Production of Granulocyte-Macrophage Colony-Stimulating Factor by Abelson Virus–Induced Tumorigenic Mast Cell Lines

S.W. Chung, P.M.C. Wong, G. Shen-Ong, S. Ruscetti, T. Ishizaka, and C.J. Eaves

We have recently described a system that supports the development of continuously growing and tumorigenic cell lines after infection of individual multilineage hematopoietic colonies with Abelson murine leukemia virus (A-MuLV). We now provide definitive evidence that these transformed lines express features characteristic of mast cells. Although these lines have been maintained in some cases for more than a year in the absence of exogenous growth factors other than those present in fetal calf serum, colony formation could consistently after 2 months, be shown to be increased several fold when pokeweed mitogen–stimulated spleen cell conditioned medium (CM) was added to the cultures. CM from the A-MuLV–transformed lines was then tested for its ability to stimulate hematopoietic colony formation by cells from both fetal and adult tissues. Four of four randomly selected cell lines produced factors that were active on erythropoietic, granulopoietic, and in some cases pluripotent progenitors. Removal of viral particles from the CM from one of the lines (27d1) by either heat inactivation or high-speed centrifugation did not alter the colony-stimulating activity detected. When CM from 27d1 cells was tested for its ability to stimulate the proliferation of interleukin 3 (IL3) granulocyte-macrophage colony-stimulating factor (GM-CSF)–dependent FDC-P1 cells, a positive result was obtained. This stimulatory activity was not reduced in the presence of neutralizing anti–IL 3 immunoglobulin (Ig), suggesting that the activity detected was GM-CSF and not IL 3. This was confirmed by the lack of expression of the IL 3 gene in 27d1 cells as determined by Northern analysis of 27d1 cell RNA. Furthermore, S1 analysis of mRNA from 27d1 cells as well as two other lines indicated that the GM-CSF gene in all three was transcriptionally active. Taken together, these data suggest that A-MuLV transformation of normal mast cells or their precursors under certain conditions commonly activates the production of GM-CSF.

A BLSON MURINE LEUKEMIA virus (A-MuLV) is an acute replication-defective transforming retrovirus containing the oncogenic sequence v-abl. It is the result of a recombination event between the replication-competent Moloney leukemia virus (M-MuLV) and a part of the host genomic c-abl. A-MuLV was originally shown to cause nonlymphoid B cell lymphoma in vivo, but is now known to cause a variety of hematologic tumor types including plasmacytomas, thymomas, mastocytomas, and myelomonocytic leukemia, depending on the specific protocol and route of inoculation. In vitro studies have shown that A-MuLV can transform cells at various stages of the B cell differentiation pathway and can do so without blocking B cell differentiation. Other in vitro studies have shown that A-MuLV infection of fetal liver cells allowed differentiating erythroid cells to proliferate and mature in the absence of erythropoietin (Ep). These cells, however, could not be passaged into continuously growing lines, indicating that A-MuLV may also alter the requirement of normal differentiating cells for their specific growth factors without their concomitant conversion to a malignant state.

In the murine hematopoietic system, a number of distinct growth factors are now recognized. These were characterized initially on the basis of differences in the progenitor cell populations on which they appeared to act and hence the kinds of colonies whose formation they supported. Several of these so-called colony-stimulating factors (CSFs) have recently been purified to homogeneity and their target cell specificity and functions studied in detail. Three, granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), and macrophage CSF (M-CSF), show specificity for cells differentiating along the granulocyte-macrophage lineage. However, both GM-CSF and G-CSF can also stimulate the limited proliferation of pluripotent progenitors. Interleukin 3 (IL 3), also referred to as multi-CSF or by one of several other names, is sufficient to support the full development of a variety of single- and multilineage colonies containing varying combinations of granulocytes, macrophages, eosinophils, megakaryocytes, erythroid cells, and mast cells. Recently, the purification of another hematopoietic growth factor, hemopoeitin I, has been reported. This latter factor is not itself a CSF, although, when present together with a known CSF, it may allow the production of recognizable progeny derived from primitive but otherwise undetectable hematopoietic progenitor cell types.

