Myeloid Differentiation-inducing Factors Produced by Pokeweed Mitogen-treated Normal G₁₀ Lymphocytes but not Chronic Lymphocytic Leukemia Cells

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ABSTRACT

HL-60 human promyelocytic leukemia cells undergo myeloid differentiation and G₁₀ specific growth arrest in response to an activity produced by pokeweed mitogen stimulated peripheral blood mononuclear cells. Elaboration of this myeloid differentiation inducing activity does not require lymphocytic proliferation or pokeweed mitogen induced B-lymphocyte differentiation. The activity is a product of initially stimulated G₁₀ lymphocytes. It is not produced by proliferating lymphocytes. The activity is not elaborated by G₁₀ peripheral blood lymphocytes of patients with chronic lymphocytic leukemia, cells which are proliferatively refractory to pokeweed mitogen stimulation.

INTRODUCTION

The growth and differentiation of myelomonocytic progenitor cells can be controlled by a variety of biological regulators. In vitro studies of these processes have been facilitated by the use of continuously cultured cell lines. One such cell line is the human promyelocytic leukemia cell line HL-60. HL-60 cells have been found to respond to colony stimulating factors needed for in vitro granulocyte-macrophage colony growth. They thus provide an opportunity for detailed study of factor-cell interactions which regulate the growth and differentiation of these leukemic cells. Human myeloid leukemia cells appear as cells blocked in the differentiation pathway; it is of significant interest to study how such blocks can be released. Significantly since such humoral regulatory factors are elaborated by lymphocytes, this cell system allows studies on regulatory effects of lymphocytic cells on a myelomonocytic progenitor cell.

HL-60 cells are a bipotent progenitor cell (13) capable of differentiating along either the monocytic or myeloid lineages. They respond to both chemically defined and cellularly elaborated inducers. Retinoic acid (5, 27, 32) and DMSO³ (9), for instance, induce myeloid differentiation, while phorbol myristate acetate (24) induces monocytic differentiation. HL-60 cells also respond to biologically derived regulatory factors. Their capability to form colonies in semisolid medium is dependent on colony stimulating activity from PHA stimulated lymphocytes, a cutaneous T-cell lymphoma cell line, human placenta, and a SV40 transformed trophoblast cell line (25). Human placental conditioned medium induces limited granulocytic-monocytic differentiation (19). Conditioned medium from PHA stimulated lymphocytes induces monocytic differentiation (26), probably due to T-cell elaborated factors (6) which initially act on S-phase HL-60 cells (28). One approximately M, 50,000 T-cell derived differentiation factor inducing myelomonocytic differentiation acts synergistically with retinoic acid (23). Another lymphocyte product, γ-interferon, induces a monocytoid differentiation (1), where in this case a recombinant DNA derived interferon was used. Conditioned medium from PWM stimulated lymphocytes induces myeloid differentiation of HL-60 cells (21). The differentiation is similar to that induced by DMSO, but possibly not as complete (21). The differentiated cells show functional capacities similar to those of granulocytes but have abnormal granule composition (21). This may be due in part to the leukemic origin of the HL-60 cells. The DIF in PWM-CM has an apparent molecular weight of 40,000 and was distinguishable from colony stimulating activity (22).

