TPA-Induced Maturation in Secretory Human B-Leukemic Cells In Vitro: DNA Synthesis, Antigenic Changes, and Immunoglobulin Secretion

By Anna P. Efremidis, Harriette Haubenstock, James F. Holland, and J. George Bekesi

The maturation of malignant cells in response to differentiating agents is interesting as a model of normal differentiation. The response of a freshly explanted neoplastic population of phenotypically well-characterized lymphosarcoma cell leukemia blasts was studied after incubation with the differentiating agent TPA (12-O-tetradecanoyl-phorbol-13-acetate). Terminal differentiation was assessed by measuring the immunoglobulin secreted in cul-
ture supernatants and the production of intracytoplasmic fluorescein-labeled monoclonal antibodies to various anti-
gens in a flow cytometer (fluorescence-activated cell sorter) and ³H-thymidine (³H-Tdr) incorporation was evaluated to measure DNA synthesis in cells grown in complete medium and TPA-supplemented medium. The events induced by TPA were characteristic of B cell maturation and included morphological changes to plasmacytoid cells, reduction in surface immunoglobulins (sIgM, sIgD, and x), enhancement of cytoplasmic immunoglobulin, and amplifi-
cation of immunoglobulin secretion. Surface antigen changes were accompanied by increased ³H-Tdr incorpora-
tion. Cell proliferation and differentiation appeared to be coupled and both were amplified by TPA treatment. These observations indicate that TPA can promote maturation of malignant secretory B cells to a terminal differentiation stage. The significance of these findings to normal B cell differentiation and their potential clinical utility is discus-
sed.

MATERIALS AND METHODS

Patient. Peripheral blood cells with B cell phenotype were obtained from a 66-year-old patient with acute lymphosarcoma cell leukemia with a serum immunoglobulin IgM component. The blood was drawn according to the standards of the Research Administration Committee of the Mt Sinai School of Medicine. The patient had presented three months prior to our study with diffuse lymphadenopathy—acute in onset—hepatosplenomegaly, and a peripheral WBC count of 80 x 10⁹/L. Lymph node biopsy showed poorly differentiated diffuse lymphoma and the patient was treated with vinblastine and chlorambucil. When he was transferred to Mt Sinai Hospital, the patient’s WBC count was 500 x 10⁹/L and leukophoresis was instituted. The circulating blast count was 99% of the total WBC count upon admission (Fig 1A).

Recently, Totterman and colleagues demonstrated that most CLL populations respond to TPA by phenotypic changes consistent with B cell differentiation. These changes include a preferential loss of IgD from the neoplastic cell surface and the accumulation of cytoplasmic IgM (cIgM). Similarly, Gordon et al demonstrated that well-characterized nonsecretory B cell populations positive for cIgM were induced to IgM secretion in vitro after treatment with TPA. Treatment with TPA augmented secretion of IgM in two of three cases with more mature Ig secreting tumor cells (secretory B cells). The same investigators suggested that the respons-
siveness of a given clone to a particular mitogen may relate to its stage of differentiation arrest, reflecting its “readiness” to receive appropriate external stimuli. Controversy exists as to whether maturation-induced phenomena are related to cessation of replication or to induction of cell prolifera-
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In the present study, we present a more detailed analysis of the sequence of TPA-induced maturation of secretory leu-

B CELL NEOPLASMS such as chronic lymphocytic leukemia (CLL) and non-Hodgkin’s lymphomas can be viewed as models of monoclonal populations restricted within discrete portions of B lymphocyte development.¹ ¹ The limited differentiation exhibited by such clones in vivo can often be overcome in vitro, if appropriate differentiating signals are presented.² ² Such in vitro systems with various agents have been used in order to study sequential events in differentiation and to elucidate cellular and biologic interrelations occurring during the process of maturation. The phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), exerts a variety of biochemical and biologic effects on hemato-
epoietic cells.³ ³ One of the more interesting properties of TPA is its ability to inhibit or to stimulate differentiation of a number of murine leukemias in vitro.⁷ TPA can also induce the differentiation of human leukemic cells in vitro, including acute myeloid leukemia (AML),⁴ ⁴ common acute lymphoblastic leukemia,⁵ ⁵ some non-Hodgkin’s lymphomata,⁶ ⁶ and non-T cell acute lymphoblastic leukemia.¹² TPA induces the differentiation of some human B cell lines,¹¹ T cell lines,¹¹ and CLL cells toward either immunoglobulin (Ig) synthesis,¹³ or the acquisition of some monocytic characteristics.¹⁶

