Role of Colony-Stimulating Activity in Murine Long-Term Bone Marrow Cultures: Evidence for its Production and Consumption by the Adherent Cells

By Jean-Michel Heard, Serge Fichelson, and Bruno Varet

The involvement of colony-stimulating activity (CSA) in murine long-term bone marrow cultures (LTBMC) was studied using bilayer agar cultures. The supernatants of LTBMC were removed, a layer of dense agar was spread over the cells adherent to the bottom of the flask, and fresh myeloid cells were plated as source of CFU-C in an upper agar layer. Large numbers of granulocytic and macrophagic colonies developed regularly when target cells were plated over adherent cells of nonrecharged and >12 wk old LTBMC that were hematopoietically inactive (i.e., producing a low number of nonadherent cells). The removal of adherent cells from the myeloid cells used as source of CFU-C did not decrease the number of colonies. This suggests that adherent cells of LTBMC release CSA that is directly active on CFU-C. This CSA was no longer detectable over adherent layers of hematopoietically active LTBMC. A close inverse relationship was demonstrated between the number of nonadherent cells harvested before the assay and the level of CSA. No inhibitor for CSA was demonstrated in the supernatant of hematopoietically active cultures. Murine exogenous CSA incubated over the adherent layer lost its activity within 24 hr, whereas in the same conditions human CSA retained its activity. These data demonstrate the production of CSA by the adherent layer of LTBMC and strongly suggest its specific in situ consumption by differentiating myeloid cells.

THE CULTURE SYSTEM described by Dexter et al. allows a sustained myelopoiesis during several months in vitro. In this system, a bone marrow adherent layer appears to provide the cellular environment necessary for the proliferation of hematopoietic stem cells and their differentiation into a variety of committed myeloid progenitors. These include granulocyte-macrophage progenitor cells (CFU-C), erythroid precursors (BFU-E), and megakaryocytes precursors. Moreover, a complete differentiation and maturation is observed only within the granulocyte-macrophage lineage, leading to the continuous production of mature granulocytes and macrophages. Megakaryopoiesis is observable to a lesser extent.

Granulocyte-macrophage colony stimulating factors (GM-CSF) or colony stimulating activities (CSA) are entities necessary for the in vitro growth of colonies derived from CFU-C and are candidate hormones of granulopoiesis and monocytopoiesis. However, the involvement of CSF in vivo in granulopoiesis is not clear. In vivo inoculation of CSF in mice leads to a slight increase in medullar and circulating granulocytes, but similar results have been observed with anti-CSF antibodies.

The long-term bone marrow culture (LTBMC) system thus appears to be an interesting tool to study the role of CSF. It is surprising to find no CSA in these cultures despite the presence of a megakaryocyte-stimulating activity. Furthermore, the addition of CSA from different sources—endotoxin lung CSF, WEHI-3B cell line conditioned medium, or L-cell conditioned medium and CSF—has no notable effect on the long-term myelopoiesis. The addition of an anti-CSF antibody directed against L cell CSF does not inhibit the continuous formation of granulocytes and macrophages. Three hypotheses might explain these results: (1) CSF is not physiologically required for granulopoiesis. (2) Granulocyte-macrophage precursors in LTBMC are CSF-independent and may be "preleukemic." This hypothesis must not be overlooked since several groups are using LTBMC to study malignant transformation induced in vitro in myeloid cells. It is very unlikely, however, because granulocyte-macrophage progenitor cells in these cultures exhibit a normal responsiveness to different CSA when tested in vitro in clonal assays. (3) CSF may be produced in a limited amount by the cells of the adherent layer, and its activity restricted locally. This latter hypothesis has been suggested to explain the lack of effect of CSF or anti-CSF added to LTBMC.

In order to further explore the role of CSF in LTBMC, we used a bilayer agar culture system. A bottom dense agar layer retained the adherent cells of a LTBMC. Fresh bone marrow cells, used as a source of progenitors of granulo-macrophagic colonies (CFU-C), are set up in an upper agar layer. The target...
CFU-C are able to form granulo-monocytic colonies only if the adherent cells produce a colony-stimulating activity.

**MATERIALS AND METHODS**

**Mice**

\((C57BL/6 \times BALB/c)F_1\), mice were obtained from our own colonies maintained in pathogen-free conditions and used at the age of 8–16 wk. Mice were killed by cervical dislocation. Femurs, removed in sterile conditions, were used as a source of myeloid cells for LTBMC and as a source of target cells for CSA-induced colonies.