The myeloid differentiation inducing capability of PWM-CM is of particular interest for several reasons. PWM elicits polyclonal peripheral blood lymphocyte proliferation and B-cell differentiation into plasmacytes (2, 7, 10). The B-cell differentiation is T-cell and monocyte dependent (11, 15, 18). As such it has been widely studied as an in vitro model of aspects of the humoral immune response. If the concentration of PWM is reduced, then the differentiative response can be uncoupled from the proliferative response (31), allowing analysis of the proliferative response independent of the differentiative aspects. The cell kinetics of the proliferative response has been analyzed in detail (33). The B-cell proliferative response in this case is T-cell and monocyte dependent (30). It is thus possible in this system to separate the cases where differentiation occurs, proliferation occurs, or lymphocyte activation prior to proliferation occurs. In the studies to be reported here this cell system stimulated with the reduced PWM concentration is used to generate conditioned medium. Thus the association of PWM-CM myeloid differentiation inducing activity with these cellular activities can be analyzed. In the cases of DIF (21) referred to above, PWM-CM containing DIF was generated by a higher dose of PWM which elicited both proliferation and B-cell differentiation and was collected after both responses would have occurred. Interestingly peripheral blood lymphocytes from patients with CLL fail to undergo proliferation or differentiation in response to PWM (8, 12, 14, 16, 17). They thus fail to be activated by PWM like normal cells. The question thus occurs as to whether DIF activity in PWM-CM requires the occurrence of differentiation, proliferation, or merely normal lymphocyte activation antecedent proliferation and whether CLL lymphocytes which fail to normally activate produce PWM-CM containing DIF activity. This communication addresses these issues.
HL-60 DIFFERENTIATION DUE TO γ-interlymphocyte factor

MATERIALS AND METHODS

Cells and Culture Conditions. HL-60 human leukemic promyelocytes from the laboratory of Dr. R. Gallo were generously provided by Drs. C. P. Bums and R. Gingrich. The cells were maintained in constant exponential growth in 25-cm² tissue culture flasks (Corning Plastics) using culture medium consisting of RPMI 1640 (Flow Laboratories) supplemented with 10% heat inactivated fetal calf serum (Kansas City Biologics) and 2 mM glutamine. No antibiotics were used in stock cultures. The cells were assayed to be Mycoplasma free (Mycoprim assay; Hana Biologics). Cells were initiated in culture at a density of 0.2 or 0.1 × 10⁶/ml and subcultured by resuspending in completely fresh medium before they reached 2.0 × 10⁶/ml. All cultures were maintained at 37°C in a 95% humidified atmosphere of 5% CO₂.

Experimental cultures were made by resuspending exponentially proliferating cells in culture medium containing 30% of various PWM-CM; 1:10,000 diluted PWM, added as 1 μl/ml medium of a 1:10 dilution in PBS of PWM (GIBCO); or 10⁻⁴ M retinoic acid (Sigma Chemical Co.). The typical PWM concentration used by others to elicit both cell proliferation and B-cell to plasmaocyte differentiation is a 1:100 PWM dilution. The serum-free culture medium used consisted of RPMI 1640 supplemented with bovine pancreatic insulin (5 μg/ml; Sigma), human transferrin (5 μg/ml; Sigma), and bovine albumin (10 mg/ml; Fraction V; Sigma) as described previously (29). Albumin was solubilized directly in the RPMI 1640 and the resulting medium was filter sterilized. Insulin was added from a 2-mg/ml stock made by solubilizing 2 mg of insulin in 0.2 ml of 0.1 N NaOH and adding 0.8 ml of PBS with 10% glycerol. Transferrin was added from a 2-mg/ml stock. It was solubilized in RPMI 1640 supplemented with 2% gum arabic. Insulin and transferrin stocks were filtered sterilized (0.45 μm; Acrodisk) and stored frozen. Albumin is not necessary to support HL-60 cell growth, and stock cultures have been maintained in this laboratory for over 1 year in RPMI 1640 supplemented with insulin (5 μg/ml) and transferrin (5 μg/ml). Cell density was assayed by multiple hemacytometer counts, using the average of at least eight counting fields. The standard deviation was routinely ±10%. Cell viability, estimated by exclusion of 0.1% trypan blue in physiological saline, was routinely at least 95%. All experiments were performed in at least triplicate repeats.