Recently, Totterman and colleagues demonstrated that most CLL populations respond to TPA by phenotypic changes consistent with B cell differentiation. These changes include a preferential loss of IgD from the neoplastic cell surface and the accumulation of cytoplasmic IgM (cIgM).¹⁷ ¹⁸ Similarly, Gordon et al demonstrated that well-characterized nonsecretory B cell populations positive for cIgM were induced to IgM secretion in vitro after treatment with TPA.¹⁹ Treatment with TPA augmented secretion of IgM in two of three cases with more mature Ig secreting tumor cells (secretory B cells). The same investigators suggested that the respons-
siveness of a given clone to a particular mitogen may relate to its stage of differentiation arrest, reflecting its “readiness” to receive appropriate external stimuli. Controversy exists as to whether maturation-induced phenomena are related to cessation of replication or to induction of cell prolifera-
tion.²¹ ²⁵

In the present study, we present a more detailed analysis of the sequence of TPA-induced maturation of secretory leu-

kemic B cells comparing morphology, DNA synthesis, antigen expression, and IgM secretion. The changes are discus-
sed in relation to current knowledge of B cell differentiation and possible clinical utility.

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density gradient at 800 g. The interface was collected and washed twice in phosphate-buffered saline (PBS), prior to all experiments. T cell and myeloid/monocytic antigens, and immunoglobulins (Igs), were measured in indirect immunofluorescence tests. Briefly, 0.5 to 1.0 × 10⁶ washed cells were treated with a 1:5 dilution of specific antibody, incubated at 4 °C for 45 minutes and washed twice with PBS. Monoclonal antibodies to human IgM, IgD, and IgG, κ and λ light chains were used, in addition to antibodies recognizing cell surface antigens designated B1 (pan-B), B2 (restricted B), J5 (common acute lymphoblastic leukemia), I2 (HLA-DR), MO1 (monocyte-leukocyte), and MO2 (adherent monocyte) (Coulter Immunology, Hialeah, Fla). In addition, the T11 (sheep erythrocyte receptor) antigen was also evaluated. Cells were then incubated with 100 μL of a 1/20 dilution of fluorescein-labeled goat anti-mouse Ig antibody (Tago, Burlingame, Calif) at 4 °C for 45 minutes and washed twice with PBS. At least 10⁴ cells were analyzed using a Becton Dickinson (Mountain View, Calif) microfluorocytometric analyzer (fluorescence-activated cell sorter [FACS]).

Staining for intracytoplasmic immunoglobulins. Cells containing cytoplasmic immunoglobulins (clgs) were stained by direct immunofluorescence technique. Cytocentrifuged (Shandon-Southern Instruments, Sewickley, Pa) preparations ofuffy coat or suspended cultured cells or cells adherent to culture plates were fixed in 95% ethanol:5% acetone at 4 °C for 20 minutes. Slides or plates were then washed in PBS three times and exposed to anti-immunoglobulin heavy chain-specific monoclonal antibodies labeled with fluorescein (Wellcome Research Laboratories, Beckenham, England) for 30 minutes at room temperature, in a humidified atmosphere. Slides were then washed three times in PBS and mounted in mounting medium (Elvanol). Stained cell preparations were scored for the percentage of positive cells and assessed for intensity by a single observer throughout the study, using a Leitz Wetzlar fluorescence microscope.