**Long-Term Bone Marrow Cultures**

The technique described by Dexter and Testa\(^1\) was used with few modifications. Briefly, the content of a single femur was flushed into a 25 cm flask (Corning, Corning, N.Y.) containing 10 ml of Fischer’s medium with 20% horse serum (Biopro, Lille, France, batch no 85220), 1-glutamine (2 m\(\text{M}\)), and hydrocortisone 10 \(\mu\text{M}\). The cultures were incubated at 33°C and 5% CO\(_2\), and half the medium removed and refed each week with an equal volume of fresh medium. Following 18–22 days from initiation, an adherent layer of fibroblasts, fat cells, and hematopoietic cells was obtained. The cultures were then either continuously weekly refed until they were assayed for CSA production, or recharged with a second fresh bone marrow in 10 ml of fresh culture medium. These recharged cultures were weekly refed and then assayed for the production of CSA.

**Single Layer Agar Culture**

Mouse bone marrow cells (7 \(\times\) 10\(^5\)) were plated in 32 \(\times\) 10 mm Petri dishes (Falcon, Becton-Dickinson, Grenoble, France) in 1 ml of alpha medium with 0.3% soft agar (Difco, Detroit, Mich.) and 15% fetal calf serum (FCS, Batch n: 29101807, France) as described by Metcalf.\(^2\) According to the experiments, this culture medium was supplemented with variable concentrations (as indicated in figures and tables) of: (A) serum from 6-hr endotoxin-treated C57BL/6 mice as a source of CSA (lipopolysaccharide E. coli 5 \(\mu\text{g}\) intraperitoneally)\(^3\); (B) L-cell conditioned medium (CM),\(^4\) (C) supernatant from LTBMC filtered through 0.45 \(\mu\text{m}\) filters (Millipore, France). After incubation for 6 days at 37°C, 5% CO\(_2\), with high humidity in air, colonies were counted under an inverted microscope. Only colonies containing more than 40 cells were scored. For identification, colonies were picked up by micropipetting, smeared by cytocentrifugation, and stained by May-Grünwald-Giemsa. Under these conditions, addition of 15% post-endotoxin mouse or 7.5% L-cell conditioned medium, respectively, induced 80–120 colonies and 70–90 colonies per 10\(^5\) plated cells.

**Bilayer Agar Cultures**

A bilayer agar culture was used for a direct bioassay of CSA production in LTBMC adherent layer at various moments following the initiation of the cultures. The culture medium of LTBMC was changed 4 days before the CSA-production assay, the supernatant was carefully removed, the cells pelleted by centrifugation, counted, and smeared for staining and identification. Three milliliters of 0.5% agar in alpha medium and 15% FCS were spread uniformly at the bottom of the flask over the adherent layer. After 30 min at laboratory temperature, a 1.2-mm thick layer of dense agar was obtained. This dense agar layer isolated the LTBMC adherent layer from fresh bone marrow cells used as target for the detection of CSA. These target cells were flushed from a femur of a \((C57BL/6 \times BALB/c)F_1\), mouse and filtered through a nylon mesh screen. Viable cells were counted by trypan blue exclusion. A quantity of 7 \(\times\) 10\(^5\) cells/ml were plated in the 3-ml second agar layer containing 0.3% of agar in alpha medium and 15% FCS. After solidification, the flasks were incubated for 7 days at 37°C, 3% CO\(_2\) with high humidity in air. Colonies were then counted in the upper layer under an inverted microscope. Only colonies containing more than 40 cells were scored and identified as described above for single layer agar cultures. No colony formation was observed when target cells were plated in control flasks without an adherent layer or when agar without target cells was layered over LTBMC adherent cells.

**Bilayer Cultures in Agarose**

In some experiments, cultures were done using a double layer of agarose (A45, IBF, France) instead of agar. Concentrations of 0.5% for the noncellular lower layer and 0.24% for the upper layer were used. In our hands, in single layer cultures with postendotoxin serum as source of CSA, the cloning efficiency was always 50% lower using agarose instead of agar.

