Alterations in enzyme expression on 12-*O*-tetradecanoylphorbol-13-acetate-induced differentiation of chronic lymphocytic leukemia cells

B.I. Sahai Srivastava and Tin Han

Grace Cancer Drug Center and The Department of Medical Oncology, Roswell Park Memorial Institute, New York State Department of Health, 666 Elm Street, Buffalo, NY 14263, USA

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Several enzyme activities were examined to establish a relationship between their expression and terminal differentiation of B-chronic lymphocytic leukemia (CLL) cells to plasma cells by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Although adenosine deaminase activity did not change significantly, thymidine phosphorylase and purine nucleoside phosphorylase increased 2–3-fold on TPA-induced differentiation of CLL cells. In addition, cytochemical reactions for non-specific esterase and acid phosphatase changed from very weak to intense on differentiation of CLL cells to plasma cells. The above markers, particularly cytochemical, could be useful for the classification of B-cell malignancies and for studying B-cell differentiation.

1. INTRODUCTION

Chronic lymphocytic leukemia (CLL[3]) is currently considered to represent a monoclonal proliferation of rather immature B-lymphocytes frozen at a given stage of maturation. Generally lower PNP and ADA activities have been reported in CLL lymphocytes although some patients show as high activity as normal B-lymphocytes [1–6]. Similarly, analyses of primary cells and cell lines representing various stages of B-cell maturation have indicated that TP, AP and NSE expression may increase during differentiation of B-cells to mature plasma cells [1,7–10]. The aim of the present work was to establish a direct relationship between ADA, PNP, TP, AP and NSE expression and differentiation of B-CLL cells to plasma cells in vitro which can be induced by TPA [11].

Abbreviations: CLL, chronic lymphocytic leukemia; B, bursa-equivalent derived cell; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; AP, acid phosphatase; NSE, non-specific esterase; PBS, phosphate-buffered saline, pH 7.2; ADA, adenosine deaminase; PNP, purine nucleoside phosphorylase; TP, thymidine phosphorylase; DP, DNA polymerase; IgM, immunoglobulin M

2. MATERIALS AND METHODS

2.1. *Induction of differentiation*

B-cell CLL patients with about 10^5 white cells/mm^3 and 93–95% lymphocytes (almost all positive for IgM) were used. The lymphocytes from the leukocyte layer of peripheral blood were separated by Ficoll-Hypaque centrifugation and washed with RPMI-1640 medium. For differentiation experiments, CLL lymphocytes were suspended at 10^6 cells/ml in RPMI-1640 medium containing 5% heat inactivated fetal calf serum, 100 units/ml penicillin and 100 μg/ml of streptomycin and incubated with or without 5×10^{-9} M TPA for 72–80 h in 75 cm^2 Falcon flasks at 37°C in 5% CO_2 and air in a humidified incubator. Cell viability determined by trypan blue exclusion at the end of the experiment was >90% in both control and TPA-treated cells. The cells in suspension were pelleted by centrifugation, washed several times with PBS and used for enzyme assays and for smear preparations.

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2.2. *Immunofluorescence staining*

Since CLL patients used were known to have IgM positive lymphocytes, the changes in the expression of surface and cytoplasmic immunoglobulin during differentiation were determined using FITC conjugated goat antihuman IgM as in [12]. Slides were examined using a Leitz Ortholux II microscope equipped with epifluorescence.

2.3. *Preparation of cell extracts and enzyme assays*

For ADA, TP, PNP and DNA polymerase determinations, the cells were homogenized with buffer A (25 mM Tris–sulfate, pH 8.0; 1 mM $MgSO_4$; 1 mM dithiothreitol and 0.1 mM EDTA) as in [9] and the homogenate was centrifuged for 30 min at 30000 × *g*. The supernatant was dialysed (4 h at 2°C against buffer A + 10% glycerol), clarified by centrifugation and used for the determination of ADA (with 0.1 mM adenosine [13]), TP (with 0.1 mM thymidine and arsenate–succinate buffer, pH 5.9 [8]) and PNP (by the coupled xanthine oxidase method [13]) activity by spectrophotometric procedures and protein by Bio-Rad reagent. DP activity in the dialysed extract was determined as in [9] using activated DNA as the template. Enzyme activity was expressed as units/mg protein where 1 unit equals 1 nmol of adenosine used/h for ADA, 1 nmol of thymine formed/h for TP, 1 nmol of labeled dGTP used/h for DP and an increase in A_{293} (1 ml) of 1.0/h for PNP.

2.4. *Cytochemistry*

Smears were stained with Wright-Giemsa for morphological examination. For AP and NSE staining the air-dried smears were fixed in formalin vapour for 4 min [14], rinsed with distilled water and incubated with AP, or NSE incubation mixture, respectively, for 1 h at 37°C. For preparing AP mixture, naphthol AS-BI phosphate (20 mg) was dissolved in 100 ml of 0.2 M sodium acetate buffer (pH 5.0) and 50 mg fast blue BB salt (diazotized-4′-amino-2′,5′-diethoxylbenzanilide zinc chloride salt) was added and the contents shaken and filtered. For NSE incubation mixture, 20 mg naphthol AS-D acetate was solubilized with 0.5 ml of acetone and 100 ml of 0.1 M potassium phosphate buffer (pH 6.8) was added. Subsequently, fast blue BB salt (50 mg) was added and the contents were shaken and filtered. The slides were scored for type and relative intensity of staining by examination under a microscope.