Stimulation studies. 10⁴/mL freshly prepared or cryopreserved cells were cultured in RPMI 1640, with 10⁴/mL, with 15% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY), penicillin (100,000 U/L), and streptomycin (100,000 U/L) (complete medium) in a humidified 7.5% CO₂ atmosphere at 37 °C. When added, the TPA (Sigma Chemical Co, St Louis; kindly provided by Dr W. Scher, Mount Sinai Hospital, New York) was present at a final concentration of 1.6 × 10⁻¹⁷ mol/L. Pairs of treated and control cultures were incubated in 3-mL or 10-mL tissue culture plates (Falcon Plastics, Oxnard, Calif). After culture for the indicated time (zero to seven days) the cells were washed free of the medium (in RPMI 1640 three times) for surface marker analysis or clg stain. In some experiments, cells were incubated in TPA-supplemented medium (for one and four hours), washed three times with complete medium, and resuspended in unsupplemented complete medium for further incubation. Cell viability was estimated by trypan blue dye exclusion and cells were counted in a hemocytometer. In all experiments, cells were shown to have a viability of >80% when cultured for three days with or without TPA.

Assay of secreted immunoglobulins. The nephelometric technique reported by Deaton et al was employed, using a Behring Laser nephelometer (Somerville, NJ) for quantification of immunoglobulins. In brief, a standard curve was established over the range of 0.5 to 20 μg/mL using serial dilutions of lyophilized human serum (Calbiochem, La Jolla, Calif, Lot No. 010374) combined with an appropriate dilution of antisera to human IgM (μ - chain specific) (Calbiochem Lot No. 010043) in 4% polyethylene glycol (Sigma Chemical Co). Light scatter readings were obtained after incubation of samples or standards with antibody for one hour at room temperature. Supernatants from cultured cells were assayed in triplicate for immunoglobulin content after centrifugation at 24 × 10³ g (Beckman Ultracentrifuge L5-65B) for 30 minutes. Immunoglobulin concentration was estimated from the standard curve and was expressed as the mean value of triplicate cultures. All antisera showed no reactivity with fetal bovine serum. Independent standard serum samples were used in each assay as a measure of reproducibil-
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Fig 2. Morphology of B-leukemic cells in TPA-treated cultures at (a) 0 time, (b) 48 hours, and (c) 96 hours. Original magnification x80; current magnification x52.

RESULTS

TPA-induced morphological and volume changes. Figures 1 and 2 show the morphological changes of human B-leukemic cells during culture with and without TPA. Control cells incubated in complete medium appeared as round free lymphocytes, and adherent cells and cell aggregates were rare. After 24 to 48 hours of continuous exposure to TPA, phase contrast microscopic examination revealed an increase in cell size, clumping, and increased adherence to the culture plate, which was maximal on day 4 of the cultures (Fig 2). Most of the cells had a dendritic appearance with long filamentous projection (Fig 1C). May-Grünwald-Giemsa-stained preparations revealed plasmacytoid morphology, as judged by the displacement of the nucleus to the periphery, a change in the chromatin network, and increased basophilia of the cytoplasm. Most of the cells resembled plasmablasts and a nuclear cytoplasmic asynchrony was prominent at day 4 (Fig 1D). Electronic analysis of cell volume corroborated the TPA-induced increase in cell size (data not shown). Morphological changes in complete and TPA-supplemented medium were reproduced in five experiments.

TPA-induced changes of surface immunologic markers. The leukemic blasts studied were classified as secretory B by their capacity to export Ig in culture that corresponded to the serum paraprotein. The phenotype of fresh cells was: sIgM, 87%; sIgD, 60%; sIgG, 3%; K, 14%; B1, 78%; I2, 32%; MO1, 1%; MO2 and T11, 0%. CIgM was detected in 98% to 100% of seeded cells (Table 1) (Fig 3). Table 1 also shows the original phenotype of cultured cells in all experiments as well as antigenic changes in both control and TPA systems at various points studied during different experiments. The number of experiments performed for each marker is indicated in the legend of Table 1. The kinetics of surface changes during a representative experiment are shown in Fig 4. The number of positive cells and the intensity of staining for sIgM, sIgD, and K were reduced dramatically after exposure of the leukemic cells to the phorbol ester for 24 hours in vitro. These isotypes remained low during the period studied (three days). The rate of decline of sIgD and sIgM was slightly different since sIgD disappeared gradually over the first 24 hours while sIgM declined faster (four hours); however, sIgD loss at 24 hours was more prominent than sIgM loss. Kappa light chain kinetics followed the same pattern of sIgM decline. Lambda light chains remained undetectable in both systems. In contrast, the expression of sIgG was induced after three days in culture in the presence of TPA (Table 1, Fig 5). Exposure of cells to TPA for short periods of time (one and four hours) produced the same loss of surface immunoglobulin markers and subsequent reacquisition of surface antigens in cells that were washed and reseeded for further culture (data not shown) (two experiments). The MO1 and MO2 antigens were evaluated in order to monitor the possible development of myelomonocytic features and they remained undetectable (<0.1%) during three experiments tested. The HLA-DR-

