Effect of Thimerosal in Leukemia, in Leukemic Cell Lines, and on Normal Hematopoiesis

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ABSTRACT

Anti-thymocyte globulin (ATG), a horse antiserum to human thymus tissue, has been shown to induce granulocytic differentiation of the HL-60 human leukemia cell line. In this paper we describe the effect of ATG on leukemic blasts and its effect on other human leukemia cell lines in vitro. The in vitro differentiation effect of ATG was observed in blasts from two patients with leukemia and the human leukemia cell line K562. The differentiation effect of ATG was attributable to its preservative, thimerosal, separable from ATG by high pressure liquid chromatography; GM-CSF, granulocyte-macrophage colony-stimulating factor.

INTRODUCTION

ATG, a horse antiserum against human thymus tissue, is an active agent in the treatment of acute severe aplastic anemia regardless of etiology (1, 2). We have previously studied the effect of ATG in vitro and observed that it directly stimulates erythroid (burst-forming units) growth and differentiation. Addition of ATG to monocytes and natural killer cells causes release of factors which stimulate normal marrow granulocyte/macrophage growth and differentiation (3). We have previously observed that HL-60 leukemic cells treated with ATG undergo terminal differentiation (4). The purpose of this study was to examine the effect of ATG on normal bone marrow and leukemic cells and to isolate the active component.

Following these observations, we have extended our research to human leukemic cells to see whether ATG also promotes their growth and differentiation in vitro. This report describes the following findings: (a) ATG induces terminal differentiation of leukemic blasts from 3 patients with acute leukemia; (b) the differentiation effect is attributable to an antimicrobial preservative, thimerosal, present in the ATG preparations; (c) thimerosal by itself induces terminal differentiation in leukemic blasts from three patients and three human leukemic cell lines (K562, KG-1, and U937) and accelerated differentiation in normal bone marrow erythroid cultures.

MATERIALS AND METHODS

Tissue Culture Technique. K562 cells, KG-1 cells, and U937 cells were obtained from the American Type Culture Collection (Rockville, MD). These lines were maintained in RPMI 1640 with 10% FCS, penicillin/streptomycin/gentamicin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, and l-glutamine (300 mg/liter) (Gibco, Grand Island, NY). They were passed twice a week.

Leukemic blasts from five patients were collected from venipuncture or leukapheresis. These cells were then separated over lymphocyte separation medium (Organon Teknika, Durham, NC), and the adherent cells were removed with overnight incubation in plastic flasks. Cells were maintained in Iscove's modified Dulbecco's media with 20% FCS, penicillin/streptomycin/gentamicin, l-glutamine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, and a 1:10 dilution of PHA-LCM (5). We have found that this dilution of PHA-LCM allows maximum plating efficiency of human leukemic cells. The cells were passed three times a week and used for experiments within 6 days from their collection. Leukemic cells from these five patients were phenotyped and were positive for CD34 (recognized by the monoclonal antibody My-10, or anti-HPCA-1). HLA typing and chromosomal analysis of leukemic blasts were performed on all patients. Three of the patients were in myelogenous blast crisis from Ph+ chronic myelogenous leukemia. Two patients had de novo acute myelogenous leukemia, French-American-British classifications M2 and M3. All cells were maintained in incubation at 37°C, 95% humidity, and 5% CO₂.

For differentiation experiments, leukemic cells from patients were first washed; viable cells were counted and resuspended in the same type medium in which the cells had been maintained. Cell lines were suspended in RPMI 1640 with 20% FCS (the concentration of FCS remained constant for all differentiation experiments). All cells were either placed into 24-well tissue culture plates (635 ml/well, 4 x 10⁴ cells/well for cell lines, 1 x 10⁵ cells/well for cells from patients) or into 75-cm² Costar (Cambridge, MA) tissue culture flasks (1 x 10⁴ cells/ml). Growth factors, differentiation agents, and control substances were added at initiation of culture. Wells were harvested by washing with Hanks' balanced salt solution (containing 4% FCS) and centrifugation to pellet. Cells were resuspended in 100 µl of FCS and microscope slides made using a Cytospin II (Shandon). Cells from flasks were used for hemoglobin assays (see below).

