Stromal Growth Factor Production in Irradiated Lectin Exposed Long-Term Murine Bone Marrow Cultures

By Thomas A. Alberico, James N. Ihle, Chi-Ming Liang, Helen E. McGrath, and Peter J. Quesenberry

Hematopoietic regulatory factors produced by adherent (stromal) cells in long-term murine bone marrow cultures have been investigated. Using an in situ double layer agar overlay system, we demonstrated that exposure of the stromal cells to 1,100-rad irradiation increased their activities in stimulating colony formation of FDC-P1, an interleukin 3 (IL 3)-responsive cell line. The colony-stimulating activities (CSAs) of the irradiated stroma also stimulated normal marrow cells to form granulocyte-macrophage, megakaryocyte, and mixed lineage colonies. Addition of the lectin pokeweed mitogen to the irradiated stroma increased the level of CSAs. The FDC-P1 CSA of the irradiated stroma was inhibited by antibodies directed against murine granulocyte-macrophage colony stimulating factor (GM-CSF) but not by those against murine IL 3.

The elicited CSAs, however, have not yet been identified and characterized.

In this paper we reported that treatment with lectin pokeweed mitogen further increased the CSAs' elaboration by irradiated adherent stromal cells. The secreted CSAs were identified by using antibodies directed against known cytokines.

MATERIALS AND METHODS

Long-term bone marrow cultures. Long-term liquid murine bone marrow cultures were established as previously described. Bone marrow obtained from the tibiae and femurs of either ICR or BDF1 12- to 20-week-old female mice (Dominion Labs, Dublin, VA and Jackson Labs, Bar Harbor, ME, respectively) were used. Cultures were established in 25 cm² Corning tissue culture flasks either from single cell suspensions or by expelling the marrow from one tibia and one femur directly onto the flask. Alternatively, cultures were carried out in 35 mm² Corning tissue culture plates or 0.32 cm² 96-well Costar flat bottom tissue culture microtiter wells from single cell suspensions. In all experiments, although variable volumes of Fishers' media, 20% horse serum, and 10⁻⁷ mol/L hydrocortisone were used, cell concentrations were established between 3 to 4 x 10⁶ cells per cc. Cultures were incubated at 33°C with 5% CO₂. They were maintained with weekly demidepopulation and replenished with cell-free fresh media.

In vitro irradiation. After three weeks of growth (one experiment was carried out to four weeks), nonadherent cells were removed, and all cultures were totally depopulated. The stromal layer was then exposed to 1,100 rad, using a gamma-cell 40 cesium 137 irradiation unit (Atomic Energy of Canada Limited, Ottawa, Canada) at a rate of 123 to 1,26 rad per minute. Control unirradiated stromal layers were included in each experiment. In one series of experiments, stromal layers were exposed to varying levels of irradiation (250, 700, 900, 1,100, 1,500, and 2,000 rad). After exposing these cultures to irradiation, they were incubated another seven days at 33°C with 5% CO₂ and 95% humidity. After seven days of incubation postirradiation, all supernatant media was removed and the stroma was assayed directly for CSA. Alternatively (see below), condition media was harvested from these cultures and assessed for CSA levels.

Stromal assay for colony-stimulating activity. Stromata were assayed for CSA using the method described by Heard et al and modified by Gualtieri et al. Seven days postirradiation all supernatant media was removed from these cultures, and a double-layer
agar system was employed to assay for CSA. Briefly, an underlayer of McCoy's 5A media with 2 mmol/L L-glutamine, 16 μg/mL L-asparagine, 8 μg/mL L-serine, 1 mmol/L sodium pyruvate, 1 x 10^{-4} mercaptoethanol, and 15% fetal calf serum, mixed in equal part with 1% agar, was added to these cultures (after all supernatant had been removed) and allowed to gel for 20 to 30 minutes. This layer separated the adherent stromal cells from the target cell population. In a number of these experiments, including variable-dose radiation experiments, potential stimulatory effect of pokeweed mitogen (GIBCO, Grand Island, N.Y.) was examined by adding this lectin at a final concentration of 2.5 μg/mL to the underlayer of both irradiated and nonirradiated cultures. After allowing this underlayer to gel, an overlayer was then added containing McCoy's media with additives plus 0.3% agar, along with the target cell population. The concentration of 2.5 g/mL served as controls.