<table>
<thead>
<tr>
<th>Time in Culture (h)</th>
<th>Culture</th>
<th>sIgM</th>
<th>sIgD</th>
<th>sIgG</th>
<th>K</th>
<th>B1</th>
<th>B2</th>
<th>CIgM Cells (%)</th>
</tr>
</thead>
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<tr>
<td>0</td>
<td>---</td>
<td>63 ± 22</td>
<td>50 ± 8</td>
<td>5 ± 2</td>
<td>66 ± 24</td>
<td>50 ± 17</td>
<td>2 ± 2</td>
<td>98.6 ± 0.9</td>
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<tr>
<td>24</td>
<td>FCS</td>
<td>70 ± 20</td>
<td>63 ± 17</td>
<td>4 ± 2</td>
<td>60 ± 10</td>
<td>62 ± 11</td>
<td>6 ± 1</td>
<td>98.3 ± 0.4</td>
</tr>
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<td></td>
<td>TPA</td>
<td>44 ± 28</td>
<td>7 ± 4</td>
<td>2 ± 1</td>
<td>24 ± 12</td>
<td>82 ± 11</td>
<td>4 ± 2</td>
<td>98.8 ± 0.7</td>
</tr>
<tr>
<td>48</td>
<td>FCS</td>
<td>50 ± 6</td>
<td>46 ± 14</td>
<td>5 ± 4</td>
<td>50 ± 8</td>
<td>35 ± 12</td>
<td>12 ± 6</td>
<td>98 ± 0</td>
</tr>
<tr>
<td></td>
<td>TPA</td>
<td>11 ± 10</td>
<td>2 ± 1</td>
<td>3 ± 3</td>
<td>9 ± 12</td>
<td>67 ± 11</td>
<td>3 ± 1</td>
<td>99.3 ± 0.4</td>
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<tr>
<td>72</td>
<td>FCS</td>
<td>38 ± 20</td>
<td>18 ± 15</td>
<td>4 ± 2</td>
<td>35 ± 20</td>
<td>55 ± 12</td>
<td>13 ± 6</td>
<td>98.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>TPA</td>
<td>18 ± 10</td>
<td>9 ± 8</td>
<td>40 ± 5</td>
<td>5 ± 5</td>
<td>60 ± 6</td>
<td>2 ± 1</td>
<td>99.6 ± 0.4</td>
</tr>
</tbody>
</table>

MO1 was 2.5 ± 4 at the time of seeding (four experiments). MO1 was 1 ± 1.4 at the time of seeding (three experiments). MO2 was <0.1% at the time of seeding (three experiments). Values represent means ± SD of four experiments for sIgM and K and three experiments for sIgD, sIgG, B1, and B2 antigens. CIgM represents the mean value of cells ± SD in four experiments. The magnitude of SD reflects changes in the initial populations in different experiments as a result of treatment and freezing.

Abbreviation: FCS, fetal calf serum.
Fig 3. Induction of clgM production by TPA treatment in B-leukemic cells preceded immunoglobulin IgM secretion. clgM stain with monoclonal anti-human antibody of the studied cells is shown on the top, at 48 hours, at 96 hours, and on the seventh day, from top to the bottom. Control cultures (left) and TPA-treated cultures (right) are shown in each line. Original magnification ×400; current magnification ×300.
three days in vitro, in a representative experiment. Rapid decline of slg coincided with the increase in ³H-Tdr incorporation (Fig 6).