The products of several viral oncogenes have been shown to be very similar to normal growth factors or their receptors. Naturally arising or induced abnormalities in the structure or expression of the cellular genes that code for these molecules might, therefore, also be expected to contribute to the malignant transformation of cells normally responsive to a particular growth factor. In a previous study, we found that A-MuLV infection of individual multilineage colonies yielded transformed tumorigenic cell lines in high frequency, but only when these were cocultured for the first 2 to 3 months in the presence of a heavily irradiated 3T3 cell.
feeder layer. This suggested that in this system, transformation may involve the acquisition of autonomy from a growth factor produced by irradiated 3T3 cells. To investigate this possibility we examined several of these A-MuLV-transformed cell lines for their sensitivity to exogenous growth factors and for their ability to produce factor(s) with normal hematopoietic cell-stimulating activity.

Preliminary characterization of the cell lines themselves showed that morphologically they resembled mast cells. In this report we provide further evidence of the mast cell characteristics of these cells and demonstrate that they constitutively produce GM-CSF. No IL-3 production could be detected.

MATERIALS AND METHODS

Cells and conditioned media. The origin of the transformed lines studied is described elsewhere. Briefly, cells from multi-lineage hematopoietic colonies were removed from methylcellulose cultures and placed together with A-MuLV on top of irradiated 3T3 cells. Cell lines were established independent of feeder cells 2 to 3 months after infection (B1, B2, B9, C3, C8, T6, and 5α), and subsequently, some were cloned (27d1, 27d2). All lines were carried in RPMI 1640 (Flow Laboratories, McLean, Va) supplemented with 20% heat-inactivated fetal calf serum (FCS) and 50 μmol/L 2-mercaptoethanol (2-ME). Conditioned media (CM) from these cell lines were collected three days after feeding cells with fresh medium. Cells were pelleted, and the clarified supernatant was concentrated eight- to tenfold, then stored at −70°C until use.

Determination of IgE binding and histamine content. Binding of mouse IgE molecules was determined by the method of Kuczycky et al. Purified normal mouse mast cells and a CSF-dependent mouse mast cell line, PT-18, were used as controls. The monoclonal mouse IgE used in the present experiments was the same preparation as that previously described. Cells at a concentration of 1 to 2 × 10⁶/mL were suspended in RPMI 1640 medium supplemented with 10% FCS and 0.01 mol/L EDTA, pH 7.6, and incubated with 10 μg/mL 125I-mouse IgE at 37°C for 90 minutes. A 100-fold excess of unlabeled IgE was added to the cells in the control tubes prior to the addition of 125I-IgE. After incubation, 0.2 mL of the cell suspension was layered over an equal volume of FCS and spun for one minute in a Beckman 152 microfuge (Beckman Instruments, Palo Alto, Calif). The radioactivity of the pellet was counted and the amount of 125I-IgE molecules bound per cell calculated assuming a molecular weight of mouse IgE of 184,000.

Histamine was extracted with 1% to 2% perchloric acid and analyzed by the automated technique of Siraganian.

Methylcellulose colony assay. Cells for assays of hematopoietic colony-stimulating activity were obtained either from BALB/c mice from Cumberland View Farm (Tenn) or from BALB/cAnN mice bred and maintained at the NIH (Bethesda, Md). Three different sources of cells were used to assay for CSFs: (1) Fetal peripheral blood was obtained from 9- to 11-day-old embryos, single-cell suspensions prepared, and cells plated at a final concentration of 3 to 4 × 10⁶ cells/mL. (2) Bone marrow cells were obtained from normal adult mice and plated at a final concentration of 3 × 10⁶ cells/mL. (3) Single-cell suspensions were prepared from the spleens of mice that had received 150 mg/kg 5-fluorouracil (5-FU) intravenously four days before. Cells were plated at a final concentration of 6 to 8 × 10⁶ cells/mL.

The culture medium used for colony assays contained 0.8% methylcellulose, 30% FCS (Flow), 1% deionized bovine serum albumin (BSA, Sigma Chemical Co, St. Louis), 2 mmol/L L-glutamine and 100 μmol/L 2-ME, and 2 U/mL of Ep. Pokeweed mitogen-stimulated spleen cell conditioned medium (SCM) was included where indicated at a final concentration of 1%. In some cases concentrated CM from A-MuLV-transformed lines were included at a final concentration of 10% (vol/vol) instead of SCM. Colonies were scored in situ under an inverted microscope after six days for embryonic peripheral blood cell cultures and after ten to 12 days for adult bone marrow and spleen cell cultures.