above. The adherent layers were then incubated in media described above. Dexter cultures were established and irradiated as stromal system described above. Unconcentrated conditioned medium was separated the adherent stromal cells from the target cell population. In a single-layer version of the agar culture experiments, potential stimulatory effect of pokeweed mitogen was employed to assay for CSA. Briefly, an underlayer or 1% agar, was added to these cultures (after all supernatant had been removed) and allowed to gel for 20 to 30 minutes. This layer

Bioassays of conditioned media. In experiments where the conditioned media was used to measure the CSA produced by the stromal cells, Dexter cultures were established and irradiated as described above. The adherent layers were then incubated in media with or without 2.5 μg/mL pokeweed mitogen for 24, 48, 96, 120, 168 hours. At these time points the conditioned media was collected and used as a stimulus in a single layer version of the agar culture system described above. Unconcentrated conditioned medium was added at various levels to stimulate both FDC-P1 and fresh murine marrow colony formation in the agar cultures. Media from Dexter flasks with no cells and with and without pokeweed mitogen at a concentration of 2.5 μg/mL served as controls.

Colony morphology. Colonies are classified as macrophage, granulocyte, macrophage/granulocyte, macrophage monocyte (see legend for Table 1) and megakaryocyte. Soft-agar cultures were fixed in 10% formalin seven days after plating, and whole-agar slide preparations were then made from these cultures. These slides were then stained for acid phosphatase and counterstained with hematoxylin. Megakaryocyte colonies were defined as aggregates of three or more acetylcholinesterase-positive cells, and mixed colonies were defined as colonies that had <90% of the predominant cell type. Degenerate colonies were scored when distinctive morphological features were absent.

Antibody blocking experiments with adherent feeder layer. Dexter cultures were established and maintained in Costar 96-well microtiter tissue culture plates, as described above, to carry out experiments that involved limited amounts of reagents (ie, antibody to IL 3). Initial concentrations in the Costar 96-well microtiter plates were 3 to 4 x 10^5 cells/mL with a volume of 0.3 mL per well. All wells were demidepopulated weekly with total depopulation and replacement with cell-free media at three weeks and exposure of the adherent layers to I mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL. Antibody to IL 3 and control rabbit IgG at 1 mg/mL.

Histochemical stains of stroma. Histochemical stains were used to evaluate the cytochemistry of the stromal cell population following XRT and pokeweed mitogen exposure. These included alpha

Sources of IL 3. IL 3 was purified to homogeneity from WEHI-3-conditioned media, as previously described. Briefly, conditioned media from WEHI-3 murine myelomonocytic leukemia cells was concentrated on a 10,000-mol wt Amicon membrane. Ammonium sulfate fractionation was then used with a 50% to 80% fraction chromatographed on DEAE cellulose. IL 3, unlike GM-CSF, was not bound to the resin and was collected in the flow-through fraction. The flow-through fraction was concentrated and further purified by hydroxylapatite column chromatography, followed by G70 Sephadex and high-pressure liquid chromatography. The IL 3 preparations were then diluted in RPMI 1640 with 10% fetal calf serum and assayed for IL 3 using thymidine incorporation into the FDC-P1 indicator cell line, as previously described. The preparation of IL 3 contained 0.2 ng/U of activity, with one unit of activity defined as the amount of IL 3 that inhibited 50% of maximal thymidine incorporation into FDC-P1 cells in culture.

Polyclonal antibodies against recombinant Escherichia coli-derived murine GM-CSF. Purified recombinant murine GM-CSF (200 μg/rabbit) in an emulsion formed by equal volume of normal saline and Freund's complete adjuvant was injected into New Zealand white rabbits (2 mL/rabbit) at multiple sites intradermally. The rabbit was boosted with the same amount of antigen in Freund's incomplete adjuvant twice at one-month intervals. Serum was obtained ten to 14 days after each boost. The anti-GM-CSF serum was partially purified by precipitation with 40% ammonium sulfate, followed by dialysis against phosphate-buffered saline (PBS), pH 7.2. The solution was then incubated at 56°C to inactivate complement. The preparation from 1 mL of rabbit serum generally yielded 10 to 15 mg of rabbit IgGs. These anti-GM-CSF antibodies neutralized the cell proliferative activity of recombinant murine GM-CSF while pre-immune serum showed no effect.