**Macrophage Elimination**

Bone marrow cells used as target for the detection of CSA were passed twice through columns of Sephadex G10, as described by Ly and Mishell,\(^2\) using 50-ml columns. Briefly, 40 \(\times\) 10\(^6\) cells suspended in 4 ml were added dropwise to the column. The nonadherent cells were eluted with 40 ml of prewarmed RPMI 1640 medium containing 10% FCS and then passed similarly through a second column. The range of cell recovery was 20%, and less than 0.1% of these cells were capable of ingesting latex particles.

**Human CSA and Bone Marrow Cells**

As control of specificity in consumption experiments, human CSA and human bone marrow cells were layered over murine LTBMC adherent layers. Human CSA was obtained from placental conditioned medium as described elsewhere.\(^2\) Briefly, placenta obtained after surgical delivery was cut, homogenized with an electric mincer, and suspended in RPMI 1640 with 5% fetal calf serum. This suspension was incubated in 75 cm\(^2\) plastic flasks (Corning, Corning, N.Y.) for 7 days at 37°C and 5% CO\(_2\). The supernatant of these cultures was then centrifuged, filtered through 0.45 \(\mu\text{m}\), and stored at \(-80^\circ\text{C}\) before use. In standard assay in single layer agar culture, after 7 days, 10% of this supernatant induced 100–150 colonies/10\(^5\) human bone marrow plated cells.

Human bone marrow cells were obtained from resected ribs of patients undergoing thoracotomy. Immediately after removal from the patient, the rib was cut into segments and immersed in alpha medium with 10 U of Liqueumine ml (Laboratoire Roche, Paris, France). The marrow was flushed into a Petri dish and gently passed through a Pasteur pipette to obtain a single cell suspension. The aggregates were removed by filtration through a nylon mesh screen. Viable cells were counted by trypan blue exclusion. Briefly, 1.5 \(\times\) 10\(^5\) cells/ml were plated in the 3-ml upper agar layer containing 0.3% agar in alpha medium, 15% FCS in conditions identical to that used in the mouse bilayer agar cultures described above.

**RESULTS**

**Lack of CSA in LTBMC Supernatants**

Supernatants were harvested from 6 LTBMCs initiated 3 wk before and not recharged, and from 5 LTBMCs initiated 6 wk before and recharged 3 wk later. Flasks were refed 5 days before harvesting. The
supernatant was filtered and checked for CSA by addition to a single layer agar culture. Several concentrations from 7.5% to 45% of LTBMC supernatant were tested. Results were constantly negative whatever the concentration used (data not shown).

**Time Course of CSA Production by the Adherent Layer of LTBMC**

The production of CSA by the adherent layer was studied at various periods following the initiation of the cultures by using the bilayer agar culture assay. Results are shown in Fig. 1. CSA was demonstrated when fresh bone marrow cells were plated over adherent layers of nonrecharged LTBMCs. Colonies were of both granulocytic and macrophagic types. Granulocytic colonies were also observed when agarose was used instead of agar (Table 1). When adherent cells were removed from the fresh bone marrow cells used as target by two successive filtrations through a G10 column, the same numbers of colonies were observed, using as a source of CSA either LTBMC adherent layers or the serum of endotoxin-treated mice (Table 2). The number of nonadherent cells harvested with the supernatant of these nonrecharged LTBMCs before the assay was always less than 2 x 10^6 cells/culture. In contrast, when adherent layers had been recharged at day 21, the colony-stimulating activity dropped abruptly, and only a few macrophagic colonies were observed. The supernatants of these cultures always contained a great number of nonadherent cells (>8 x 10^6/culture). After 12 wk, when the number of nonadherent cells produced by LTBMCs decreased, CSA was again detectable. This result and the sustained production of CSA by the adherent layer of nonrecharged cultures demonstrated that the drop in CSA production was not due to the aging of the adherent layer. The results suggested a relationship between the ability to detect CSA and the number of nonadherent cells harvested with the supernatant before the assay.