Cell proliferation assays. DA-1 cells were obtained from J. Ihle (Frederick Cancer Research Facility, Frederick, Md). FDC-P1 cells were obtained from J. Pierce (National Cancer Institute, NIH, Bethesda, Md). To evaluate the proliferation of these cells, 2 × 10⁴ cells in 50 μL of RPMI plus 10% FCS were placed in each well of a 96-well microtiter plate. Appropriate dilutions of IL-3 or CM were then added and the plate incubated for 22 hours at 37°C. One μCi of 3H-thymidine (Amersham Corp, Arlington Heights, Ill) in 20 μL of medium was then added to each well and the plates incubated for an additional six hours. Cellular DNA was harvested onto glass fiber filter paper and the amount of 3H-thymidine incorporation determined. To measure inhibition of factor-induced cell proliferation, wells were set up in the same way with the additional inclusion of an anti-IL-3 immunoglobulin preparation (anti-IL-3 Ig, kindly provided by J. Ihle) in the final culture medium.

Molecular analyses. High-molecular weight DNA preparations from A-MuLV-transformed lines, WEHI-3 cells, and BALB/c mouse thymus were prepared and approximately 10 μg of DNA digested with EcoRI, then electrophoresed, transferred to nitrocellulose, and hybridized to 32P-labeled IL-3 cDNA probes. Two different IL-3 probes were used. One was a 0.8-kb PstI fragment representing the 3' end of IL-3 cDNA (from J. Ihle). The other was a full-length IL-3 cDNA from K. Arai (DNAX, Palo Alto, Calif). After hybridization, the filter was washed for half an hour with 2 × sodium chloride-sodium citrate (SSC)/0.1% sodium dodecyl sulphate (SDS) and then at 65°C for one hour with 0.1 × SSC/0.1% SDS. The total RNA from A-MuLV-transformed lines, WEHI-3 cells, as well as pokeweed mitogen-stimulated spleen cell extracts and about 15 μg per lane electrophoresed, transferred, and hybridized to the same probes described. S1 analysis was carried out as described by Ley et al.

Assay for focus-forming units. The titers of A-MuLV in the virus stock and in the concentrated CM were measured using the procedure described by Scher and Siegler. Briefly, early-passage 3T3 cells were seeded in 60-mm plastic Falcon dishes (10⁴/dish) (Falcon Labware, Oxnard, Calif) in medium with 10 μg/mL of Polybrene. The next day the medium was replaced with 0.5 mL viral stock or CM and incubated at 37°C for one hour with occasional rocking of the dishes. Medium was changed again after five days and transformed foci scored on the tenth day using an inverted microscope.

RESULTS

Mast cell characteristics of A-MuLV-transformed cell lines. Table 1 shows the results of toluidine blue staining, histamine content determinations, and IgE binding studies of cells from two clones (27d1 and 27d2) of a typical A-MuLV-transformed cell line. Comparison of these to fresh and cultured mast cells from another source showed all to be similar. An additional four lines tested were shown to contain histamine, although the amount varied slightly (data not shown). The amount of 125I-IgE that bound to the 27d1 and 27d2 cells was similar to what bound to normal mouse peritoneal mast cells and the IL-3-dependent PT-18 mast cell line (Table 1). Under the same conditions we have found...
Experiment 2 was plated 5 months after viral infection at 1.0 cells/mL. The numbers in average colony number obtained per 0.5 mL of culture.

2-ME and 2 mmol/L 1-glutamine. Experiment 1 was plated 2 months after virus infection at a final concentration of 5.0 x 10^6 cells/mL, whereas that other cell lines showed no binding. These include a cloned line of inducer T lymphocytes (Cl, Ly1 + 2 -/9), YAC lymphoma cells, and 33 of 34 lymphocyte cell lines with natural killer (NK)-like or CTL activity. The majority of lymphocyte cell lines with lymphoma transformed cell lines. The plating efficiency of these lines on these cells had a high affinity for the immunoglobulin 27d2 cells remained after washing, indicating that the receptors on these cells had a high affinity for the immunoglobulin.

Effect of SCM on the cloning efficiency of A-MuLV-transformed cell lines. The plating efficiency of these lines was studied at various times after their separation from the 3T3 feeders. As shown in Table 2, cells removed from feeders 2 months after infection gave rise to very few colonies when plated in methylcellulose cultures containing FCS but no other known source of specific growth factors (eg SCM). At this time, the plating efficiency was increased three- to fourfold if either partially purified IL 3 (obtained as described by Iscove) or crude SCM was added to the assay medium. At later passages, higher plating efficiencies were sometimes encountered, and a difference in colony number was no longer consistently observed when cultures with or without SCM were compared. For example, experiment 2 in Table 2 shows results for some of the same lines used in experiment 1 assayed 3 months later. It can be seen that in this experiment the plating efficiency was approximately 100-fold higher than in experiment 1 and the previously observed effect of added SCM was not seen.