Normal volunteers donated normal marrow for in vitro blast-forming units-erythocyte colony growth experiments, after giving informed consent. Nonadherent mononuclear cells were obtained as above. Using a positive panning technique, described previously (3), the HPCA-1-positive cells were removed and plated at 5 x 10⁴ cells/well in Iscove's modified Dulbecco's medium with 0.8% methylcellulose, 30% FCS, penicillin/streptomycin, PHA-LCM 1:10 dilution, and erythropoietin (2 units/ml). Cells were examined 5–10 days later under inverted microscope and after Wright's staining of cytopsin preparations from harvested wells.

In tritiated thymidine incorporation experiments, 1 µCi/ml (20 Ci/ mmol; New England Nuclear) was incubated with cells (1.5 x 10⁴ cells/well) for 20 h in 96-well plates, washed, and then transferred to glass fiber filter using a semiautomated harvester (Titertek, Rockville, MD) filled with distilled water. Scintillation counting was performed on a Beckman liquid scintillation counter (LS 200). Various concentrations of TMS with and without DTT (both from Sigma, St. Louis, MO) and DTT alone were added to the wells at the initiation of culture. The experiments were done in triplicate.

Separation of TMS from ATG. K562 leukemic cell differentiation, enhanced by staining of cytopsin slides, was used as an assay to determine differentiation activity of various preparations of ATG (Upjohn, Kalamazoo, MI) and other solutions. They included preimmunization horse IgG from Upjohn prepared identically to ATG except for the thymus immunization, preimmunization horse IgG also from Upjohn prepared without the preservative TMS, horse IgG from Cappell without preservative (West Chester, PA), anti-HPA-1 (anti-CD34) antibody with azide (0.1%), (Becton-Dickinson, Sunnyvale, CA), human...
IgG without preservative (Cutter, Emoryville, CA), and horse serum without preservative (Gibco). Lastly, pure TMS was added to K562 cultures and compared to different quantities of ATG that contained equivalent amounts of TMS.

Six ml of ATG were dialyzed against three 1500-ml exchanges of phosphate-buffered saline over 36 h at 4°C. The protein concentration was measured, using Bio-Rad protein assay, before and after dialysis. The dialyzed ATG was then tested for differentiation activity in K562 cells.

In separate experiments, ATG was diluted 1:2 in phosphate-buffered saline and chromatographed on a TSK G 3000 SW 0.8- x 30-cm HPLC column (LKB). For three separate runs, fractions were collected each minute for 1 h. Three 280 nm absorbance peaks were observed, one large and two small. Protein concentrations of the three peaks and several other fractions were measured using Bio-Rad protein assay (Richmond, CA). All fractions were tested for differentiating activity in K562 cells and the degree of effect compared to an equivalent protein concentration of unfractionated ATG. TMS concentrations were estimated on the basis of mercury levels found in solutions containing known quantities of TMS. Mercury assays were measured by atomic absorption on protein-digested samples (UV Mercury Monitor, LDO).

Effect of Dithiothreitol on TMS. DTT was used to test the inhibition of differentiation induced by TMS (6). In these experiments, DTT (1 mm) was added to K562 cultures 1 h before, 2 min before, and 1 h after the addition of TMS at three different concentrations (1.5, 2.5, and 7.5 μM). Cell morphology was examined on cytospin slides.

Hemoglobin Analysis. Cells were cultured in the presence of ATG or TMS. Erythropoietin (Step III; Connaught, Willowdale, Ontario, Canada), 1-2 units/ml, was added to some of the cell line cultures and all of the patient's cell cultures. Only patient's cells obtained via leukapheresis contained enough pure blasts to perform hemoglobin assay. Because of the higher cell density (10^6) used in some of these assays, higher concentrations (10^6) of TMS or ATG were necessary. The assay is based on peroxidation reaction using diaminofluorine and quantitated by a spectrophotometer at 610 nm (7). Diamminofluorine is more specific for hemoglobin and reportedly less carcinogenic than benzidine (7). Repeat experiments with patient's cells were limited by longevity of cells. Experiments with K562 were done in duplicate and those with KG-1 in triplicate.