**Relationship Between the Production of CSA by the Adherent Layer and the Number of Nonadherent Cells Harvested Before the Assay**

In order to look for such a relationship, cultures were initiated every 4 days from day 48 to day 12 before the assay for CSA. Flasks were thus recharged or not in pairs, 20 days after initiation, i.e., on day 24, 20, 18, 16, 12, 8, and 4, respectively, before the assay. The goal of this experimental schedule was to assay, for the production of CSA, all the adherent layers on the same day using myeloid cells from a single animal as targets for the colony formation. The results indicated that the number of cells harvested from each culture before the assay was inversely related to the number of myeloid colonies scored in the layer of fresh bone marrow cells used for the detection of CSA, as shown in Table 3 and Fig. 2 (r = -0.81 p < 0.001). This relationship remained significant within the group of nonrecharged cultures (r = -0.67, p < 0.01). Thus, the detected CSA decreased when the number of cells harvested with the supernatant before the assays increased, the number of cells in the supernatant being related to the number of precursors differentiating in the adherent layer. This result suggested two hypotheses: (1) the production of a CSA inhibiting factor

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Table 1. Induction of Granulo-macrophagic Colonies From Murine Bone Marrow Cells in Agar and Agarose Bilayer Cultures by Postendotoxin Mouse Serum and LTBMC Adherent Layer

<table>
<thead>
<tr>
<th>Source of CSA</th>
<th>Colonies/10^6 Plated Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherent layer of 2-wk-old nonrecharged LTBMCs</td>
<td>135 ± 39</td>
</tr>
<tr>
<td>5% postendotoxin mouse serum added in the lower agar layer</td>
<td>144 ± 17</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD of four experiments.

Table 2. Generation of Colonies From an Adherent-Cell-Depleted Bone Marrow in a Bilayer Agar Assay

<table>
<thead>
<tr>
<th>Source of CSA</th>
<th>Number/10^5 Cells*</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherent layer of 2-wk-old nonrecharged LTBMCs</td>
<td>238 ± 41†</td>
<td>Granulocytes and macrophages</td>
</tr>
<tr>
<td>5% postendotoxin mouse serum added in the lower agar layer</td>
<td>247 ± 5†</td>
<td>Granulocytes and macrophages</td>
</tr>
</tbody>
</table>

*Whole bone marrow cells were passed twice through a Sephadex G10 column before plating (see Materials and Methods).
†Mean ± 1 SD of four experiments.
Table 3. Relationship Between the CSA Detected by the Induction of Colonies Over the Adherent Layer and the Number of Nonadherent Cells in the Supernatant of the Cultures Before the Assay

<table>
<thead>
<tr>
<th>Days of Culture</th>
<th>Nonrecharged Cultures</th>
<th>Recharged Cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colonies/10^3 Cells Plated in the Upper Layer</td>
<td>Number of Cells Harvested Before the Assay (x 10^-1)</td>
</tr>
<tr>
<td>12</td>
<td>177</td>
<td>3.25</td>
</tr>
<tr>
<td>16</td>
<td>155</td>
<td>3.2</td>
</tr>
<tr>
<td>20</td>
<td>130</td>
<td>7.1</td>
</tr>
<tr>
<td>24</td>
<td>132</td>
<td>0.5</td>
</tr>
<tr>
<td>28</td>
<td>140</td>
<td>1.2</td>
</tr>
<tr>
<td>32</td>
<td>141</td>
<td>5</td>
</tr>
<tr>
<td>36</td>
<td>134</td>
<td>4.8</td>
</tr>
<tr>
<td>40</td>
<td>170</td>
<td>1.1</td>
</tr>
<tr>
<td>44</td>
<td>107</td>
<td>6.3</td>
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<tr>
<td>48</td>
<td>82</td>
<td>25</td>
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<td></td>
<td>65</td>
<td>1.4</td>
</tr>
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<td></td>
<td>64</td>
<td>6.6</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>27</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between numbers of nonadherent cells harvested with the supernatant and the LTBMC adherent cell CSA production detected in bilayer agar cultures. (▲) Nonrecharged cultures; (▼) cultures recharged with fresh bone marrow cells at day 20. Each point represents one experiment.

Fig. 3. CSA dose–response curves in monolayer agar cultures supplemented with filtered supernatant of LTBMC (●—●) or fresh culture medium (○—○). (A) Postendotoxin CSA dose–response curves. The final concentration of supernatant or fresh medium was 35%. (B) L-cell CSA dose–response curves. The final concentration of supernatant or fresh medium was 25%. Each point represents the mean ± S.D. three experiments.
by the myeloid cells differentiating in the adherent layer; (2) the in situ consumption of CSA by the granulocyte-macrophage progenitor cells.