Cells were incubated in TPA-supplemented or complete medium for three days, B₁ levels were 16% and 1% above the initial level, respectively. Expression of the restricted B₂ antigen was increased after culture in vitro. This increase appeared earlier when cells were treated with TPA. After 24 hours in culture, cells incubated under either condition demonstrated a 24% increase of B₁ expression above the initial level, and after four-hour exposure to TPA and returned to close to the original level at 24 hours and remained low, while cells incubated in unsupplemented medium showed peak expression of B₂ antigen (15% above the initial level) at day 3. TPA-treated cells expressed slgG related 12 antigen varied significantly and did not follow a constant pattern.

Changes in B₁ and B₂ are shown in Table 1. A representative experiment is also shown in Fig 5. Expression of both the B₁ and B₂ antigens was increased after culture in vitro. This increase appeared earlier when cells were treated with TPA. The B₁ antigen increased above the initial level after a four-hour incubation with TPA. After 24 hours in culture, cells incubated under either condition demonstrated a 24% increase of B₁ expression above the initial level, and after cells were incubated in TPA-supplemented or complete medium for three days, B₁ levels were 16% and 1% above the initial level, respectively. Expression of the restricted B₂ antigen increased 18% after a four-hour exposure to TPA and returned to close to the original level at 24 hours and remained low, while cells incubated in unsupplemented medium showed peak expression of B₂ antigen (15% above the initial level) at day 3. TPA-treated cells expressed slgG on day 3 in contrast to cells cultured without TPA, in which this antigen remained undetectable (Table 1, Fig 5). In order to exclude the possible effect of T cells on the maturation events of B cells, we serially followed expression of the T11 antigen during culture. This antigen remained undetectable (<1% in the TPA cultures) when serially followed in three experiments.

TPA-induced changes in IgM production and secretion. After exposure to TPA for 24 hours, the cells positive for cytoplasmic IgM displayed a morphology ranging from lymphoplasmacytoid to plasmacytoid. Although the number of cells positive for clgM was approximately 100% in both systems (Table 1), cells from control cultures expressed less intense immunofluorescence than those in TPA-treated cultures. Table 1 shows the mean number of cells ± SD positive for clgM during culture in both systems for four experiments. TPA-treated cells contained more clgM since they were larger than untreated cells.

Figure 3 shows the sequential changes of clgM at 0, 2, 4, and 7 days in a representative experiment. In Table 2, IgM secretion of fresh cells cultured with and without TPA is expressed as the mean values of two experiments performed on fresh cells and run in triplicate. Figure 6 shows IgM secretion in parallel to ³H-Tdr incorporation and cell viability evaluated simultaneously in one experiment. A rapid surge in extracellular IgM secretion was induced after 24 hours of exposure to TPA and secretion continued to increase over the seven-day period observed. In unsupplemented cultures, IgM was first detectable after 48 hours in culture. The rate of secretion was parallel in both systems. The surge in IgM secretion did not result from cell death and lysis, since cell viability decreased earlier in the control cultures while IgM secretion occurred earlier in TPA cultures. In none of the experiments was either IgG or IgA detected at a level above the sensitivity of the assay (1 µg/mL).

The loss of slgD (Fig 4) and the beginning of IgM secretion may be correlated, since IgM secretion in both culture systems occurred after complete loss of slgD. No relationship was noted between the magnitude of induced IgM secretion and the quantitative expression of any other cell surface antigen investigated.