Evaluation of Differentiation. Morphological evaluation of Wright's-stained slides were performed on all cell lines and cells from patients. Cells were harvested after incubation for 1 h to 7 days. The effects of TMS on K562 was compared with known differentiating agents, such as 1-β-D-arabinofuranosylcytosine (0.1-100 μM), bleomycin (2.4-4.8 μM), actinomycin D (0.5-80 μM), and hemin (0.1 μM). KG-1 cells were also examined for hemoglobin, naphthol AS-D chloroacetate esterase, and α-naphthyl acetate esterase. With U937, TMS was combined with or compared to macrophage-colony-stimulating factor (Genetics Institute), GM-CSF, interleukin 3 (Amgen), γ-interferon (Genentech), and burst-promoting factor (PHA-LCM, 1:20 dilution). Cytospin slides were stained with naphthol AS-D chloroacetate esterase or α-naphthyl acetate esterase (with and without fluoride inhibition) and 200 cells/slide scored. All assays were done in duplicate or triplicate. Data are reported as the arithmetic mean.

RESULTS

Effect of ATG on K562 Cells (Table 1). The K562 human leukemic cell line, was derived from a patient with Ph+ chronic myelogenous leukemia in blast crisis (8). K562 leukemic cells differentiated in the presence of ATG in vitro at a concentration of 0.2–0.6 mg/ml when cell density was 2 × 10⁴ cells/ml and 2–4 mg/ml when cell density was 1 × 10⁵ cells/ml At the higher concentrations, nearly 100% of blasts underwent terminal differentiation. The differentiated cells exhibited the morphological continuum of erythrocytic maturation with moderate karyorrhexis and synthesized hemoglobin in culture (Table 1). Hemoglobin concentration of K562 cells increased until day 4 or 5 and could be augmented by addition of erythropoietin. The cell numbers remained stable through the measured time period, indicating that selective survival of only a few cells is not occurring and that growth is inhibited.

Separation of TMS from ATG (Tables 2 and 3). In an attempt to test whether the ATG effect on leukemic cell differentiation was related to the anti-human antibody property of the agent, a number of preparations produced by different laboratories were obtained. Our results indicate that immunization against the thymus was not necessary for the activity. K562 could be induced to differentiate by preimmunized horse IgG identically to ATG, as long as it contained TMS as a preservative. The same IgG prepared without TMS (0.2–0.4 mg/ml), however, showed no activity. Horse IgG from Cappell (0.2–0.4 mg/ml), monoclonal antibody to HPCA-1 (CD34) containing azide (30 μl of 200 μg/ml), horse serum (1:10 dilution), and human IgG without preservative (0.2–0.4 mg/ml) were also inactive.

Two methods were used to see whether TMS was the active component for differentiation in ATG (Table 2). (a) Diazylated ATG was compared with nondialyzed ATG for effects on K562 cells. Using diazylated ATG at concentrations below 400 μg/ml there was slight activity above baseline (14% differentiation into nucleated RBC). At a higher concentration of 600 μg/ml, only 20% differentiation was observed. Although losing most of its differentiating activity, the diazylated ATG still retained its full growth stimulation activity for normal bone marrow. (b) HPLC separated ATG into one major and two adjacent minor peaks. The differentiation activity of the peak fractions (protein concentration, 0.6 mg/ml) were reduced by 87–95% when compared with ATG (0.6 mg/ml) before HPLC separation. Mercury

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Table 1: Hemoglobin content and cell counts of K562 cells incubated with ATG

<table>
<thead>
<tr>
<th>Condition</th>
<th>Absorbance at 610 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Hb conc.</td>
<td>0.146</td>
</tr>
<tr>
<td>Cell count</td>
<td>2.1 × 10⁴</td>
</tr>
<tr>
<td>ATG (2 mg/ml)</td>
<td>Hb conc.</td>
</tr>
<tr>
<td>Cell count</td>
<td>0.9 × 10⁴</td>
</tr>
<tr>
<td>ATG (4 mg/ml)</td>
<td>Hb conc.</td>
</tr>
<tr>
<td>Cell count</td>
<td>1.0 × 10⁴</td>
</tr>
<tr>
<td>RBC (1 × 10^6 assay)</td>
<td>Hb conc.</td>
</tr>
<tr>
<td>Control</td>
<td>Hb conc.</td>
</tr>
<tr>
<td>Cell count</td>
<td>1.4 × 10⁴</td>
</tr>
<tr>
<td>ATG (2 mg/ml); no epo</td>
<td>Hb conc.</td>
</tr>
<tr>
<td>Cell count</td>
<td>1.0 × 10⁴</td>
</tr>
<tr>
<td>ATG (2 mg/ml); epo (1 unit/ml)</td>
<td>Hb conc.</td>
</tr>
<tr>
<td>Cell count</td>
<td>1.1 × 10⁴</td>
</tr>
</tbody>
</table>

* Absorbance at 610 nm/1 × 10⁴ cells and are the mean of duplicate experiments.