**Lack of Inhibiting Activity for CSA in the Supernatant of Hemopoietically Active LTBMC**

In order to look for CSA inhibiting factor, pooled filtered supernatants from 5 LTBMCs was added to single agar layer cultures containing increasing concentrations of exogenous CSA. These supernatants were harvested from hemopoietically active cultures, i.e., recharged 3 wk previously and containing more than 7 x 10^6 cells in the supernatant. CSA dose–response curves were compared with those of control cultures. In the latter, complete fresh medium for growing LTBMC (Fischer's medium, 10% horse serum, 10⁻⁷ M hydrocortisone) was added. CSA dose–response curves were established with both postendotoxin mouse serum and L cell conditioned medium. In the presence of 35% LTBMC supernatant, no inhibitory effect was observed either on postendotoxin mouse serum or on L-cell CM dose–response curves, as shown in Fig. 3, A and B. A slight but not significant enhancing effect was shown in cultures supplemented with LTBMC supernatant. These results, using two different sources of CSA, showed the lack of detectable inhibitory effect to CSA in supernatants of hemopoietically active LTBMC.

**Consumption of Murine CSA by Adherent Layers of Hemopoietically Active LTBMCs**

To show whether CSA was indeed consumed in situ by the adherent layer of hemopoietically active LTBMCs, we used bilayer agar cultures to which CSA was added in the lower agar layer, thus maintained in close contact with the adherent cells. LTBMCs were 6 wk old and had been recharged 3 wk previously. They produced more than 7 x 10^6 cells/culture. The dense agar layer supplemented with postendotoxin mouse serum at a final concentration of 7% was incubated upon the adherent layer for 24 hr before plating the second agar layer containing the fresh bone marrow cells. Controls were made by incubating postendotoxin serum supplemented 0.5% agar layer in empty flasks for 24 hr. Table 4 shows that no granulocytic colony and few macrophagic colonies were obtained when the CSA-containing agar was previously incubated over a LTBMC adherent layer. In control cultures, the number of colonies was almost the same as that in mono-layer agar cultures containing a nonincubated CSA. In

**Table 5. Inactivation of Human and Murine CSA by the Adherent Layer of Hemopoietically Active Murine LTBMC**

<table>
<thead>
<tr>
<th>Origin of the Exogenous CSA and of the Bone Marrow Cells Used as Targets</th>
<th>Colonies/10⁶ Cells After Incubating Exogenous CSA for 24 hr Over*</th>
<th>Percent of Exogenous CSA Inactivated†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murine</td>
<td>LTBMCAdeherent Cells</td>
<td>Control-Cell-Free Flask</td>
</tr>
<tr>
<td>Macrophagic</td>
<td>34 ± 9</td>
<td>258 ± 30</td>
</tr>
<tr>
<td>Granulocytic and macrophagic</td>
<td>101 ± 5</td>
<td>131 ± 6</td>
</tr>
</tbody>
</table>

*Data represent the mean ± 1 SD of four experiments. Serum from LPS-treated mice was used as source of murine CSA at a final concentration of 7%. Conditioned medium from placental cells was used as source of human CSA at final concentration of 10%.

†Percent of inactivation of the exogenous CSA = 1 – Number of colonies after inhibiting CSA over LTBMC adherent layer/Number of colonies after incubating CSA into a cell free flask x 100.
contrast, the activity of exogenous CSA was scarcely preserved when incubated over adherent layers of either nonrecharged or 12-wk-old recharged cultures. These results suggested that the adherent layer of hematopoietically active LTBMC was able to consume large amounts of CSA.

**Lack of Consumption of Human CSA by Hematopoietically Active Murine LTBMC**

The former experiments might also be explained by the release of substances able to inactivate CSA by cells of the adherent layer. In order to rule out a nonspecific inactivation of the mouse CSA, we took advantage of the species specificity of CSA and we repeated the same type of experiments with human CSA. Human CSA was incubated for 24 hr over adherent layers of murine LTBMCs recharged 3 wk previously. Human myeloid cells were plated as target in the upper layer. Results shown in Table 5 demonstrated that human CSA persisted, whereas in the same experimental conditions the activity of murine CSA was sharply reduced.