Assay for HL-60 Myeloid Differentiation. HL-60 myeloid differentiation was assayed as described previously (27, 32) by the PMA (Sigma) induced superoxide production characteristic of mature myeloid cells. Cytoplasmic superoxide was detected by its ability to reduce soluble NBT (Sigma) to a blue-black precipitate, formazan. Cell differentiation in experimental cultures was assayed by withdrawing 0.2 ml of cell suspension from culture, centrifuging (200 × g, 10 min, room temperature) to form a cell pellet and resuspending the pellet in 0.2 ml of PMA-NBT stock, incubating for 30 min in a 37°C water bath, and scoring the fraction of cells containing the cytoplasmic blue-black precipitate, formazan, using a hemacytometer. At least 200 cells were scored for each determination. Standard deviations are typically approximately within 10%. PMA-NBT stock was made as 50 mg of NBT in 25 ml of Dulbecco’s PBS containing 0.01 ml of PMA stock. The PMA stock was made as 1 mg PMA solubilized in 2 ml ace tone yielding a final 0.5 mg/ml of PMA stock. The PMA-NBT stock containing 2 mg NBT/ml and 2 × 10⁻⁴ mg PMA/ml was stored protected from light, refrigerated at 2° C. The PMA stock was stored protected from light at −20°C.

Assay for Cell Cycle Distribution. The relative numbers of cells in G₁, S, and G₂ + M were assayed by flow cytometry using propidium iodide stained nuclei of the cells as described in detail before (30–32). One ml of cell suspension was withdrawn from experimental cultures and centrifuged (4°C) to yield a cell pellet. The cell pellet was resuspended in 2 ml of hypotonic propidium iodide (Sigma) staining solution and stored refrigerated, protected from light. After 24 h the stained HL-60 nuclei were resuspended in 1 ml of 70% ethanol (4°C), stored refrigerated, protected from light, and then resuspended in staining solution prior to flow cytometric analysis. The hypotonic propidium iodide solution used to prepare isolated nuclei was 0.05 mg/ml propidium iodide, 0.1% sodium citrate, and 0.1% Triton X-100 (Sigma). The solution was stored refrigerated and protected from light. Both the fluorescence and narrow angle light scatter were simultaneously measured using a multiparameter fluorescence activated cell sorter (FACS IV; Becton-Dickinson Corp.). Incident light at 488 nm was provided by an argon ion laser (Model 164-05; Spectra Physics) operated at 0.3 watt in the light stabilized mode. Emitted fluorescence was passed through a DM560 dichroic mirror and a DF625/35 six-cavity band pass filter and measured by a PMT operated at 500 V. Fluorescent microspheres (Duke Scientific) were used to confirm instrument stability throughout each run when the samples of an experiment were analyzed. The distribution of number of nuclei versus relative fluorescence intensity was analyzed to yield the fraction of cells in G₁, S, and G₂ + M as reported previously (30). Briefly this method assumes that cellular DNA content is compartmentalized with values ranging from G₁ DNA content to G₂ DNA content. Each compartmentalized cellular DNA content is assumed to produce a normally distributed, emitted fluorescence intensity. The summed distribution of fluorescence intensities is then fitted to the observed to derive the number of cells with each DNA content. Each data point is due to a fluorescence distribution generated by the measurement of at least 10,000 nuclei. Standard deviations in DNA histogram derived cell cycle phase fractions are typically 10%.

