Human Erythroleukemia Cell Line (HEL) Undergoes a Drastic Macrophage-Like Shift With TPA

By Thalia Papayannopoulou, Betty Nakamoto, Takashi Yokochi, Alan Chait, and Reiji Kannagi

We investigated the effect of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) on the human erythroleukemia cell line, HEL, and found that TPA addition (10^{-6} - 10^{-4} M) to HEL cell cultures induces morphological, functional, and biochemical changes in HEL cells that are characteristic for macrophage-like cells. Apart from the drastic changes in morphology, the cells greatly enhance their phagocytic ability and acquire receptors for binding and degradation of chemically modified lipoproteins. At the biochemical level, changes characteristic of nonproliferating monocyte/macrophage-like cells. Similar changes have also been described when leukemic cells from patients with chronic myelogenous leukemia (CML), acute myelomonocytic leukemia (AMML), and acute monocytic leukemia (AMoL) were treated with phorbol esters, but not when cells from acute lymphocytic leukemia (ALL) or chronic lymphocytic leukemia (CLL) patients were similarly treated, although these cells were able to bind TPA equally well. In addition, murine erythroleukaemia cells have shown either inhibition or induction of erythroid differentiative functions after treatment with phorbol diesters, but have not reverted to a macrophage-like phenotype. These data have led to the suggestion that the phenotypic response following 12-O-tetradecanoyl-phorbol-13-acetate (TPA) treatment is primarily dictated by the target cells and probably mediated through postreceptor binding events.

PREVIOUS STUDIES have indicated that the tumor-promoting phorbol diesters elicit pleiotropic effects on cultured cells in vitro. These effects include stimulation of proliferation of various cell types and either induction of terminal differentiation with growth cessation or inhibition of terminal differentiation in a variety of cells from several species. In addition, phorbol diesters have been shown to interact synergistically with several classes of polypeptide growth factors in vitro, such as epidermal, fibroblast, and platelet-derived growth factors, as well as insulin and colony-stimulating factor (CSF), to bring about an enhancement in cellular proliferation. There is growing evidence in the literature that all these diverse effects elicited by phorbol diesters are preceded by their binding to a specific cellular receptor, or that they are mediated through interaction or interference with other types of cellular receptors.

In this context, it is of interest that, apart from the effects of tumor promoters on transformed cells, it has been recently found that normal human cells respond to phorbol diester binding by modulation of their specific cellular functions.

As far as the human hemopoietic cell lines are concerned, it has been shown that those of myelomonocytic origin, such as HL-60, KG-1, ML-3 or U937, respond to phorbol diester treatment by phenotypic changes characteristic of nonproliferating monocyte/macrophage-like cells. Similar changes have also been described when leukemic cells from patients with chronic myelogenous leukemia (CML), acute myelomonocytic leukemia (AMML), and acute monocytic leukemia (AMoL) were treated with phorbol esters, but not when cells from acute lymphocytic leukemia (ALL) or chronic lymphocytic leukemia (CLL) patients were similarly treated, although these cells were able to bind TPA equally well. In addition, murine erythroleukaemia cells have shown either inhibition or induction of erythroid differentiative functions after treatment with phorbol diesters, but have not reverted to a macrophage-like phenotype. These data have led to the suggestion that the phenotypic response following 12-O-tetradecanoyl-phorbol-13-acetate (TPA) treatment is primarily dictated by the target cells and probably mediated through postreceptor binding events.

We investigated the response of a human erythroleukaemia line (HEL cells) to a phorbol diester (TPA). This agent induces a dramatic macrophage-like shift in these cells, comparable to or more pronounced than the one previously described for myelomonocytic cells or cell lines. The data are interpreted to suggest the unmasking by TPA of an inherent monocyte-like phenotype in the HEL cells.

