
<td>432 ± 202</td>
<td>33 ± 9</td>
</tr>
<tr>
<td>Leukemic</td>
<td>86 ± 17</td>
<td>328 ± 185</td>
<td>93 ± 26</td>
</tr>
</tbody>
</table>

In each DC 2 × 10^5 normal separated bone marrow cells were mixed with 3 × 10^6 granulocytes isolated from blood of the bone marrow donor, from another healthy individual, or from a leukemic (CML) patient. The chambers were implanted in mice (NMRI) preirradiated with 600 rads, and the cells were harvested after 13 days. Means ± SE for three different experiments are given as per cent of control values obtained in DC where no granulocytes had been added, nine to ten chambers in each group.

cell types harvested were as follows: proliferative granulocytes 55 ± 15, all granulocytes 77 ± 21, and macrophages 3.3 ± 1.7 (6 experiments).

Mature leukemic blood granulocytes (3 × 10^6) added to human bone marrow cells (2 × 10^5) depressed granulopoiesis less (p < 0.001, Table 5) than autologous and allogeneic granulocytes in two experiments (A and B). In these two experiments the normal and leukemic granulocytes had approximately the same survival times in DC (Fig. 5). In a third experiment, where leukemic granulocytes inhibited granulopoiesis almost to the same extent as normal granulocytes, the leukemic cells survived longer than control cells (Table 5, experiment C; Fig. 5C). The separated leukemic cells contained 0.4 (A), 1.6 (B), and 2% (C) myelocytes, respectively, and the majority of these myelocytes had disappeared after 3–4 days.

The number of macrophage-like cells in the controls amounted to only 1%, 2% of total cell number, as compared to 15%–20% in cultures of mouse bone marrow. Normal and leukemic granulocytes stimulated macrophage formation approximately to the same extent (Table 5), but the effect varied considerably.

DISCUSSION

The present experiments showed that mature syngeneic or xenogeneic granulocytes added to DC cultures of bone marrow cells depressed granulopoiesis (Figs. 1–3). The effect was caused by a diffusible factor, capable of passing
through Millipore filters (Table 2). The factor(s) may be released from dying granulocytes, but a continuous production and release by surviving cells is probably important, since leukemic granulocytes with prolonged survival in DC inhibited more than rapidly dying leukemic cells (Table 5, Fig. 5).

Mature granulocytes did not affect blastoid transformation and proliferation of lymphocytes. Further, syngeneic lymphocytes, unlike granulocytes, did not inhibit granulopoiesis in single or double DC. Thus the inhibition induced by mature granulocytes was probably specifically directed towards granulopoiesis. The inhibition caused by human lymphocytes (Fig. 3), which was eliminated by irradiation or mitomycin treatment, appeared to be a cytotoxic effect elicited by xenogeneic immune cells, probably combined with some inhibitory effect of contaminating platelets.

Mouse granulocytes inhibited granulopoiesis less (Fig. 2) than human granulocytes (Fig. 1). This difference could be due to a more rapid elimination of mouse peritoneal granulocytes than of human blood granulocytes, although the considerable difference may suggest that the human granulocytes were producing inhibitor at a higher rate than mouse cells.

The added granulocytes had no immediate inhibitory effect on cells already committed to granulopoiesis. Proliferative granulocytes (Fig. 3) as well as CFU-C (Fig. 4) increased at the same rate in the control and the granulocyte co-culture group during the initial period. A rapid decrease of proliferative granulocytes after day 5 was preceded by a similar reduction of CFU-C 1 day earlier. The nature of this suppression was not evident, but its delayed appearance may suggest an effect on the multipotent stem cell (CFU-S). The number of CFU-S was increased (Fig. 4) in the granulocyte co-culture group, although to a variable extent in two strains of mice. Nevertheless, this increase may reflect enhanced self-replication due to inhibited differentiation along the granulopoietic pathway. The consequence would be reduced replenishment of the CFU-C compartment and decreased production of granulocytes. Further investigations are necessary to verify this possibility.

The lack of immediate effect on proliferative granulocytes (Fig. 3) and CFU-C (Fig. 4) may appear somewhat in disagreement with previous findings. MacVittie and McCarthy observed a decreased DC cellularity\(^2\) and CFU-C content\(^2\)\(^4\) (\(-30\%\)) already from the first days of culture, when the host animals were repeatedly injected with medium conditioned by large numbers (30 \times 10^6 per dose) of peritoneal granulocytes. A possible explanation for this discrepancy is that the amount of factors released by the granulocytes added in our experiments was insufficient to inhibit mitotic multiplication, but nevertheless capable of affecting the differentiation pattern of CFU-S.

The granulocyte depression effect and the macrophage-stimulating effect are probably exerted by separate mechanisms. The enhanced macrophage formation starts before any effect on proliferative granulocytes is noticed (Fig. 3). Further, syngeneic granulocytes stimulate macrophage proliferation (Fig. 2), but fail to do so in double DC (Table 2). However, it cannot be totally excluded that, if the differentiation pattern of a common progenitor cell towards granulopoiesis is blocked,\(^2\)\(^5\) it could produce macrophages rather than granulocytes.
Mature leukemic granulocytes depress granulopoiesis less than normal granulocytes (Table 4, Table 5, A, B), indicating that granulocytes in CML are deficient in their ability to produce inhibitors. This finding could partially or wholly explain the increased granulocyte production in CML. In one experiment, where leukemic cells showed prolonged survival in DC (Fig. 5C), they inhibited granulopoiesis to the same extent as normal cells (Table 5C), probably because the duration of the inhibitory effect on progenitor cells was then extended.

Although it appears that the granulocyte-inhibiting effect is cell-line specific, it remains to show conclusively that it has any physiologic role in homeostatic control of granulopoiesis.

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REFERENCES

11. Laerum OD, Maurer HR: Proliferation kinetics of myelopoietic cells and macrophages in diffusion chambers after treatment with granulocyte extracts (chalone). Virchows Arch (Zellpathol) 14:293, 1973
18. Böyum A: Separation of leucocytes from...


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