Phorbol Esters Induce Changes in Adenosine Deaminase, Purine Nucleoside Phosphorylase, and Terminal Deoxynucleotidyl Transferase Messenger RNA Levels in Human Leukemic Cell Lines

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ABSTRACT

We have studied the expression of mRNA encoding adenosine deaminase (ADA; EC 3.5.4.4), purine nucleoside phosphorylase (PNP; EC 2.4.2.1), and terminal deoxynucleotidyl transferase (TdT; EC 2.7.7.31) in different leukemic cell lines of B- and T-cell lineage. Incubation of leukemic cells in the presence of the phorbol esters, 12-O-tetradecanoylphorbol-13-acetate or phorbol 12,13-dibutyrate, resulted in reduction of ADA and TdT mRNA levels, while PNP mRNA levels increased under the same treatment. The effect of TPA on the activity of these enzymes correlated well with its effects on their mRNA levels. TPA caused a 40% decrease in ADA and a 60% decrease in TdT enzyme activity, after 6 h of treatment. In contrast, PNP activity increased up to 200% after 12 h of incubation with the phorbol ester.

The changes induced by the phorbol esters in the levels of mRNA of ADA, PNP, and TdT, and their enzyme activities in human leukemic cell lines mimic the changes in the activities of these enzymes in developing T-lymphocytes during differentiation in vitro, suggesting a role for protein kinase C in the regulation of ADA, PNP, and TdT gene expression during lymphoid cell differentiation.

INTRODUCTION

Immunodeficiency is often associated with inborn errors of purine metabolism. Deficiency of either one of the two enzymes which catalyze sequential reactions in purine catabolism, adenosine deaminase (EC 3.5.4.4) and purine nucleoside phosphorylase (EC 2.4.2.1), leads to severe combined immunodeficiency (1, 2) and T-cell immunodeficiency (3, 4), respectively. ADA catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxynosine; and PNP catalyzes the conversion of inosine, guanosine, and their respective deoxy derivatives to hypoxanthine (5). During the development of lymphocytes the activity of both enzymes change markedly. During T-lymphocyte differentiation ADA activity decreases and PNP activity increases (6–8). The enzyme activities in ALL patients resemble those of immature thymocytes (9).

Terminal deoxynucleotidyl transferase (EC 2.7.7.31) catalyzes the polymerization of DNA in the absence of a template. Its activity is restricted to populations of immature lymphocytes and malignant lymphocytes of immature phenotype (10, 11). The function of TdT is unclear; however, it has been proposed that this enzyme plays an important role in the generation of diversity of immunoglobulin and T-cell antigen receptor molecules during gene rearrangements (12, 13). These observations led to the postulation that TdT, ADA, and PNP play a role in lymphocyte differentiation (14).

We previously demonstrated that the ratio of ADA and PNP activities plays a role in the regulation of intracellular deoxy-nucleoside triphosphate pools in human thymocytes, which are the substrates for TdT activity (15). It is therefore expected that the expression of TdT, ADA, and PNP mRNAs may be coordinately regulated during T-cell antigen receptor gene rearrangement. Indeed, we have recently shown that the expression of TdT, ADA, and PNP mRNAs is coordinately regulated by phorbol esters in fresh isolated human thymocytes (16). The phorbol ester TPA is an active modulator of mammalian cell differentiation (17–19), a phenomenon that may be of relevance in developmentally lymphoid leukemic cells, arrested in early stages of differentiation (20–23). Thus, the activities of these enzymes can serve as markers of lymphoid cell differentiation. In order to investigate whether this pattern of regulation exists in undifferentiated malignant cells, we have studied the effect of phorbol esters on TdT, ADA, and PNP gene expression in human acute lymphoblastic leukemic cell lines of T- and pre-B-cell lineage.