Assay of natural murine GM-CSF (GM-CSA-2) or recombinant GM-CSF for FDC-P1 colony-stimulating activity. GM-CSA-2 was partially purified from supernatants of concanavalin A (Con A)-activated splenic T lymphocytes. Briefly, this involved removing BALB/c spleens, gently mashing them, and washing these cells in RPMI 1640. This suspension was cleansed of RBCs and was cultured with 5 μg/mL of Con A for 48 hours at 37°C. Cells were removed by centrifugation, and the supernatant was used for purification. The purification of the supernatant included ammonium sulfate precipitation with subsequent dialysis against a phosphate buffer. This dialysate was clarified by centrifugation at 10,000 RPM for 30 minutes and then applied to a diethyl aminoethyl (DEAE) cellulose column. The GM-CSA-2 activity was eluted from the column at 0.1 mol/L NaCl. This GM-CSA-2 purified in this manner is serologically and biochemically distinct from IL 3 and is not inhibited by antibody to IL 3. One unit of activity is defined as the amount of GM-CSA-2 that in 1 mL gives 50% maximal stimulation of a GM-CSA-responsive cell population. In a single-layer soft-agar system consisting of McCoy's 5A media plus additives and 0.3% agar (at a concentration of 80 U/mL), GM-CSA-2 was assayed for FDC-P1 CSA. These cultures were incubated for seven days at 37°C, 5% CO2. After seven days they were removed from the incubator, fixed with formalin, and colonies of 50 cells or greater were scored. Alternatively, recombinant E coli-derived murine GM-CSF was tested.

Sources of IL 3. IL 3 was purified to homogeneity from WEHI-3-conditioned media, as previously described. Briefly, conditioned media from WEHI-3 murine myelomonocytic leukemia cells was concentrated on a 10,000-mol wt Amicon membrane. Ammonium sulfate fractionation was then used with a 50% to 80% fraction chromatographed on DEAE cellulose. IL 3, unlike GM-CSF, was not bound to the resin and was collected in the flow-through fraction. The flow-through fraction was concentrated and further purified by hydroxylapatite column chromatography, followed by G70 Sephadex and high-pressure liquid chromatography. The IL 3 preparations were then diluted in RPMI 1640 with 10% fetal calf serum and assayed for IL 3 using thymidine incorporation into the FDC-P1 indicator cell line, as previously described. The preparation of IL 3 contained 0.2 ng/U of activity, with one unit of activity defined as the amount of IL 3 that inhibited 50% of maximal thymidine incorporation into FDC-P1 cells in culture.

Histochemical stains of stroma. Histochemical stains were used to evaluate the cytochemistry of the stromal cell population following XRT and pokeweed mitogen exposure. These included alpha
naphthyl acetate (nonspecific) esterase, acid phosphatase, and alkaline phosphatase. Stromal cells were stained 48 hours after exposure to XRT and pokeweed mitogen. Over 500 cells were counted per 35-mm plate, and percent positivity was scored qualitatively 0 to 4+. Cells in the 2 to 4+ range were scored as positives.

RESULTS

In situ stromal assay for growth factor production. The capacity of nonirradiated and irradiated 3- to 4-week-old Dexter stroma to produce a growth factor capable of stimulating proliferation of FDC-P1 cells is shown in Fig 1. Total colonies of 50 cells or greater were scored at seven days. There was an 18.2-fold increase in the numbers of FDC-P1 colonies stimulated by the irradiated stroma as compared to the nonirradiated stroma. However, colony growth in this system did not approach that seen with IL 3-rich WEHI-3-conditioned media; in these preparations FDC-P1 colonies were too numerous to count. Pokeweed mitogen (PWM) stimulates IL 3 production from T lymphocytes. Thus we added this to the adherent Dexter cell underlayer to evaluate its effect on the irradiated and nonirradiated stromal growth factor production (Fig 2). Addition of pokeweed mitogen at a concentration of 2.5 μg/mL to nonirradiated stroma did not increase colony formation of the FDC-P1 cell line in the agar overlays as compared to nonirradiated stroma without pokeweed mitogen. However, when the pokeweed mitogen was added to the underlayer of cultures with irradiated stroma, we found an 11.4-fold increase in colony numbers as compared to irradiated stroma without pokeweed mitogen. Different levels of irradiation were also assessed with and without the addition of PWM. As before, there is a marked increase in FDC-P1 CSA with the addition of PWM, with activity reaching a plateau at 700 rad (Fig 3). Although detectable, FDC-P1 CSA is lower without the addition of PWM, and there is a similar increase in FDC-P1 CSA with...
irradiation alone, which plateaus at 1500 rad. These data suggested that the FDC-P1-stimulating activity from lectin-stimulated irradiated stroma might be IL 3. However, an antibody to IL 3 that effectively blocked pure IL 3 stimulation of clonal growth of FDC-P1 cells in a double-layer agar system did not block irradiated PWM stromal-induced colony formation; in fact, both the IgG control and anti-IL 3 antibody increased colony formation (Fig 4).