³H-Tdr incorporation and clgM secretion. There was an increase of ³H-Tdr incorporation by 24 hours after TPA treatment, which continued to rise, reaching a maximum at

![Fig 4. Kinetics of disappearance of slgM, slgD, and x in control (left) and TPA-treated (right) B-leukemic cells, over a period of three days in vitro, in a representative experiment.](image)

![Fig 5. Kinetics of B₁, B₂, and slgG in control (left) and TPA-treated (right) B-leukemic cells, over a period of three days in vitro, in a representative experiment.](image)

<table>
<thead>
<tr>
<th>Time in Culture (h)</th>
<th>Culture</th>
<th>cpm ± SD</th>
<th>IgM (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>FCS</td>
<td>2,213 ± 181</td>
<td>0</td>
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<tr>
<td></td>
<td>TPA</td>
<td>4,805 ± 412</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>FCS</td>
<td>388 ± 17</td>
<td>2.9 ± 0.04</td>
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<td></td>
<td>TPA</td>
<td>2,832 ± 219</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>48</td>
<td>FCS</td>
<td>557 ± 76</td>
<td>4.5 ± 0.16</td>
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<tr>
<td></td>
<td>TPA</td>
<td>12,006 ± 1,374</td>
<td>6.55 ± 0.005</td>
</tr>
<tr>
<td>72</td>
<td>FCS</td>
<td>3,490 ± 96</td>
<td>3.85 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>TPA</td>
<td>68,310 ± 5,230</td>
<td>6.55 ± 0.005</td>
</tr>
</tbody>
</table>

Results shown represent means ± SD on two experiments done in triplicate.
72 hours. Incorporation was also observed in control cultures, but at a lower rate (Table 2). The rapid increase of $^3$H-Tdr uptake during TPA treatment coincided chronologically with the continued loss of surface IgD and IgM when tested concurrently in two experiments.

**DISCUSSION**

The monoclonal populations in B cell malignancies provide a model for studying maturation sequences after treatment with differentiating agents. Within this framework, the possibility of correlating changes in these cells and their relationship to normal differentiation pathways may lead us to a better understanding of normal maturation as well as leukemogenesis.

The qualitative changes observed after treatment of leukemic cells with differentiating agents are related to the degree of maturation of the cells before treatment. The present study investigated sequences of maturation of secretory B cells with respect to TPA. Ig secretion, which is the most reliable index of functional maturation, was greatly increased in the production of extracellular IgM was observed following exposure of the lymphosarcoma cells to TPA. Increased cell aggregation and a shift toward plasmacytoid morphology accompanied the increase of IgM and IgG secretion induced by TPA. Immunofluorescence studies of Ig expression of the leukemic cells showed a rapid decrease of IgD, IgM, and $\kappa$ after four hours of culture with TPA, although these immunoglobulins could still be detected after three days. In similar studies, normal human peripheral blood and tonsil B lymphocytes responded more slowly to TPA treatment with a decrease of IgD and IgM over a 24-hour period and after three days, respectively. Normal B cells evaluated seven days after activation with pokeweed mitogen (PWM) also lost IgD followed by IgM. Most CLL cells with well-characterized "pre-B," "early B" and "intermediate" or "mature B" cell populations lost IgD and showed decreased IgM after three days in cultures with TPA. These differences may reflect the maturity of the populations of cells studied or the time frame of antigenic determinations. The re-expression of Ig markers observed after short exposure to TPA followed by incubation in unsupplemented medium suggests that continued exposure of the leukemic cells to TPA not only strips off the Ig but may also decrease their synthesis. Loss of Ig did not reflect general toxicity since the HLA-DR-related Ig antigen was unchanged and the B1, B2, and IgG antigens were induced by TPA treatment.

The pan-B cell marker B1, after a slight initial increase in both systems which occurred earlier in TPA-treated cells, decreased slightly during the three-day culture, a phenomenon also observed in the TPA-treated and PWM-stimulated normal B cells. This was not a reflection of the mitogenic effect of TPA, since FACS analysis indicated that B1 induction preceded the volume changes. The B1 antigen remained high on cultured cells in both systems, indicating that a loss of B1 may not play a functional role in IgM secretion. The expression of B2 increased transiently on the TPA-exposed cells at four hours but was clearly absent by day 3. In contrast, in the control system, B2 antigen was expressed slowly over a three-day period. This antigen detects restricted subpopulations of B cells and is also induced transiently in similar experiments with normal B cells. While it has been suggested that the B2 antigen is expressed together with IgD, Aman et al showed that B2 can be expressed after the loss of IgD. This pattern was observed in our study of cells cultured in unsupplemented medium, in which the disappearance of IgD coincided with the maximal increase of B1 on the third day.