Demonstration of hematopoietic growth factor activity. To assay for colony-stimulating activity, we first tested the ability of concentrated CM from these transformed mast cell lines to stimulate progenitors present in the peripheral blood of 9- to 11-day old mouse embryos. As indicated in Table 3, CM from two A-MuLV-transformed cell lines stimulated the formation of large erythroid colonies, erythroid-mixed colonies, and a number of large nonerythroid colonies consisting mostly of granulocytes and/or macrophages. CM from two other lines (B9 and B2) supported the formation of large erythroid and nonerythroid colonies, but no erythroid-mixed colonies were detected in these assays.

Waneck and Rosenberg have reported that Abelson virus itself can stimulate early hematopoietic progenitor cells from fetal liver to produce macroscopic colonies containing large numbers of differentiating erythroid as well as myeloid elements in the absence of exogenous growth factors. Since the transformed cell lines being examined here were productively infected by A-MuLV (Table 3), we reexamined the colony-stimulating activity present after treatment of CMs with heat or high-speed centrifugation to remove viral particles. Results for 27d1 cell CM are shown in Table 3. It can be seen that a 1,000-fold reduction in viral particles did not significantly alter the colony-stimulating activity of this CM. Neither the number nor the size or erythroid cell content of the colonies obtained appeared to have been affected. Addition of an A-MuLV stock with a titer roughly equivalent to that of the 27d1 cell CM showed some colony-stimulating activity, but the erythroid colonies stimulated were small and were eliminated when the viral stocks were inactivated by heating. The nonerythroid colonies observed after heat inactivation of the A-MuLV stock were probably due to GM-CSF produced by the virus-infected 3T3 cells and present in the virus stocks as reported by Koury and Pragnell.

Similar results were obtained when normal bone marrow cells or spleen cells from 5-FU-treated mice were tested in methylcellulose cultures containing Ep but no other known growth factor (Table 4). Colony formation was again

### Table 1. Characterization of A-MuLV-Transformed Mast Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Toluene Blue per 10^6 Cells</th>
<th>Histamine per 10^6 Cells</th>
<th>IgE Bound per Cell Before Wash</th>
<th>After Wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>27d1</td>
<td>+</td>
<td>332 ng</td>
<td>2.91 x 10^6</td>
<td>2.33 x 10^6</td>
</tr>
<tr>
<td>27d2</td>
<td>+</td>
<td>328 ng</td>
<td>2.19 x 10^6</td>
<td>1.97 x 10^6</td>
</tr>
<tr>
<td>PT-16</td>
<td>+</td>
<td>278 ng</td>
<td>3.33 x 10^6</td>
<td>3.30 x 10^6</td>
</tr>
<tr>
<td>Mouse mast cells</td>
<td>+</td>
<td>10.3 μg</td>
<td>3.59 x 10^6</td>
<td>3.31 x 10^6</td>
</tr>
</tbody>
</table>

*After incubation with ^3H-IgE for 90 minutes, the cells were washed three times with RPMI 1640 medium and cell-bound radioactivity counted.

†Mouse peritoneal mast cells were obtained from BALB/c mice and purified as described elsewhere.

### Table 2. Effect of SCM on the Cloning Efficiency of A-MuLV-Transformed Lines

<table>
<thead>
<tr>
<th>Lines</th>
<th>Experiment 1 + SCM</th>
<th>Experiment 1 - SCM</th>
<th>Experiment 2 + SCM</th>
<th>Experiment 2 - SCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB*</td>
<td>(113 ± 18.3)</td>
<td>(42.3 ± 5.9)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.45%</td>
<td>0.17%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B9*</td>
<td>(69.9 ± 9.5)</td>
<td>(27.6 ± 4.4)</td>
<td>(213.7 ± 9.8)</td>
<td>(229 ± 9.7)</td>
</tr>
<tr>
<td></td>
<td>0.28%</td>
<td>0.11%</td>
<td>42.7%</td>
<td>45.8%</td>
</tr>
<tr>
<td>B1*</td>
<td>(40.7 ± 7.0)</td>
<td>(15 ± 2.5)</td>
<td>(179 ± 13.5)</td>
<td>(169.3 ± 7.0)</td>
</tr>
<tr>
<td></td>
<td>0.16%</td>
<td>0.06%</td>
<td>35.8%</td>
<td>33.9%</td>
</tr>
<tr>
<td>C3</td>
<td>—</td>
<td>—</td>
<td>(179.3 ± 5.6)</td>
<td>(160 ± 5.5)</td>
</tr>
<tr>
<td></td>
<td>35.9%</td>
<td>32%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>T6</td>
<td>(6.3 ± 1.2)</td>
<td>(1.7 ± 1.2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.03%</td>
<td>0.01%</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*A partially purified SCM was used (see text).