Derivation of Lymphocyte Conditioned Media. Conditioned media resulting from PWM stimulation of peripheral blood lymphocytes, performed as described previously (29–31, 33), were derived as follows. Mononuclear cells were separated from peripheral blood of normal adults or patients with CLL. Blood was collected in heparinized containers, diluted 1:1 (or more for CLL blood) with PBS, layered on a Ficoll-Hypaque (Lymphocyte Separation Medium; Litton Bionetics) cushion and centrifuged at 400 × g for 30 min at 4°C after the method of Boyum (3). Mononuclear cells recovered from the interface were washed twice in RPMI 1640 and resuspended in RPMI 1640 supplemented with 15% heat inactivated fetal calf serum, 4 mM glutamine, 1% penicillin-streptomycin antibiotic mixture (Flow Laboratories) and containing PWM (1 μl/ml; GIBCO) diluted 1:10 in PBS. This results in medium containing PWM at a final dilution of 1:10,000. The final cell density was 1.0 × 10⁶ cells/ml. Serum free medium used to culture lymphocytes was RPMI 1640 supplemented with glutamine, insulin, transferrin, and albumin as described above. The cell suspension was inoculated into plastic replicate minewolf plates (Linbro; No. 76-033-05) with 1 ml/well. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂. Population sizes were determined by repeated cell counts using a hemacytometer. Cell viability was measured as the percentage of cells excluding 0.1% trypan blue. Cultures initiated as described above were assayed for the relative distribution of cells among the G₁, G₂, and G₂ + M cell cycle phases at indicated times. Cells were harvested by vigorous pipet trituration of minewolf contents. Minewells were observed by phase microscopy to be free of residual cells after harvesting. Cell counts were performed with a hemacytometer. Cell densities recorded represent the average of trypan blue excluding cells from triplicate minewells. Cell cycle phase distribution of the cells was determined by flow cytometry of propidium iodide stained cells as described previously. Triplicate wells were harvested for each reported determination. Cultures initiated as described above, but without PWM, showed no cells leaving G₂/M to enter S phase or proliferate. No detectable spontaneous mitogenic stimulation by the culture conditions therefore occurred. For large cultures used to generate batches of conditioned media, the above cell suspension was inoculated into 75-cm² tissue culture flasks with 20–30 ml/flask. At 72 h the cell suspension was harvested. Cells were removed by centrifugation (200 × g, 10 min, room temperature), resuspended in an equal volume of fresh culture medium containing PWM, and incubated as before in the same flask until 240 h, when the cell suspension was harvested again as at 72 h. Thus two conditioned media, designated ePWM-CM and 1PWM-CM, were made. ePWM-CM was derived from 0 to 72 h of culture. 1PWM-CM was derived from 72 to 240 h of culture, using fresh medium with PWM.
was verified that replacing the culture medium at 72 h did not inhibit the lymphocyte proliferative response (see Chart 1). Supernatant were clarified by centrifugation (800 x g, 20 min, 4°C) and stored frozen (−70°C) until assayed for activity on HL-60 cells, as described above. Various control conditioned media were generated as follows. Control conditioned medium without PWM from unstimulated MNC were generated as described above but with PWM deleted from the culture medium. Control conditioned medium with PWM were generated as described above but with PWM deleted from the culture medium during incubation with the cells and subsequently added to the harvested conditioned medium.

Different lots of conditioned media from various normal donors were generated and assayed. All contained similar activity. Ten cases of peripheral blood from patients with CLL were provided by the Hematology-Oncology Division at the University of Iowa Hospitals. The patients were currently untreated. The conditioned media generated from these samples all behaved similarly in showing the same lack of myeloid differentiation inducing activity shown in "Results."

RESULTS

Lymphocyte Conditioned Media: ePWM-CM and 1PWM-CM. Conditioned medium from PWM stimulated lymphocytes has been reported to induce myeloid HL-60 differentiation, bearing similarities to the process induced by DMSO (21). In this case conditioned medium was harvested from lymphocytes stimulated with the typically used dose of PWM after 6 days. By this time T- and B-cell proliferation and B-cell to plasmacyte differentiation have occurred. The conditioned medium thus represents cell proliferative and differentiative activity. By reducing the PWM concentration the differentiative portion of the cellular response is eliminated, leaving only a proliferative response (31). This response shows no S-phase cells until after 72 h of stimulation (31, 33). The proliferative kinetics of lymphocytes so stimulated has been characterized in detail (33). It is thus possible to generate conditioned media associated with lymphocyte proliferation, but without differentiation, and with lymphocyte activation, but without proliferation yet.

Conditioned media, ePWM-CM (early PWM induced conditioned medium) and 1PWM-CM (late PWM induced conditioned medium), were generated, as described in "Materials and Methods," using PWM stimulated peripheral blood lymphocytes (PBL). ePWM-CM is the conditioned medium harvested after stimulating the cells from 0 to 72 h. 1PWM-CM is the conditioned medium generated from culturing lymphocytes in fresh PWM containing medium from 72 to 240 h of stimulation. The PBL were stimulated with a dose of PWM which was 1:1000 of that typically used to elicit proliferation and differentiation, as described previously (30, 31, 33). After 72 h the resulting conditioned medium (ePWM-CM) was harvested and replaced with fresh PWM containing culture medium. At this time no significant recruitment of cells from G₁₀ to S had occurred, as reported previously (33). Chart 1 shows the distribution of cells with respect to nuclear DNA content. Essentially all cells had G₁ DNA. The medium conditioned from 72 to 240 h (1PWM-CM) was subsequently harvested. During this time the stimulated cells underwent proliferation. Chart 1 shows the distribution of cells with respect to nuclear DNA content at 120 h. Significant numbers of cells were in S and G₂ + M, demonstrating cell proliferative activity during this interval. 1PWM-CM is thus conditioned medium representing cell proliferation without differentiation. ePWM-CM is conditioned medium representing mitogenic lymphocyte activation but still without proliferation.