**Assays of conditioned media.** As stated previously, we (and others) have had difficulty in detecting significant levels of myeloid growth factors from Dexter culture conditioned media. We also have had repeatedly negative results when conditioned media was assayed for IL 3 using tritiated thymidine incorporation into the FDC-P1 line. It was for this reason that the in situ assay method was used. However, since high levels of activity were seen when the irradiated stroma was exposed to PWM, we again evaluated media conditioned by the irradiated lectin-induced stromal cells. Figure 5 demonstrates high levels of CSA in this conditioned media, stimulating clonal proliferation of both FDC-P1 and fresh murine BDF1 marrow cells. There was no stimulation of the FDC-P1 cell line by media conditioned by irradiated stroma without pokeweed mitogen, but in conditioned media exposed to pokeweed mitogen had increased levels of CSF for both marrow and FDC-P1 cells. However, the threshold for stimulation was lower for the murine-marrow target cell population compared to the FDC-P1 target cells. Figure 5 shows the effect of the conditioned media after 48 hours of culture. These results were confirmed in two other experiments, and the same phenomenon was noted at time points of 24 and 168 hours in one of these experiments.

We have previously shown that CSF-1, as determined by radioimmunoassay, was present in cm from Dexter cultures and that the levels were not increased with irradiation. We have repeated these studies here with similar results and have also shown that CSF-1 levels from irradiated or nonirradiated stroma were not increased by exposure to pokeweed mitogen (data not shown). In addition, we have cultured FDC-P1 cells with pure CSF-1 (courtesy of Dr Abdul Waheed and Dr Richard Shadduck) over a range of 78 to 10,000 U/mL and have shown that CSF-1 does not stimulate any clonal proliferation of these cells in soft agar culture.

**Colony morphology.** Table 1 shows types of murine marrow colonies observed in assays of conditioned media. The conditioned media obtained from irradiated lectin-treated stromal cells increased the numbers of all types of colonies, with up to a 70-fold increase in granulocyte-colony formation.

**Assay of GM-CSA-2 for FDC-P1 colony-stimulating activity.** Table 2 shows the effects of partially purified GM-CSA-2 from Con A spleen cm on FDC-P1 clonal growth in soft agar culture. GM-CSA-2 at a concentration of 80 U/mL stimulated FDC-P1 colony formation, but this did not approach the stimulation observed with IL 3. The blank containing McCoy's 5A media plus additives and agar
showed no colony formation. Recombinant GM-CSA also stimulated proliferation of FDC-P1 cells as assayed by \(^{3}H\)TdR incorporation or clonal growth in agar (data not shown).

Effect of anti-GM-CSF antibodies on PWM/1,100 rad cm stimulation of FDC-P1 and murine marrow colony formation. Anti-GM-CSF antibody blocked the stimulation of both FDC-P1 and murine marrow colony growth by PWM/1,100 rad cm (Table 3). The inhibition was less for the marrow growth (53% \(\pm\) 4.8%) but this is consistent with the presence of CSF-1, which is not inhibited by anti-GM-CSF (data not shown) and which does not stimulate FDC-P1 cells (see above). Thus the residual growth seen with murine marrow after GM-CSF blocking of PWM/1,100-rad cm is presumably due to CSF-1 in these preparations. The more complete inhibition of FDC-P1 proliferation by anti-GM-CSF suggests that GM-CSF is the primary stimulator of this cell type found in cm from PWM/1,100-rad stroma. In addition, an antibody to recombinant IL 3 was assessed for its ability to block FDC-P1 or murine marrow soft agar colony formation stimulated by PWM/1,100 rad cm in a fashion similar to that described for the anti-GM-CSF. No effect on marrow-(one experiment) or FDC-P1-(one experiment) stimulated growth was observed.

Histochemical stains of stroma. Histochemical stains of irradiated stroma with and without exposure to PWM at 2.5 \(\mu\)g/mL showed no shift in cell types. We have previously reported on the staining characteristics of stroma exposed to 950 rad, and the results with 1,100 rad alone were similar to our previously reported data except for a higher percentage of alkaline phosphatase positive cells in the present study.