A-MuLV-transformed cells were plated in 1% methylcellulose in α-minimum essential medium (α-MEM) with 36% FCS, 1.2% BSA, 100 μmol/L 2-ME, and 2 mmol/L 1-glutamine. Experiment 1 was plated 2 months after virus infection at a final concentration of 5 x 10^6 cells/mL, whereas experiment 2 was plated 5 months after viral infection at 10^5 cells/mL. The numbers in parentheses are mean values ± SEM where the mean is the average colony number obtained per 0.5 mL of culture.

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erythroblasts; erythroid-mix colonies, containing capable of stimulating DA-l cells to proliferate (data not shown). Most colonies, containing >50 cells with no hemoglobinized erythroblasts and consisting mainly of granulocytes and/or macrophages. Values ± shown are means ± SEM from data pooled from two to five experiments and are expressed as the number of colonies scored per 10^5 cells.

observed when either treated or untreated 27d1 CM was added. The number of macroscopic multi-lineage colonies stimulated was, however, relatively lower in normal bone marrow cell cultures than in 5-FU spleen cell cultures.

Absence of IL 3 but production of GM-CSF. We next sought to determine whether 27d1 CM contained IL 3. The effect of 27d1 CM on DA-1 cells (initially thought to be exclusively IL 3 dependent) was therefore examined. As positive controls WEHI-3 cell CM, SCM, and pure IL 3 (from WEHI-3 cell CM) were also tested. These were all capable of stimulating DA-1 cells to proliferate (data not shown). As further indicated in Table 5, addition of anti-IL 3 Ig to wells containing pure IL 3 resulted in a significant inhibition of IL 3-induced proliferation. CM from 27d1 cells was also found to stimulate DA-1 cells, and this activity was not affected by heat inactivation of the virus present in the CM. However, in contrast to the results obtained with pure IL 3, the addition of anti-IL 3 Ig had no effect on the stimulatory activity of 27d1 cell CM.

We next examined the IL 3 gene itself and looked for evidence of its expression in a number of our A-MuLV-transformed cell lines. Figure 1 shows a Southern blot of EcoRI-digested high-molecular weight DNA from normal BALB/c mice, WEHI-3 cells, and the 27d1 cell line. The blot was hybridized with a 0.8 kb-PstI fragment representing the 3' end of the cDNA of the IL 3 coding region (from J. Ihle). As found previously by others, the IL 3 gene was seen to be altered in WEHI-3 cells. However, in 27d1 cells as in normal mouse cells, no alteration was detected. The same result was observed when the blot was hybridized with a full-length IL 3 cDNA probe. Two other A-MuLV-transformed lines gave similar results (data not shown). Figure 2 shows the results of Northern analysis using the 0.8-kb PstI fragment of IL 3 cDNA. A strong band about 1.0 kb was detected in WEHI-3 cells and the 27d1 cell line, but not in normal mouse cells.

Table 4. Effect of 27d1 Conditioned Media on Colony Formation in Cultures of Adult Bone Marrow and 5-FU-Treated Spleen Cells

<table>
<thead>
<tr>
<th>Conditioned Medium</th>
<th>BFU-E</th>
<th>CFU-GEMM</th>
<th>CFU-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>27d1 CM</td>
<td>5.7</td>
<td>2.2</td>
<td>5.1</td>
</tr>
<tr>
<td>27d1 CM (spun)</td>
<td>5.7</td>
<td>2.2</td>
<td>5.1</td>
</tr>
<tr>
<td>A-MuLV</td>
<td>5.7</td>
<td>2.2</td>
<td>5.1</td>
</tr>
<tr>
<td>A-MuLV</td>
<td>5.7</td>
<td>2.2</td>
<td>5.1</td>
</tr>
<tr>
<td>R20</td>
<td>5.7</td>
<td>2.2</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Abbreviations: BFU-E, producing a hemoglobinized erythroblast colony consisting of more than three clusters of erythroblasts; CFU-GEMM, producing a colony containing erythroblasts, macrophages, granulocytes, and/or megakaryocytes; CFU-C, producing all nonerythroid colonies containing >50 cells, primarily macrophages, granulocytes, or macrophages and granulocytes.