Acquisition of IgG was a late event in TPA-supplemented cultures and was not observed in controls. It could be argued that the appearance of IgG-positive cells could represent expansion of IgG-bearing normal lymphocytes, which may be present at the time of the seeding. However, a doubling time of less than one hour would have been required in order to explain this phenomenon. Although the untreated leukemic cells secreted IgM in vitro without TPA, it may not be correct that all of the cells were committed to secretion. The cells that developed IgG may have derived from an uncommitted subpopulation which was positive for IgM. The expression of an additional heavy chain in the TPA system may be explained by the recent report that the onset of synthesis of $\gamma$-chain may reflect messenger RNA (mRNA) processing rather than DNA splicing. Switching of IgM to IgG production has been reported to occur on secretory B-leukemic cells after treatment with PWM in the presence of T cells. The physiologic basis for this phenomenon requires further investigation.

In normal B cells, the peak of TPA-induced synthesis of DNA followed IgM secretion detected between two and three days in culture. In contrast, PWM-stimulated lymphocytes initiate Ig secretion after the peak of DNA synthesis. In our study, TPA-induced secretion of IgM was detected before maximum DNA synthesis occurred and may have reflected a more differentiated subpopulation among
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the malignant peripheral blood lymphocytes. These data indicate that terminal differentiation of leukemic B cells with TPA may not involve the early loss of proliferative capacity as suggested by previous studies in which proliferation was assessed by cell enumeration, but is in agreement with more recent studies in which proliferation of normal B cells was assessed by \(^{3}H\)-Tdr incorporation. The absence of MO\(_{2}\) expression during our experiments indicates that the observed differentiation phenomena were independent of accessory monocytes. The presence of T cells at a low concentration at the time of seeding is highly unlikely to play a significant role in the TPA-induced B cell differentiation. This corroborates studies that show that the effect of TPA on human peripheral B cells is unlikely to be mediated by accessory monocytes or monocyte products. These observations contradict those of other investigators, who showed T cell and macrophage dependence of Ig secretion after five and six days of treatment with TPA. In these experiments, however, the concentration of TPA used was much higher than the concentration used in our studies and it may well be that TPA at various concentrations exerts a different response on cells.

Treatment of normal human B cells with anti-IgM induced DNA synthesis in the presence of B cell growth factor. A high concentration of anti-IgM antibody is sufficient to stimulate normal B cell proliferation in the absence of accessory cells or accessory cell factors. Surface antigen changes induced by an appropriate concentration of TPA may initiate signals for DNA synthesis by the same mechanism, since the rapid increase of \(^{3}H\)-Tdr incorporation coincided with the initiation of slgM and slgD loss in our study.

In conclusion, changes in surface antigens detected by monoclonal antibodies suggest that TPA both accelerated activation and induced terminal differentiation of malignant human secretory B cells in vitro. The rapid surface immunoglobulin loss was followed by DNA synthesis, suggesting that proliferation and differentiation were coupled. Maturation was also manifested by development of plasmacytoid morphology and accelerated secretion of IgM. The sequence of these events is similar to the sequences reported when normal B cells are stimulated by PWM or TPA and indicates that the malignant cells had retained the capacity to follow a normal differentiation pattern when induced appropriately in vitro. These findings add to previous ones that TPA induces maturation of most “nonsecretory” and some “secretory” CLL populations in vitro.

The capacity of TPA to induce a high rate of IgM production by these cells may have clinical utility. Antibodies directed against the idiotype portion of paraprotein M component produced by lymphoid tumors can be viewed as tumor-specific and can be used to target tumor cells in B cell leukemia and lymphoma. The in vitro production of clonally restricted idiotypic Ig produced by malignant B cells in response to TPA may provide a rapid way to obtain tumor-specific immunogens necessary for production of antiidiotypic antibodies useful in clinical trials.

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Tpa-induced maturation in secretory human B-leukemic cells in vitro: DNA synthesis, antigenic changes, and immunoglobulin secretion

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