MATERIALS AND METHODS

Cell Lines. A number of cell lines were used in this study: they include T-cell acute lymphoblastic leukemic cells; JURKAT, MOLT-3, CEM, and HSB-2, which were obtained from Dr. Hans-Michael Dosch (Division of Immunology, The Hospital for Sick Children, Toronto, Ontario, Canada), and PEER, DND-41, and SKW-3 from Dr. T. W. Mak (Ontario Cancer Institute, University of Toronto). Pre-B ALL cell lines, HYON, HOON, and NALM-6, were generously supplied by Dr. Michelle Letarte (Division of Immunology, The Hospital for Sick Children, Toronto). The cells were maintained in a logarithmic growth phase in RPMI 1640 medium supplemented with 10% fetal bovine serum, at 37°C in a humidified incubator with 5% CO2 atmosphere. Mycoplasma contamination was excluded by periodic microbiological tests.

Materials. [α-32P]dCTP (3000 Ci/mmol) was purchased from Du Pont New England Nuclear (Lachine, Quebec, Canada). Phorbol esters: TPA, 4α-phorbol-12,13-didecanoate, 4β-phorbol-12,13-didecanoate, and phorbol-12,13-dibutyrate, sodium chloride, bovine serum albumin fraction V, sodium citrate, and salmon sperm DNA were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions of phorbol esters (0.1 mM) were prepared in dimethyl sulfoxide (Fisher). Nylon transfer membranes (1.20 μm) were purchased from PALL BIODINE Ultrafine Filtration Co. (Glen Cove, NY). The TdT cDNA (24) was supplied by Dr. Bollum (Uniformed Services University, Bethesda, MD), ADA cDNA (25) was provided by Dr. J. J. Hutton (Children’s Hospital Medical Center, Cincinnati, OH), and PNP cDNA (26) was obtained from Dr. D. W. Martin, Jr. (Genentech, Inc., San Francisco, CA).

RNA Isolation and Northern Blot Analysis. The total RNA was extracted in guanidinium isothiocyanate according to the method of Chomczynsky and Sacchi (27). RNA concentrations were measured with a double beam Beckman DU-40 spectrophotometer. Total RNA (10 μg) was electrophoresed under denaturing conditions on a 0.9% agarose and 0.66 M formaldehyde gel according to the method of Chomczynsky and Sacchi (27).
Lehrach et al. (28). The gel was stained with 0.5 µg/ml of ethidium bromide to check the integrity of RNA. RNA was transferred onto nylon PLUS membranes as described by Thomas (29). Nylon membranes were allowed to dry at room temperature and were baked for 1 h at 80°C in a vacuum oven. Blots were prehybridized for 12 h at 42°C in 50% formamide, 5x SSC (1x SSC is 0.15 M NaCl-0.015 M sodium citrate, pH 7.0); 5x Denhart’s solution (1x Denhart is 200 µM Ficoll, 200 µg/ml polyvinyl-pyrrolidone, and 200 µg/ml bovine serum albumin fraction V); 25 mM sodium phosphate, pH 6.5, 1.0% sodium dodecyl sulfate, and 200 µg/ml denatured salmon sperm DNA. Hybridizations were carried out by using the same prehybridization solution for 16 h by adding [α-32P]dCTP-labeled probes with the use of hexamer deoxyoligonucleotides as random primers and the Klenow fragment of DNA polymerase I (Pharcma Fine Chemicals, Upplasa, Sweden). The probes were EcoRI cDNA fragment encoding TdT (24), the ADA cDNA insert was excised with EcoRI and Ddel (25), and the PNP cDNA insert was excised with PstI (26). Probes were isolated from low melting agarose gels. After hybridization blots were stringently washed for 2 h with three changes of 0.1x SSC, 25 mM sodium phosphate, and 0.2% sodium dodecyl sulfate at 68°C, and were then autoradiographed at −70°C by using a screen intensifier. Controls for equal amounts of mRNA were performed by using the cDNA probe for the constitutively expressed gene β-actin, which is a HinIII fragment of a plasmid obtained from the American Type Culture Collection (Rockville, MD).