Maximum value if only one colony was observed in each experiment.

Colonies were scored under an inverted microscope 10 to 14 days after initiation of cultures from bone marrow (BM) and 5-FU-treated spleen cells. Values given are means ± SEM from data pooled from two to four experiments and are expressed as the number of colonies scored per 10^5 cells for bone marrow and 5-FU-treated spleen, respectively.
Table 5. Effect of CM on the Proliferation of IL 3-Dependent DA-i Cells

<table>
<thead>
<tr>
<th>CM</th>
<th>Cell Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CM Alone</td>
</tr>
<tr>
<td>IL-3‡</td>
<td>40,411 ± 2,186</td>
</tr>
<tr>
<td>10% 27d1 CM</td>
<td>47,857 ± 656</td>
</tr>
<tr>
<td>10% Δ 27d1 CM</td>
<td>45,688 ± 861</td>
</tr>
<tr>
<td>10% A-MuLV</td>
<td>11,646 ± 854</td>
</tr>
<tr>
<td>10% R20</td>
<td>8,253 ± 514</td>
</tr>
<tr>
<td>R10§</td>
<td>9,536 ± 1,530</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM. Numbers are 3H-thymidine incorporation into cellular DNA measured in counts per minute.

*The amount of anti-IL 3 Ig added was sufficient to neutralize one third of the input IL 3.
†An amount of normal rabbit Ig equivalent to that present in the anti-IL 3 containing wells was used.
‡Pure IL 3 at a final dilution of 1/200,000.
§RPMI 1640 medium supplemented with 10% FCS.

kb in size was observed in lanes containing mRNA from both WEHI-3 cells and pokeweed mitogen–stimulated mouse spleen cells. No band could be detected in the 27d1 cell mRNA. Similar results were again obtained with two other lines (data not shown). Thus it appears that the IL 3 gene is not involved in the generation of any of the activities detected in the CM of our A-MuLV–transformed cell lines.

Since GM-CSF has been reported to stimulate the initial division of pluripotent hematopoietic progenitors and since some IL 3–dependent cell lines can respond to GM-CSF, we looked also for evidence of GM-CSF production by our A-MuLV–transformed cell lines. Figure 3 indicates the results of S1 analysis of RNA from three of the lines. The probe used was the Xhol-TaqI fragment in the pCD vector containing the full length cDNA of GM-CSF. This fragment contains 258 nucleotides of the 5' end of GM-CSF cDNA (Fig 3). RNA from pokeweed mitogen–stimulated spleen cells and from all three transformed mast cell lines (27d1, 27d2, and 5A) examined contained the 258-bp protected fragment, indicating the presence of GM-CSF mRNA. The same size fragment could not be detected in WEHI-3 cells, which do not produce GM-CSF, even when as high as 100 μg of RNA was used for hybridization (Fig 3).

We then tested the 27d1 CM for its ability to stimulate FDC-P1 cells, which have been previously shown to be responsive to GM-CSF as well as IL 3. The results presented in Fig 4 indicate that FDC-P1 cell proliferation was stimulated by 27d1 CM and this stimulation was not reduced by the addition of neutralizing anti-IL 3 Ig. In contrast, the level of stimulation by pure IL 3 was markedly reduced when anti-IL 3 Ig was added to the medium. Additional titration studies indicated that the amount of 27d1 CM used in Fig 4 was equivalent to the amount of IL 3 added in terms of measured thymidine incorporation (data not shown). A slight stimulation of thymidine incorporation seen with increasing amounts of anti-IL 3 Ig or normal rabbit Ig (Fig 4) was also seen without added IL 3 or CM (data not shown).

DISCUSSION

In an earlier paper, we described a method for transforming murine hematopoietic cells with A-MuLV in vitro.