**DISCUSSION**

CSA is a group of substances defined by their biologic activity in vitro: the ability to induce the differentiation of granulocyte-macrophage progenitors in a semisolid medium into colonies containing mature granulocytes and macrophages. Our results clearly demonstrate that the adherent layer of LTBMCs is able to produce high concentrations of CSA and strongly suggest that this CSA is further consumed by differentiating myeloid cells. These conclusions are based on the following main findings. (1) Adherent layers of nonrecharged or very old LTBMCs induced granulocytic and macrophagic colonies in a bilayer agar assay. This technique excluded any contact between cells of the adherent layer and target myeloid precursors plated in the upper layer, demonstrating the soluble nature of the substance responsible for this granulo-macrophagic differentiation. The persistence of colony formation after removing the adherent cells from the bone marrow cells used as source of CFU-C indicates that the factor inducing colonies is a genuine CSA and not a factor that stimulates the production of CSA by the added normal monocytes-macrophages. (2) A highly significant inverse relationship was observed between the number of nonadherent cells present in the supernatant of LTBMCs and the level of CSA detected by the bilayer agar technique over the adherent layer of the same LTBMCs. One may suppose that this indicates a similar relationship between the number of differentiating cells in the adherent layer and the amounts of detectable CSA. Indeed, works by Dexter et al. strongly suggested that cells in the supernatant were generated from the progenitors present in the adherent layer. (3) The disappearance of exogenous CSA incubated over the adherent layer of hematopoietically active LTBMCs suggested that the drop in CSA was due either to its consumption by the cells of the adherent layer or to its inhibition by products of these cells. The absence of an inhibitor for CSA in the filtered supernatant of these hematopoietically active LTBMCs argued against the latter hypothesis, but did not exclude the short range secretion of such an inhibitor within the adherent layer. However, this is very unlikely, since human CSA is not inactivated by incubation over adherent layers of hematopoietically active LTBMC. Indeed, this specific decrease of murine CSA incubated over murine myeloid cells fits very well with the known species specificity of CSA. Nevertheless, the local secretion of an inhibitor specific for murine CSA cannot be absolutely ruled out.

Since it was previously shown that agar was mitogenic for murine B cells in the presence of β-mercaptoethanol and was able to cause the release of CSA when incubated with human leukocytes, one could question whether the CSA we detected was really a spontaneous product of the adherent cells of LTBMC or a product induced by the stimulation of those cells by agar. This seems very unlikely since: (1) according to Trang Hoang et al.'s results, agar extracts do not induce colonies when directly added for 7 days to bone marrow cells plated in an inert semisolid medium; and (2) in our experiments, CSA was detected over adherent layers of LTBMC when agar was replaced by agarose. Indeed, agarose is a pure preparation of galactose polymers and differs from crude agar by the absence of sulphated polysaccharides and proteins that are presumably responsible for CSA induction.

Our results are not in conflict with the previously negative assays for CSA in LTBMC. Indeed, one can suppose that the negative assays of Dexter et al. and Williams et al. were performed on LTBMCs producing a great number of cells in the supernatant; in similar conditions, our assays were also negative. The only previously positive result in the search for CSF in LTBMC was apparently obtained by Stanley (quoted by Dexter and Shadduck) using a radioimmunoassay specific for CSF, but no precisions about the timing of this assay and the nature of the detected CSF are available. The lack of CSA in the supernatant, at least during the first weeks of culture before recharging, is probably explained by a too low concentration of CSA. Indeed, CSA was detected during the first 3 wk of culture by Queessenberry (personal communication) after concentrating the supernatant several-fold. The
absence of stimulatory effect on the cultures after adding exogenous CSF was explained by Dexter and Shadduck and Williams and Burgess by a cell-to-cell transmission of a CSF-like activity between the cells of the adherent layer. The lack of inhibitory effect of anti-CSF added to the cultures is another argument for a direct cell-to-cell transmission of CSF. It recalls the lack of in vivo effect of similar anti-CSF. Our results are consistent with this hypothesis, but suggest that a close cell-to-cell transmission of CSA does not exclude a long-range activity. This long-range activity is only detectable when the number of differentiating precursors within the adherent layer, and therefore the amounts of locally consumed CSA, are low.

A major conclusion to be drawn from this study is that LTBMC is no longer an exception to the general rule that CSA is required for the in vitro differentiation of granulocyte-macrophage progenitors. This strongly argues for a physiologic role of CSA as hormones of granulopoiesis. Furthermore, this strengthens the use of LTBMC as a suitable tool to study the long-term effects in vitro of oncogenic factors on normal myeloid cells.

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