Hb, hemoglobin; epi, erythropoietin.

Number of cells per ml of culture media; all cultures started with 1 × 10⁶ cells/ml.

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levels were used to determine the concentration of TMS in different HPLC fractions. The peak fractions retained only 22% of the mercury (TMS) levels as compared with prechromatographed ATG. This loss of mercury parallels the loss in differentiating activity. The retained mercury (22%) in the peak fractions was still associated with the IgG, presumably by disulfide cross-links.

Upjohn ATG (containing TMS, 0.25 mM) was then compared with samples containing the same concentrations of pure TMS. Pure TMS (0.25–2.5 μM) increased differentiation over ATG (0.05–0.5 mg/ml) containing the similar concentrations of TMS (Table 3). Morphological changes were similar between Upjohn ATG and pure TMS. Differentiation effect was present whether media contained serum or not (Fig. 1). The evidence that TMS is responsible for the leukemic cell differentiation effect of ATG is strong.

The efficacy of TMS as a differentiation agent was compared to known differentiating agents such as 1-β-D-arabinofuranosylcytosine, bleomycin, and actinomycin D and the effect of hemin on K562 cells (9). Although hemin-induced differentiation was morphologically similar to TMS, the percentage of cells differentiating was much lower (maximum of 25% by morphology, day 4, 0.1 mM). In K562 cells, the concentration of chemotherapeutic agents listed above required for morphological differentiation was near cytotoxic levels (1-β-D-arabinofuranosylcytosine, 17% differentiation at 60 μM; actinomycin D, 39% at 30 nM; and bleomycin, 17% at 10 μM). At these levels, cell and nuclear fragmentation, cell loss, and only few normal appearing nucleated RBC were observed. With higher doses, even fewer recognizable cells were noted.

Effect of TMS and ATG on Leukemic Blasts from Patients (Table 4; Fig. 2). ATG was added to the non-adherent mononuclear blast cells from patients with CML in blastic crisis (patients 1 and 2). Pure TMS was tested in blast cells from three patients with CML in blast crisis (patients 1, 2, and 4) and two patients with AML (patients 3 and 5). Leukemic blasts from these five patients expressed HPCA-1 antigen (CD34). Regardless of the cell surface phenotype, karyotype, HLA type, or previous treatment, the morphological changes were similar. A concentration- and time-dependent transformation was seen from undifferentiated blasts to erythroblasts, to nucleated RBC, and then to anucleate RBC. TMS concentration-dependent changes, as calculated from Wright’s cytospin preparations, are illustrated in Fig. 2. When compared to K562 cells, leukemic cells from patients exhibited more karyorrhexis and a greater sensitivity to TMS. For leukemic cells from patients, noticeable differentiation began at 25 μg/ml for ATG or 0.2 μM for TMS, 50% differentiation at 75 μg/ml ATG or 0.4 μM TMS, and close to 100% differentiation at 300 μg/ml of ATG or 1.25 μM TMS. For K562 cells, these values were 0.3, 1.0, and 2.5 μM TMS, respectively (cell density, 4 x 10^4 cells/ml). Hemoglobin synthesis on days 3 and 5 of culture with TMS in three patients is shown in Table 4.

Response of KG-1 (Table 5) and U937 (Figs. 3 and 4) to TMS. When TMS was tested in the KG-1 cell line (10) for differentiation, the morphological changes were similar to both K562 and patients’ cells. Increased hemoglobin synthesis was likewise observed (Table 5). After TMS treatment, a small number of cells stained weakly for esterase [8% using TMS (0.75 μM), 2% in control, n = 3].