Slot Blot and Densitometry Scanning of mRNA Levels. After 4 h of incubation with 10 nM TPA, the cell lines were harvested by centrifugation and total RNA was extracted as described above. RNA aliquots (10 µg total) were spotted onto nitrocellulose, using a Schleicher & Schuell slot blotter apparatus and following manufacturers instructions. Nitrocellulose was allowed to dry at room temperature and then baked for 2 h at 80°C in a vacuum oven. Slot blots were prehybridized, hybridized, washed, and autoradiographed as described for Northern blots. Autoradiograms were scanned in a Beckman DU-40 spectrophotometer. The value of the cell sample incubated with no additions was normalized to 1.0 unit of relative absorbance, and other experimental points were calculated as the mRNA ratio (TPA/control). The results are representative of three or four experiments for each cell line.

Quantitation of Enzyme Activities and Protein. Specific activities were determined in duplicate by using radiochemical methods as previously described for ADA (30), for PNP (31), and for TdT (32) in HYON cell line, and the results are representative of one or two experiments. Total protein concentration was measured by the method of Bradford (33).

RESULTS

The effects of phorbol esters on TdT, ADA, and PNP expression in human leukemic cells of T- and B-cell lineage were analyzed by Northern blots. We chose to study HYON as a representative of B-cell lineage and PEER as a representative of T-cell lineage (Figs. 1 and 2). Incubation of these cell lines with the phorbol esters TPA or phorbol-12,13-dibutyrate decreased the mRNA levels of ADA and TdT. In contrast, the mRNA levels of PNP were increased under the same conditions, in both B- and T-lymphoid cell lines. The quantitation of ADA, PNP, and TdT mRNA levels in a number of leukemic lines of B- and T-cell lineages treated with phorbol esters is summarized in Table 1. Incubation of the cell lines for 4 h in the presence or absence of the phorbol ester TPA (1.0 × 10−8 M), caused a reduction of ADA and TdT mRNA levels, while PNP mRNA levels increased in all leukemic cell lines tested.

Phorbol esters have a structure similar to diacylglycerol, the physiological activator of PKC, and activate PKC directly in vivo and in vitro. There are several lines of evidence suggesting that PKC is the receptor for phorbol esters (17). Their effect depends on their chemical configuration, i.e., the β configuration is known to have high potency on PKC activation, whereas the α configuration is totally ineffective (17). In order to determine whether the effect of phorbol esters on human leukemic cells is consistent with the activation of PKC, the effect of inactive or active forms of phorbol esters on TdT, ADA, and PNP mRNA levels was tested. As shown in Figs. 1 and 2 the phorbol ester with β configuration caused a decrease in the mRNA levels of TdT and ADA, and an increase in the mRNA levels of PNP, while that with α configuration did not modify the mRNA levels of these enzymes. Thus, there was good correlation between the structure of phorbol esters required for PKC activation and their effects produced on TdT, ADA, and PNP mRNA levels.

To investigate whether the changes produced by phorbol esters on specific mRNA levels are followed by comparable effects on enzyme activities, we compared the enzymatic activities of ADA, TdT, and PNP in cell-free extracts from the control and phorbol ester-treated cell cultures. Cultures containing 1 × 106 cells/ml of the pre-B-cell line HYON were incubated in the presence or absence of 10 nM TPA and samples were taken at 6, 12, 24, 50, and 80 h; the enzyme activity was determined by using radiochemical analysis, as described in “Materials and Methods.” The data in Fig. 3 show that after 6 h of treatment with TPA, there was a rapid fall in the enzymatic activity of both TdT and ADA, reaching 60 and 20% of control values, respectively. PNP enzyme activity, on the other hand, increased up to 200% after 6 h of incubation in the presence of the phorbol ester. These results correlate well with the effects of phorbol esters on TdT, ADA, and PNP mRNA levels (Figs. 1 and 2).
REGULATION OF PURINE ENZYME mRNA LEVELS BY PHORBOL ESTERS

Fig. 2. Northern blot analysis of TdT, ADA, and PNP mRNA in pre-B-cells treated with phorbol esters. Human leukemic pre-B-cells (HYON) were incubated in the presence or absence of phorbol esters as is indicated in Fig. 1 legend.