Effect on HL-60 Myeloid Differentiation. The conditioned medium ePWM-CM induces HL-60 myeloid differentiation and G₁₀ specific growth arrest while 1PWM-CM does not. Initially exponential HL-60 cells were cultured in the presence of either 30% ePWM-CM, 30% 1PWM-CM, or 10⁻⁶ M RA or in culture medium without these additives. Chart 2 shows the behavior of control cells in culture medium without additives. There was no significant phenotypic differentiation assayed by induced cellular superoxide production (Chart 2, top) which is characteristic of mature myeloid cells. The development of oxidative metabolism is one of the earliest differentiation markers HL-60 cells acquire (20). This widely used assay of functional differentiation has been found to be correlated with a variety of other differentiation markers, although a few markers of mature granulocytes do not develop with it, as discussed previously (4, 20, 27). The distribution of cells throughout the cell cycle was stable (Chart 2, middle) and without evidence of G₁₀ specific growth arrest. Population size increased exponentially with time (Chart 2, bottom). In contrast cells exposed to 10⁻⁶ M RA showed progressively increasing numbers of phenotypically differentiated cells (Chart 2, top). This was associated with G₁₀ specific growth arrest (Chart 2, middle) as evidenced by the enrichment in the relative number of G₁₀ cells. Consistent with this, population size growth was inhibited within two population doublings (Chart 2, bottom). When cells were cultured in the presence of 30% ePWM-CM, there was phenotypic differentiation, assayed by the development of oxidative metabolism, G₁₀ specific growth arrest.
HL-60 DIFFERENTIATION DUE TO G10 LYMPHOCYTIC FACTOR

Table 1
Morphological differentiation (%)

<table>
<thead>
<tr>
<th></th>
<th>Promyelocyte</th>
<th>Metamyelocyte</th>
<th>Banded neutrophil</th>
<th>Segmented neutrophil</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 0 h</td>
<td>99</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T = 72 h; ePWM-CM</td>
<td>56</td>
<td>19</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>T = 72 h; RA</td>
<td>34</td>
<td>19</td>
<td>22</td>
<td>25</td>
</tr>
</tbody>
</table>

Chart 2. Response of HL-60 cells with respect to (top) percentage of functionally differentiated cells, (middle) percentage of cells in G10 (O, •) and S (O, Δ), and (bottom) cell population density as a function of time (h, abscissa). The cells were either untreated controls (O, •) or treated with 10^{-8} M RA (Δ, Δ).

Chart 3. Response of HL-60 cells, depicted as in Chart 2, for cells cultured in 30% ePWM-CM (early pokeweed mitogen induced conditioned medium).

Chart 4. Response of HL-60 cells, depicted as in Chart 2, for cells cultured in 30% 1PWM-CM (late pokeweed mitogen induced conditioned medium).

and inhibition of population size growth (Chart 3). ePWM-CM thus contained a phenotypic differentiation and growth arrest inducing factor(s). The extent of differentiation was pronounced but was not as great as that induced by RA, which is one of the strongest differentiation inducers (4). Table 1 shows the associated morphological differentiation. In contrast when cells were cultured in the presence of 30% 1PWM-CM, there was no significant phenotypic differentiation, G10 specific cell cycle arrest, or inhibition of population size growth (Chart 4). While ePWM-CM, conditioned medium derived from 0 to 72 h of lymphocyte stimulation, induced differentiation, 1PWM-CM, conditioned medium derived from 72 to 240 h of stimulation, failed to induce differentiation.