Table 1. Morphology of Colonies From Conditioned Media Assayed for Hematopoietic CSA Elaborated From Irradiated Dexter Stroma

| Percent | Colony Morphology (colonies/10\(^{5}\) cells)
<table>
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<tbody>
<tr>
<td>Conditioned Media</td>
<td>Granulocyte</td>
</tr>
<tr>
<td>A. PWM CM (1,100 rad)*</td>
<td>23</td>
</tr>
<tr>
<td>16</td>
<td>24.3 ± 1.6</td>
</tr>
<tr>
<td>9</td>
<td>25.3 ± 1.3</td>
</tr>
<tr>
<td>4.5</td>
<td>19.5 ± 7.5</td>
</tr>
<tr>
<td>2.3</td>
<td>14.3 ± 1.4</td>
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</table>

B. CM (1,100 rad)*

| Percent | Colony Morphology (colonies/10\(^{5}\) cells)
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</thead>
<tbody>
<tr>
<td>Conditioned Media</td>
<td>Granulocyte</td>
</tr>
<tr>
<td>23</td>
<td>8.3 ± 2.3</td>
</tr>
<tr>
<td>16</td>
<td>1.6 ± .9</td>
</tr>
<tr>
<td>9</td>
<td>.33 ± .33</td>
</tr>
<tr>
<td>4.5</td>
<td>.6 ± .6</td>
</tr>
<tr>
<td>2.3</td>
<td>.5 ± .5</td>
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*Agar preps stained with acetylcholinesterase-hematoxylin. Numbers indicate absolute colony counts of one time point in one of three representative experiments. Three petri dishes were scored per group with counts \(\pm 1\) SEM.
†Gran/Mac = Granulocyte/macrophage.
§Mac/Mono = Macrophage/monocyte - Typical macrophages intermixed with mononuclear cells with relatively high nuclear-cytoplasmic ratio and irregular cytoplasmic margins.
∥Meg = megakaryocyte.
¶Deg = Degenerative: Cells in these colonies were disrupted or fragmented or colony type not interpretable.

Table 2. Effect of GM-CSA-2 on FDC-P1 Clonal Growth in Soft-Agar Culture

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>FDC-P1 Colonies/10(^{5}) Cells*</th>
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<tbody>
<tr>
<td>GM-CSA-2†</td>
<td>139 ± 16.7</td>
</tr>
<tr>
<td>IL 3‡</td>
<td>TNTC</td>
</tr>
<tr>
<td>Blank§</td>
<td>0</td>
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*In each group, three to four petri dishes were scored. These results are from three separate experiments.
†Preparations used in these studies were partially purified from supernatants of Con A-activated splenic lymphocytes (see "Methods"). One unit of activity was defined as amount of GM-CSA-2 in 1 mL, which was 50% of maximal stimulation of a GM-CSF-responsive cell population.
‡Interleukin 3 (IL 3) was used as a positive control in all experiments yielding colonies that were too numerous to count (TNTC). Preparations used in these studies had 0.2 ng of IL 3 per unit of activity. One unit of activity was defined as the amount of IL 3 that in 1 mL gives 50% of maximal tritiated thymidine incorporation into FDC-P1 cells in culture.
§Blank media containing only McCoy’s with additives (see "Methods") and 0.3% agar were used as negative controls and yielded no clonal growth.

DISCUSSION

Our present studies show that radioresistant adherent cells from murine Dexter cultures produce growth factors that stimulate FDC-P1 cells and murine marrow granulocyte-macrophage-megakaryocyte progenitors to proliferate.

The growth factor acting on FDC-P1 cells, which is increased by stromal irradiation and lectin exposure, appears to be GM-CSF. While CSF-1 is present in conditioned media from normal stroma, its levels are not increased by irradiation and/or lectin exposure, and, as noted above, pure CSF-1 does not stimulate FDC-P1 proliferation. The anti-GM-CSF antibody used in these studies blocks PWM/1,100-rad cm stimulation of FDC-P1 cells but does not block CSF-1 stimulation of marrow growth. Finally, we have confirmed the results of Hapel et al. showing that GM-CSF (recombinant or partially purified from Con A spleen cm) stimulates FDC-P1 proliferation as assessed by clonal agar culture or tritiated thymidine incorporation in liquid culture. The fail-
This was confirmed in two separate experiments testing two different cell colonies in Terasaki plates were counted at day 4 and BDF 1 murine experiments, six separate assessments). ± assessing two different lots of PWM/1 other separate experiments using both BDF Linbro plates and assessing replicate wells. This was confirmed in three colonies).