3. RESULTS AND DISCUSSION

The CLL cells after 3 days in culture showed only weak reaction for surface immunoglobulin (IgM) which became negative on culture in the presence of TPA. On the other hand, the cells from all 4 patients which were negative for cytoplasmic IgM at the start of 3 days of culture became strongly positive for cytoplasmic IgM after treatment with TPA (fig.1). In addition to immunologic differentiation, nearly 90% of cells from each patient showed morphological differen-

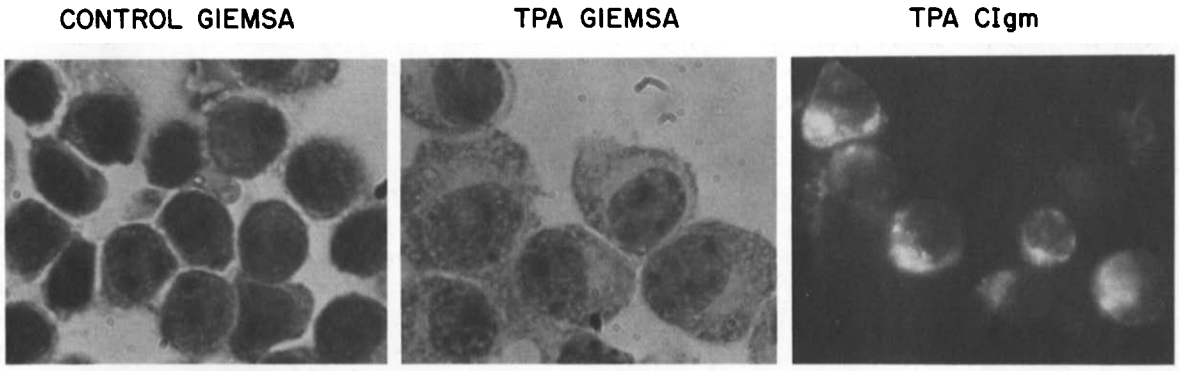

Fig.1. Typical Wright-Giemsa (control and TPA-treated) and cytoplasmic IgM (TPA-treated) staining of untreated control and 5×10^{-9} M TPA-treated cells after 72 h incubation at 37°C. Control cells (not shown) gave negative staining for cytoplasmic IgM.

Table 1

Enzyme activities in extracts (dialysed 30000 × *g* supernatant) of untreated and 72-hr TPA (5×10^{-9} M)-treated cells from CLL patients

Patient	Thymidine phosphorylase		Purine nucleoside phosphorylase		Adenosine deaminase		DNA polymerase	
	Control	TPA	Control	TPA	Control	TPA	Control	TPA
1	515	1030	21.7	59.7	1171	1020	2.3	10.4
2	883	1840	49.5	60.3	1083	1251	1.0	3.1
3	714	2262	31.1	60.5	726	960	–	–
4	1009	2724	–	–			–	–

Enzyme activity was expressed as units/mg protein where 1 unit equals 1 nmol of adenosine used/h for ADA, 1 nmol of thymine formed/h for TP, 1 nmol of labeled dGTP used/h for DP and an increase in A_{293} (1 ml) of 1.0/h for PNP

tiation in the presence of TPA to plasma cells with large basophilic and frequently vacuolized cytoplasm and an eccentrically located nucleus (fig.1). Control cultures without TPA showed only few (~10%) plasmacytoid cells. Similar changes in B-CLL cells on TPA treatment have been reported in [11] and [15]. In addition, TPA-treated CLL cells at 3 days had higher DP (primarily α) activity compared to the control cells (table 1). Although there were variations in ADA, TP and PNP activities between different CLL patients, the ADA activity did not change significantly, whereas TP and PNP activities increased 2–3-fold on TPA-induced differentiation of CLL cells (table 1). No significant differences have been found in ADA between B-CLL cells and normal B-lymphocytes in most of the studies [1–6] or between pre-B, B- and plasma cell lines [9]. On the other hand, low PNP activity has been generally found in B-CLL cells compared to normal B-lymphocytes [1–6]. Similarly, low TP activity is found in pre-B acute lymphoblastic leukemia cells [9] and B-CLL cells [8] compared to normal B-cells. The results presented here indicate that PNP and TP increase during maturation of B-CLL cells. Variations in the activities of these enzymes in CLL patients from low to overlap with normal B-cells could result from differences in degree of maturation of B-CLL cells in different patients. Although TP and PNP appear to be useful markers for the maturation of B-CLL cells the variation in their activity between patients limits their clinical utility.