Morphological changes seen in U937 cell line (11) were different from those for K562 and KG-1 cells. During the first

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### Table 2 Comparison of the differentiating effects between control ATG and dialyzed ATG, ATG separated over HPLC, horse IgG, and equivalent concentrations of TMS

<table>
<thead>
<tr>
<th>Substance added to the culture</th>
<th>% of differentiation at following ATG concentration</th>
<th>Mercury concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ATG</td>
<td>84% 94%</td>
<td>1.2 μg/mg protein</td>
</tr>
<tr>
<td>Horse IgG</td>
<td>3% 4%</td>
<td>Not present</td>
</tr>
<tr>
<td>Dialyzed ATG</td>
<td>14% 20%</td>
<td>Not tested</td>
</tr>
<tr>
<td>HPLC ATG</td>
<td>5% 13%</td>
<td>0.27 μg/mg protein</td>
</tr>
<tr>
<td>TMS (0.8 μg/ml, 1.2 μg/ml)</td>
<td>94% 96%</td>
<td>TMS (1.0 μg/ml) yielded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.65 μg/ml mercury</td>
</tr>
</tbody>
</table>

* Percentage of differentiated K562 cells; cultures evaluated on the 4th day.

### Table 3 Comparison of the percentage of differentiation induced by ATG and TMS on K562 cells*

<table>
<thead>
<tr>
<th>ATG concentration (mg/ml)</th>
<th>% of differentiation</th>
<th>Equivalent TMS concentration (μM)</th>
<th>% of differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>58%</td>
<td>2.5</td>
<td>91%</td>
</tr>
<tr>
<td>400</td>
<td>57%</td>
<td>2.0</td>
<td>94%</td>
</tr>
<tr>
<td>300</td>
<td>47%</td>
<td>1.5</td>
<td>74%</td>
</tr>
<tr>
<td>200</td>
<td>45%</td>
<td>1.0</td>
<td>62%</td>
</tr>
<tr>
<td>100</td>
<td>18%</td>
<td>0.5</td>
<td>15%</td>
</tr>
<tr>
<td>50</td>
<td>12%</td>
<td>0.25</td>
<td>9%</td>
</tr>
</tbody>
</table>

* Percentage of differentiated K562 cells; cultures evaluated on the 3rd day.
stimulating factor (1 × 10^3 units/ml), interleukin 3 (5–50 ng/ml), tumor necrosis factor (250–500 units/ml), and γ-interferon (200–500 units/ml) alone and in many combinations were tested with or without TMS. γ-Interferon and tumor necrosis factor increased α-naphthyl acetate esterase staining from 11% to 20% and 15% to 35%, respectively. Only GM-CSF and PHA-LCM improved differentiating effects of TMS. No enhancement, however, in naphthol AS-D chloroacetate esterase (granulocyte-specific) staining was noted in these TMS-treated cells.

The sensitivity of the cell lines to TMS was similar (Fig. 2). Fifty % differentiation occurred at TMS concentrations of 0.7 μM for KG-1 cells, 1.0 μM for K562 cells, and 1.3 μM for U937 cells. Human leukemic cells from patients demonstrated an increased sensitivity to TMS by a factor of 0.5 μM. Thus, TMS induces differentiation in a wide spectrum of cells in two lineages of hematopoietic differentiation.

Effects of TMS on Normal Erythroid Colonies (Fig. 5). When TMS (0.2–0.8 μM) was added at initiation of normal bone marrow cultures, erythroid clusters formed on day 5 and matured by day 10. Formation of large colonies was inhibited in cultures where greater than 0.4 μM TMS was added. When TMS (0.2–1.6 μM) was added on day 8 to ongoing erythroid cultures, an enhanced differentiation was observed on day 10 (Table 6) showing a more rapid hemoglobinization and reduction in the nuclear size of normoblasts (Fig. 5).

Reversal of TMS-induced Differentiation by DTT. TMS inhibits sulfhydryl groups, and this is responsible for its germicidal property (12). In order to examine whether the TMS-induced leukemic differentiation is mediated via its sulfhydryl group interaction, DTT was used to see whether the differentiation induction could be reversed. When DTT (1 mM) was added simultaneously with TMS (1.5 μM) the differentiation effects decreased from 74% to 5%. DTT inhibition could be overcome by increasing TMS to higher concentrations (e.g., 70% differentiation with TMS, 7.5 μM; and DTT, 1 mM). When DTT was added 1 h after TMS, differentiation continued unhindered.