GM-CSF was 87.3, ± difficult) to 0 (no growth). PWM/1 marrow colonies (>50 cells) at day 8 of culture.

PWM/1,100-rad cm. The mean % inhibition (± SEM) of PWM/1,100-rad cm stimulation of murine marrow colony formation by anti-GM-CSF was 87.3 ± 7. In the third experiment, growth was scored on a scale of 4 + (colonies so numerous that accurate counting was very difficult) to 0 (no growth). PWM/1,100-rad cm stimulation of marrow cell proliferation indicates that IL 3 is not the stromal-derived marrow stimulatory growth factor. Thus a radiosensitive stromal cell appears to be stimulated by lectin exposure to release GM-CSF, which stimulates both FDC-P1 and a range of marrow progenitors. Finally, it should be noted that GM-CSF (with or without CSF-1) is capable of stimulating differentiation of all the differentiated hematopoietic lineages seen in Dexter marrow cultures: macrophages, granulocytes, and megakaryocytes.28

The identity of the radiosensitive cell type generating these in vitro growth factors remains speculative. Two critical adherent cell types capable of supporting liquid culture hematopoiesis are seen after in vitro29 or in vivo irradiation.30 One cell type has the phenotype of a macrophage (phagocytic, acid phosphatase, and nonspecific esterase positive), while the other cell is a large alkaline phosphatase-positive epithelioid cell. Both cell types were Factor VIII negative and a small (<3%) fraction of cells did not fit either phenotype. In these studies we found no apparent shift in cell types with lectin exposure (Table 4). Thus the lectin responsive GM-CSF-producing cell types seem most likely to be either macrophages or the large alkaline phosphatase positive cells.

Previous studies from our laboratory have suggested that increases in myeloid stimulatory factors seen with irradiation of Dexter culture adherent cells may be due to removal of the negative feedback exerted by mature myeloid cells or removal of a population of cells that rapidly bind, utilize, or degrade these growth factors.30 As with previous studies, our data on radiation dose response suggest an inverse correla-

<table>
<thead>
<tr>
<th>Staining Stain*</th>
<th>1,100 rad</th>
<th>1,100 rad + PWM (2.5 μg/mL)</th>
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<tbody>
<tr>
<td>Alpha naphthyl acetate (nonspecific esterase)</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>95</td>
<td>97</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>34</td>
<td>34</td>
</tr>
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</table>

*Results were derived from one experiment, evaluating the stroma at day 2 post-XRT and PWM exposure. Eight hundred cells were scored for each stain.
†Cells stained at 2 to 4+ intensity on a scale of 0 to 4+ were scored as positive.
tion with FDC-P1 growth factor production and total amount of mature granulocytic elements present in the stromal layer.

The mechanism by which pokeweed mitogen stimulates stromal cells to produce increased levels of GM-CSF is not known. It seems probable that pokeweed mitogen selectively binds and stimulates a specific cell type remaining in the stromal layer after the exposure to irradiation, thereby increasing levels of GM-CSF. Alternatively, the pokeweed mitogen may act by eliminating an inhibitory phenomenon that may also increase colony formation. Studies by Gualtieri et al. demonstrated that conditioned medium from normal or irradiated stroma (without pokeweed mitogen) did not show differing levels of inhibitors. Also, in preliminary studies using conditioned media from irradiated cultures with and without pokeweed mitogen, we have found that when adding either of these conditioned media to purified CSF-1 there is no inhibition of growth. In fact, the conditioned medium from pokeweed mitogen-stimulated irradiated stroma, when added to pure CSF-1, actually increases both size and numbers of hematopoietic colonies, suggesting that a synergistic activity may be present in this conditioned medium. We have recently described an adherent cell line derived from murine Dexter cultures, termed TC-1, which produces a CSF-1-dependent synergistic activity. At present it is uncertain whether this latter synergistic bioactivity may in part be responsible for the increased FDC-P1 and marrow colony formation seen with cm from irradiated lectin-exposed stroma.

In summary, we have shown that a radioresistant cell present in the adherent layers of long-term Dexter cultures responds to the lectin pokeweed mitogen by generating GM-CSF. CSF-1 is also produced by these adherent cells. These two regulators are sufficient to account for the differentiated hematopoietic progeny seen in this culture system: macrophages, granulocytes, and megakaryocytes.

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