On the other hand, the cytochemical reactions for NSE and AP were consistently weak in B-CLL cells from all patients whereas on TPA-induced differentiation to plasma cells they gave strong reactions for NSE and AP, particularly for the latter enzyme (table 2 and fig.2). Normal B-cells also gave weak reaction for NSE and AP [7,10] whereas strong reactions for plasma cells have been observed [7,10]. These results indicate that NSE and AP, which increase on maturation of B-CLL cells to plasma cells, could be useful markers involving inexpensive technology for the classification of B-cell malignancies and for studying the B-cell maturation.

Table 2

Cytochemical reaction of cell smears for acid phosphatase and non-specific esterase (pH 6.8) from untreated and 72-h TPA (5×10^{-9} M)-treated cells from CLL patients

Patient	Acid phosphatase		Non-specific esterase	
	Control	TPA	Control	TPA
1	1^+	$2^+ \sim 3^+$	±	1^+
2	1^+	3^+	±	$1^+ \sim 2^+$
3	1^+	3^+	±	$1^+ \sim 2^+$
4	1^+	$2^+ \sim 3^+$	±	1^+

The cells were scored for relative intensity of staining. Almost all of the cells were stained

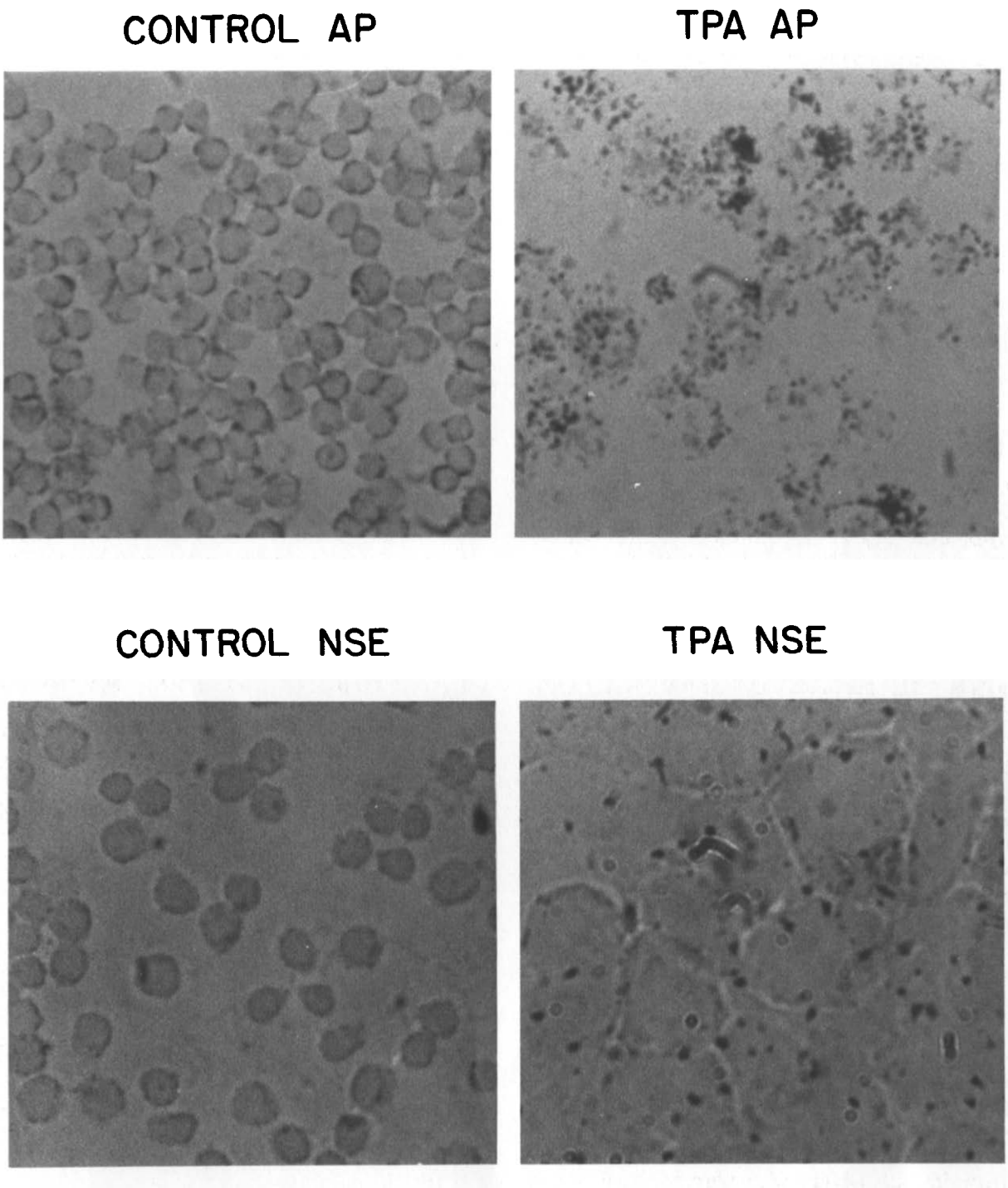

Fig.2. Typical acid phosphatase (AP) and non-specific esterase (NSE) activity of untreated control and 5×10^{-9} M TPA-treated CLL cells after 72 h incubation at 37°C.

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