Other compounds that inhibit sulfhydryl group-dependent enzymes, such as p-hydroxymercuribenzoate, inhibit nucleoside transport through the cell membrane (13). Similarly, TMS was found to inhibit the uptake of [3H]thymidine by K562, KG-1, and U937 cells. This effect was seen beginning at 0.25 μM, with graded increases of inhibition (Table 7). The inhibitory effect of TMS (1–2 μM) could be partially reversed by the addition of DTT (0.6 mM) at initiation of culture. The reversal of TMS effects on differentiation and nucleoside incorporation by DTT suggests that differentiation is controlled by a sulfhydryl group-dependent process.

**DISCUSSION**

Thimerosal induces cell differentiation in HPCA-1 (CD34)-positive leukemic blasts from 3 patients with chronic myelogenous leukemia in blastic crisis and 2 patients with AML at 50% effective dose concentrations of 0.20 to 0.55 μM. It is also active in three leukemic cell lines, K562, KG-1, and U937, at concentrations of 0.7–1.3 μM. TMS-treated cells reach their terminal stage of differentiation and were incapable of resuming cell division. TMS induction of differentiation is different from agents previously reported in the literature in its very broad spectrum of activity encompassing both fresh leukemic blasts from patients and three human leukemic cell lines. Evidence of differentiation induction is usually apparent within 24 h of
Fig. 3. U937 cells after 4 days of culture. A and B, Wright's-stained cells, x 680. C and D, stained for α-naphthyl acetate esterase. x 400. A and C were cultured without TMS, and B and D were incubated with TMS (1.0 μM).

Fig. 4. α-Naphthyl acetate esterase staining of U937 cells after 4 days of culture with TMS (C), with TMS plus burst promoting factor (BPF, which is PHA-LCM, 1:20 dilution) (D), and with TMS plus GM-CSF, 500 units/well (Δ). ○, ■, △, TMS doses inducing cell loss and cytotoxicity. Each symbol represents the mean of three experiments; bars, SD.

incubation with TMS, and the effect reaches its maximum in 3–5 days. The degree of differentiation is concentration and time dependent. Growth factors such as erythropoietin PHA-LCM, or GM-CSF potentiate the TMS-induced differentiation effect, although not universally. TMS-induced differentiation can be blocked by a sulphydryl-protective agent, dithiothreitol. This observation suggests that the mechanism of differentiation is a sulphydryl group-dependent process.

As has been noted by others (14, 15), mature leukemic cells do not contain similar quantities of specific proteins found in normally differentiating cells. This is illustrated in our case by the modest increases in hemoglobin occurring with marked morphological changes. While TMS induces morphological neutrophil differentiation in HL-60 cells similar to effects of ATG, low percentages of differentiation and difficulties with our neutrophil assay preclude inclusion of these data. It is believed that our previous findings are due to the presence of TMS in the ATG preparation. ATG may be necessary in vivo to carry TMS directly to the leukemic cell population.

TMS has been used as a relatively nontoxic antimicrobial preservative for some topical and parenteral drug preparations. Its toxicity has previously been tested in rodents and in humans (12, 16–18). Its 50% lethal dose in mice is 120 mg/kg. Up to 2 g has been given to humans p.o. without “toxic effects” (12).
EFFECT OF THIMEROSAL IN LEUKEMIA

Since it is well tolerated, TMS has potential usefulness as a differentiation agent clinically. This compound has been reported to have many different activities and recently it has become a useful tool in analyzing enzyme function. The known biological effects of TMS that are purported to be active for differentiation are: (a) inhibition of Na+/K+ ATPase by modifying the intracytoplasmic subunit of the enzyme (19). Ouabain, which blocks this enzyme, is a known inducer of erythroid differentiation; (b) inhibition of acyl-CoA:lysophatide acyltransferase with subsequent increase in arachidonic acid (20). Prostaglandin E₂ is a known erythroid inducer, and a lipoxygenase product has recently been implicated in U937 monocytic differentiation (21). Blockade of this single pathway would explain both erythroid and monocyte differentiation found in this report; (c) inhibition of nucleoside transport through the cell membrane (13). Compounds which inhibit nucleoside transport have been shown to induce or potentiate differentiation. Other effects of TMS which include induction of calcium release (22), inhibition of 2′,3′-cyclic nucleotide 3′-phosphodiesterase (23), and production of a vasorelaxation factor (24) may also be related to differentiation. Therefore, an in depth study of actions of this interesting agent on cell differentiation may help define mechanisms of neoplastic cell differentiation.

REFERENCES

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