PWM by itself does not induce HL-60 differentiation or growth arrest. In control experiments initially exponential HL-60 cells were exposed to PWM at the same concentration as used to stimulate lymphocytes. There was no resulting phenotypic differentiation, G10 specific arrest, or inhibition of cell growth, as shown in Chart 5.

The differentiation inducing activity in ePWM-CM requires lymphocyte activation by PWM. Two cases of control conditioned media were tested. In one case lymphocytes were cultured as for ePWM-CM and 1PWM-CM but with PWM deleted from the culture medium. The lymphocytes were thus unstimulated by PWM. Conditioned medium resulting from 0 to 72 h of culture showed no phenotypic differentiation or growth arrest as seen in Chart 6, left. Likewise such conditioned medium resulting from 72 to 240 h of unstimulated lymphocyte culture induced no phenotypic differentiation or growth arrest (data not shown). Generation of differentiation inducing activity in PWM-CM thus required the presence of PWM. In the second case of control conditioned medium tested, conditioned media from unstimulated lymphocytes was produced as described above except that PWM was added to the harvested conditioned media. The conditioned medium produced from 0 to 72 h of culture resulted in no HL-60
HL-60 DIFFERENTIATION DUE TO G1/0 LYMPHOCYTIC FACTOR

Chart 5. Response of HL-60 cells, depicted as in Chart 2, for cells cultured in medium containing PWM.

Chart 6. Functional differentiation (top) and cell population growth (bottom) of HL-60 cells cultured in (left) 30% conditioned medium from unstimulated lymphocytes and (right) 30% conditioned medium from unstimulated lymphocytes with PWM added after removal of cells.

phenotypic differentiation or growth arrest as shown in Chart 6, right. Likewise such conditioned medium resulting from 72 to 240 h of lymphocyte culture failed to induce HL-60 phenotypic differentiation or growth arrest (data not shown). Generation of differentiation inducing activity in ePWM-CM thus required that the lymphocytes be stimulated by PWM.

Taken together these results indicate that HL-60 cells undergo myeloid differentiation in response to a factor(s) elaborated by normal lymphocytes stimulated with PWM while the lymphocytes are still in G1/0. Once actively proliferating the stimulated lymphocytes do not exhibit this activity.

Effect of Conditioned Media from CLL Lymphocytes. Conditioned media generated by PBL from patients with CLL fail to induce differentiation or G1/0 growth arrest. Conditioned media were generated as described above for ePWM-CM and 1PWM-CM except that CLL lymphocytes were used. The conditioned medium resulting from 0 to 72 h of culture was harvested and its differentiation inducing activity was assayed as for ePWM-CM. Initially exponentially growing HL-60 cells were cultured in 30% of this conditioned medium. The HL-60 cells did not differentiate, undergo G1/0 cell cycle arrest, or have inhibited population growth in response to the conditioned medium. The results are shown in Chart 7. When the conditioned medium from 72 to 240 h of culture was assayed, there was also no differentiation or G1/0 specific arrest inducing activity. The response of the HL-60 cells was indistinguishable from that shown in Chart 7 (data not shown). In all 10 cases of CLL derived lymphocytes were assayed. All patients had elevated peripheral blood leukocyte counts which minimized the potential contamination of the presumed leukemic cells by residual normal cells. The patients were not on treatment when their blood was collected. In all cases there was no detectable differentiation or G1/0 cell cycle arrest inducing activity elaborated by these cells when PWM stimulated.

The response of the HL-60 cells in each case was indistinguishable from that shown in Chart 7.

Consistent with the reported observations of proliferative non-response for PWM stimulated CLL cells, the PWM stimulated CLL cells here retained G1/0 DNA content throughout PWM stimulation. The cause of their failure to proliferate is unknown. The above results indicate that stimulated elaboration of myeloid differentiation inducing activity is dependent on some early characteristic of normal G1/0 lymphocytes which is aberrant in the CLL G1/0 lymphocytes. For instance the lack of an essential normal lymphocyte subpopulation may be involved.