Table 1 Effects of TPA on ADA, TdT, and PNP mRNA levels in human leukemic cells

<table>
<thead>
<tr>
<th>mRNA ratio TPA/control</th>
<th>ADA</th>
<th>PNP</th>
<th>TdT</th>
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<tr>
<td>T-cell lines</td>
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<td></td>
<td></td>
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<td>JURKAT</td>
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<td>0.50</td>
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<td>MOLT-3</td>
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<tr>
<td>PEER</td>
<td>0.42</td>
<td>10.40</td>
<td>NE</td>
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<td>0.67</td>
</tr>
<tr>
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</tr>
<tr>
<td>SKW-3</td>
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<td>NE</td>
</tr>
<tr>
<td>Pre-B-cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>0.50</td>
</tr>
<tr>
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<tr>
<td>NALM-6</td>
<td>0.44</td>
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</table>

* NE, nonexpressed; ND, not done.

DISCUSSION

During the course of lymphocyte differentiation, the enzyme activities of ADA and TdT decrease while the activity of PNP increases (6–9). Recently, we have shown that the expression of ADA, TdT, and PNP mRNA are coordinately regulated in fresh isolated human thymocytes by phorbol esters. Phorbol esters are active modulators of mammalian cell differentiation (17–20), a phenomenon that may be relevant in developmentally arrested leukemias arrested in early stages of differentiation (20–23). We have shown in the present communication that incubation of human leukemic cells of T- and B-cell lineage in the presence of various phorbol esters caused a decrease in TdT and ADA mRNA levels, while PNP mRNA levels increased (Table 1). These change in mRNA levels correlated well with changes in the enzymatic activities (Fig. 3). Moreover, the changes induced by phorbol esters on the expression of these enzymes are consistent with variations of the TdT, ADA, and PNP enzyme activities during lymphocyte differentiation in vivo (6–9). Our results on the regulation of ADA, TdT, and PNP expression by phorbol esters in leukemic cells, reported in the present work, together with our previous studies in human thymocytes (16), strongly suggest a role for PKC activation in the coordinate regulation of TdT, ADA, and PNP mRNA expression during lymphocyte differentiation.

Fig. 3. ADA, PNP, and TdT activities in TPA-treated cells. Cells (1 × 10⁶/ ml) of the pre-B-cell line HYON were incubated with or without 10 nM TPA. At the indicated times, samples were taken and the enzyme activities were determined as described in “Materials and Methods.” The enzyme activities are represented in percentage of change with respect to control. The basal levels of enzyme activities for the control cells were: 53.5 nmol/min/mg for ADA, 25.5 units/min/mg for TdT, and 20.0 nmol/min/mg for PNP.

The vast majority of ALL cells express TdT enzymatic activity, exclusively present in immature lymphocytes, and leukemic cells with immature phenotype only (10, 11). TdT is thought to participate in the synthesis of N-regions during gene rearrangement of immunoglobulins and T-cell antigen receptor genes (12, 13). TdT activity is present in nondividing thymocytes, and is active in the absence of de novo deoxynucleotide synthesis operative during the S phase of the cycle cell (11, 12). We have previously shown that under these conditions, the ratio of the purine deoxynucleotide degrading enzymes ADA and PNP largely control the levels of dATP and dGTP pools needed for TdT activity (15), and our previous observations of coordinate regulation of TdT, ADA, and PNP mRNA levels in freshly isolated thymocytes is consistent with this hypothesis (16). Here we have extended these observations to leukemic cells of both B- and T-cell lineages. The results reported here further support the hypothesis that the expression of TdT, ADA, and PNP are coordinately regulated during both B- and T-cell differentiation, indicating a common functional role of their enzyme activities during lymphocyte differentiation.

ACKNOWLEDGMENTS

The authors are thankful to Drs. J. J. Hutton, D. W. Martin, Jr., and F. J. Bollum for providing ADA, PNP, and TdT cDNA probes, respectively, and to Dr. Paul J. Doherty for reviewing the manuscript.

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