MATERIALS AND METHODS

Cells

HEL cells, K562 cells, and HL-60 cells are maintained in our laboratory in medium RPMI 1640 supplemented with glutamine, antibiotics, and 10% fetal calf serum. TPA (Sigma Chemical Co., St. Louis, MO) was dissolved in dimethyl sulfoxide (DMSO), and it was added to suspension cultures at final concentrations from 10^{-6} to 10^{-12} M. Control cultures contained equivalent amounts of DMSO (0.01%). TPA-treated cells were observed daily for a period of 10 days. Both tissue culture treated and nontreated surfaces were used for growth (35 mm TC dishes 3001 and non-TC dishes 1008, Falcon, Oxnard, CA). Enumeration of nonadherent cells was done daily and at specified times for adherent cells, as outlined in results. Fixed preparations from adherent and nonadherent cell populations were

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Scanning Electron Microscopy

Unreated and TPA-treated cells (nonadherent and adherent) were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer for 30 min at room temperature, followed by 1% OsO₄ in the same buffer for 30 min. Before fixation, nonadherent cells were deposited on coverslips, through centrifugation in a Shandon Cytospin. Preparations were stained with 2% uranyl acetate, dehydrated in increasing concentrations of ethanol, embedded in Medcast, and viewed in JEOL SEM 35C scanning electron microscope.

Phagocytic Activity

The phagocytic activity of TPA-treated cells and controls was tested by incubating the cells (12–24 hr) in the presence of 2μ latex beads or in the presence of sensitized (IgG-coated) ox red cells (EA cells) or sheep red cells coated with IgG and complement (EAC cells), according to previously described methodology.

Binding and Degradation of Native and Acetylated Low-Density Lipoprotein (LDL) in TPA-Treated Cells

The receptor activity of native and acetylated low-density lipoprotein (LDL) was evaluated in TPA-treated cells and controls by binding and degradation of 125I-labeled acetyl and native LDL. Native LDL or LDL that has been acetylated was iodinated by the iodine monochloride method, as modified for lipoproteins. The binding of 125I-LDL or 125I-acetyl-LDL was determined by incubation of cells with the respective iodinated lipoprotein at 4°C for 2 hr. At this temperature, internalization of the lipoprotein does not occur. At the end of the incubation, the cells were washed extensively and then dissolved in 1.0 M NaOH. An aliquot was counted for 125I radioactivity and another aliquot was used for the determination of cell protein content by the Lowry technique.

Specific binding was calculated by subtracting binding measured in the presence of a 25-fold excess unlabeled lipoprotein from total 125I-LDL or acetyl-LDL binding.

For the measurement of degradation of 125I native or acetylated LDL, the lipoprotein was added to cells in serumless medium at a concentration of 5 μg/ml for either 4 or 24 hr at 37°C. At the end of the incubation, an aliquot of medium was removed for determination of trichloroacetic acid (TCA)-soluble degradation products, which were not due to free iodide, by the method of Bierman et al. The determination of LDL degradation gives an integrated measure of LDL binding. Non-cell-associated LDL degradation was measured under identical conditions in cellfree dishes and was subtracted from total LDL or acetyl-LDL degradation to give a measure of lipoprotein degradation by cells. Values for high affinity LDL degradation were obtained by subtracting values for 125I-LDL or acetyl-LDL degradation in the presence of 25-fold excess unlabeled lipoprotein from total degradation.

Surface Antigens

Expression of surface antigens was studied through the use of monoclonal antibodies in indirect immunofluorescence assays. The list of monoclonal antibodies used, with their established or presumed specificity and their laboratory of origin, is provided in Table 2 (see below). For indirect immunofluorescence, approximately 2 × 10⁶ cells were incubated for 30 min at room temperature (or at 4°C) with predetermined optimal dilutions of the different antibodies. After 2 washes, the cells were stained with an affinity-purified F(ab'), preparation of anti-mouse antibodies or with anti-human IgM labeled with FITC (Tago, Inc., Burlingame, CA). After labeling, the cells were washed twice and held on ice until they were viewed under the fluorescent microscope or they were used for flowfluorometric analysis in a fluorescence-activated cell sorter (Cytotruorograf, Model 50H, Ortho Diagnostic Systems, Westwood, MA). For detection of transferrin receptors, rabbit anti-human transferrin was purchased from DAKO-immunoglobulins, Denmark, and anti-rabbit IgG from Tago, Inc. Ulex europaeus-I fluorescein lectin was purchased from E.Y. Labs, Inc., San Mateo, CA.