The studies reported here have not addressed the molecular nature of the cell elaborated activity described. As might be expected for a lymphokine it appears to be present in minute quantities. All of the conditioned media from PWM stimulated normal cells, unstimulated cells, and PWM treated CLL cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 4–16% gradient gels, as described previously (32). In these cases the conditioned media were generated in serum free medium containing RPMI 1640 plus insulin, transferrin, and albumin, thus eliminating the many proteins of serum from the conditioned medium. The gels were stained with a

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\begin{align*}
\text{Chart 7. Response of HL-60 cells, depicted as in Chart 2, for cells cultured in} \\
\text{30% ePWM-CM, CLL (early pokeweed mitogen induced conditioned medium from} \\
\text{chronic lymphocytic leukemia lymphocytes).}
\end{align*}
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sensitive silver stain and densitometrically scanned. No protein could be identified the presence of which correlated with the presence of biological activity. Nor could any newly appearing protein species attributable to lymphocyte stimulation be observed. When the conditioned media were passed over a Blue-Sepharose column to remove albumin, thereby allowing more conditioned media to be loaded per polyacrylamide gel electrophoresis lane, a protein species attributable to lymphocyte stimulation and correlated with the presence of biological activity still was not detected. The moieties responsible for biological activity, assuming that they are proteinaceous like many lymphokines, are thus undetectable by this method.4

**DISCUSSION**

PWM stimulated lymphocytes elaborate a factor(s) which induces myeloid differentiation of HL-60 human leukemic promyelocytes (21). Such lymphocytes have undergone activation, cell proliferation, and cell differentiation (31). The results presented here show that stimulated cellular elaboration of this biological activity (a) does not require B-cell differentiation, (b) does not require lymphocyte proliferation, and (c) is a characteristic of normal PWM stimulated lymphocytes in G1,0. Indeed conditioned medium from proliferating lymphocytes do not exhibit this activity. This suggests that proliferating cells no longer elaborate this activity. However, alternative explanations are possible. For example proliferating cells might consume or degrade the relevant factors while still producing them. Nevertheless the data do indicate that elaboration of the activity is an early response of stimulated lymphocytes. This suggests a focus on this period for further studies on the characterization of such factor(s). Although this activity is elaborated by normal PWM stimulated G1,0 lymphocytes, it is not elaborated by CLL derived G1,0 lymphocytes. While it is known that CLL lymphocytes usually fail to proliferate in response to PWM, the cell physiological basis of this relatively late response is not known. Significant work has been done on describing aberrant cellular capabilities of CLL cells (8, 12, 14, 16, 17). For example an aberrant T-helper activity has been found, as well as B-cell abnormalities. The data here indicate that CLL cells are aberrant in this early response as well. This defines another pathological aberration of CLL cells. Further studies are required to determine the cause of this defect. For instance lack of a circulating subset of lymphocytes, an inactive subset, or suppression may be involved. Such studies, motivated in part by the present ones, may eventually present a clearer description of the cellular defects in this disease. From the present studies it is apparent that production of these myeloid differentiation inducing factors is a component of normal PWM activated lymphocyte early response which is not part of CLL lymphocyte response.

While HL-60 promyelocytes undergo myeloid differentiation in response to a factor(s) elaborated by PWM stimulated G1,0 lymphocytes, they undergo monocytic differentiation due to a factor(s) elaborated by PHA stimulated lymphocytes (28). It thus appears that different mitogens elicit production of different regulatory factors. This could be due to stimulation of different lymphocyte subsets. Indeed while PHA is a predominantly T-cell mitogen, PWM is a B- and T-cell mitogen (33). Alternatively it may be that different mitogens elicit different activities from the same lymphocyte subset. A comparison of the PHA and PWM elicited responses in this cell system may thus provide insight into how the variety in the repertoire of elaborated myelomonocytic regulatory activities is generated.

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**REFERENCES**


CANCER RESEARCH VOL. 45 SEPTEMBER 1985 4065

A. Yen and R. Van Sant, unpublished observation.
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