![Fig 1](image1.png)

**Fig 1.** Southern analysis of EcoRI-digested high-molecular weight DNA extracted from left to right: BALB/c mouse thymus, WEHI-3 cells, and 27d1 cells. The blot was hybridized to a 0.8-kb PstI fragment of an IL 3 cDNA probe (3' end). An extra band of about 4.4 kb was observed only with WEHI-3 cells.

![Fig 2](image2.png)

**Fig 2.** Northern analysis of total RNA extracted from left to right: 27d1 cells, PWM-stimulated spleen cells (PWM-SC), and WEHI-3 cells. The blot was hybridized to the same probe as in Fig 1. A prominent 1.0-kb band was observed with PWM-SC and WEHI-3 RNA, but no band could be detected in 27d1 RNA.
A-MuLV–TRANSFORMED MAST CELLS PRODUCE GM-CSF

that allows the rapid emergence of tumorigenic cell lines that morphologically resemble mast cells. In the present study we provide definitive evidence that these cells express mast cell characteristics. Although we showed previously that these lines arose from cells in multilineage colonies of established single-cell origin, this protocol did not allow identification of the type of cell initially infected and transformed. Since 10% to 20% of the cells present in the type of multilineage colonies selected were morphologically identifiable as mast cells, it is possible that mast cells or their precursors were the primary targets. More recent preliminary findings suggest that early hematopoietic progenitors may be targets and that the subsequent phenotype to emerge may be influenced by the conditions used for the selection of transformants (Wong, PMC, Chung SW, Raefsky E, Eaves CJ, and Nienhuis AW, unpublished observations). In any case our data confirm the recent finding that mast cells are related to cells of the myeloid lineages.\(^42\) The availability of rapidly growing mast cell lines and a reproducible method for their generation should be useful for future investigations of the development and differentiation of mast cells.

Recently several groups have also reported the isolation of tumorigenic factor-independent mast cell lines following A-MuLV infection of hematopoietic cells in vitro.\(^43,44\) However, in none of these has evidence of production of a hematopoietic growth factor been found. In our experiments, an irradiated 3T3 cell feeder rather than an exogenous supply of IL 3 was used initially to select A-MuLV–transformed lines capable of continuous growth. Because, during early passages, these lines showed an increased plating efficiency in the presence of factors produced by spleen cells, we undertook experiments to investigate more extensively the factor-producing properties of these A-MuLV–transformed cells. CM from four randomly selected cell lines were found to contain activities that stimulated erythroid as well as granulocyte-macrophage and in some cases also multilineage colony formation in methylcellulose assays of murine progenitors from a variety of embryonic and adult sources. However, Northern blots and immunologic analysis indicated that these activities were not attributable to IL 3. From SI analyses, we found that in three of three lines studied GM-CSF mRNA was produced at a relatively high level (100 copies/cell). Additional evidence of GM-CSF release was obtained when we tested the ability of the CM from one cell line to stimulate the proliferation of FDC-P1 and DA-l cells. FDC-P1 cells are known to be responsive to GM-CSF as well as IL 3, and we have recently found that
DA-1 cells are also responsive to pure recombinant murine GM-CSF (Wong PMC, Chung SW, Broder T, and Nienhuis AW, unpublished data; and Krystal G, Farber N, and Eaves CJ, unpublished data). Since the FDC-P1 stimulatory activity present in the transformed cell CM was not neutralized by anti-IL 3 Ig, it seems likely that it was GM-CSF. These findings differ most strikingly from those reported by Cook et al10 who found little or no GM-CSF to be produced by the tumorigenic cells obtained following A-MuLV infection of FDC-P1 cells. Whether differences between the target cells involved or differences in the conditions used to allow A-MuLV transfectants to emerge account for these differences in growth factor expression must await further study.

In summary, we have clearly documented the production of at least one hematopoietic growth factor (ie, GM-CSF) by tumorigenic mast cell lines derived from A-MuLV-transformed hematopoietic cells. Because these cells could at an early stage in their development be shown to respond to factors present in pokeweed mitogen–stimulated spleen cell conditioned media, it is possible that their ability to produce factor(s) themselves, which we detected subsequently, may have played a role in the early evolution of a malignant phenotype. Although such a model awaits further study, it is interesting to note that the conversion of FDC-P1 cells to GM-CSF production by retroviral transfer of the GM-CSF cDNA sequence was recently shown to confer autonomous growth and tumorigenic potential on these cells.16

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SW Chung, PM Wong, G Shen-Ong, S Ruscetti, T Ishizaka and CJ Eaves