Cell Surface Glycoprotein Profile

The expression of major surface glycoproteins was studied by the galactoperoxidase titrated sodium borohydride surface labeling technique, as previously described.

Globin Synthesis

Presence of globin chains in single cells was evaluated by immunofluorescence using anti-γ chain-specific monoclonal antibodies. Globin synthesis before and after induction by hemin or other inducers was studied by labeling the cells with 3H-leucine in leucine-free media for 12–24 hr. Radiolabeled cell lysates were subjected to isoelectric focusing in NP40 urea gels, followed by fluorography as previously described.

RESULTS

Induction of Adherence

Unreated HEL cells grow in suspension cultures as free single cells, but a few adherent cells are also present. Two hours following TPA addition, a significant difference in the ability of HEL cells to adhere to tissue culture surfaces is apparent; 24 hr after TPA addition, the majority of the cells have adhered firmly to the plastic surface and display a considerable cytoplasmic spread (Fig. 1, A–C). Induction of adherence is TPA concentration dependent (10⁻⁶–10⁻⁸ M TPA); at TPA concentrations of 10⁻⁹ M and less, HEL cells behave as control cultures. Adherence is less pronounced in non-tissue-culture-treated surfaces (Fig. 2, A and B), as usually is the case with other adherent cells in culture, i.e., fibroblasts or monocytes. Once maximum adherence is induced (about 24 hr after TPA addition), it is maintained for several days, even in the absence of TPA. Presence of serum in the culture medium is not necessary for induction of adherence. Comparison of adherence induction between HEL cells and concurrently studied K562 and HL-60 cells is provided in Table 1.

Cell Viability and Cell Proliferation

Adherent TPA-treated HEL cells remain viable and attached as long as 2 wk in culture, at which time vacuolization and cell engorgement become more apparent. However, cell detachment was not observed.
Fig. 1. HEL cells in suspension cultures, before and after TPA addition. (A) Control cells grow freely in suspension and appear as round, smooth cells. (B) Two hours following TPA (10^-6 M) addition, the cells show numerous cytoplasmic pseudopodia as they attempt to adhere. (C) Twenty-four hours following TPA addition, most of the cells adhere firmly and show a considerable cytoplasmic spread (magnification x 190).

Table 1. TPA Induced Adherence in HEL Compared to K562 and HL-60 Cells

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>No. of Adherent Cells/10 cu mm Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 2 hr</td>
</tr>
<tr>
<td>HL-60</td>
<td>29</td>
</tr>
<tr>
<td>K562</td>
<td>10</td>
</tr>
<tr>
<td>HEL</td>
<td>10</td>
</tr>
</tbody>
</table>

*Counting of adherent cells was done after thorough removal of nonadherent cells, fixation, and staining of remaining adherent cells. Same initial inoculum was used for all three cell lines.

The ability of TPA-treated cells to proliferate was evaluated by cell counts of nonadherent free cells. The data (Fig. 2, A and B) indicate that virtually no cell proliferation occurs at concentrations of TPA from 10^-6 to 10^-8 M, although viability in these cells is largely unimpaired (over 80% viable cells). In a separate experiment, both adherent and nonadherent cells were counted on two occasions (1 day and 5 days) after TPA addition (data not shown). The adherent cells were counted after cooling (4°C) and detachment from the plastic surface; in these cells, as in the nonadherent cells, no evidence of proliferation was found.
Fig. 2. Viable cell counts of nonattached cells performed daily in cultures without or with several concentrations of TPA.

**Morphological Changes**

Control HEL cells in suspension cultures appear as round free cells; adherent cells and cell aggregates are rare. Following TPA treatment, most of the HEL cells adhere firmly, and they stretch their cytoplasm in a variety of ways so that cells with a thin spindle-like or dendritic appearance with long filamentous projections or very flat cells with indistinct cytoplasmic borders are seen (Fig. 1C). In addition, some round, rather flat cells are loosely attached on the adherent cell layer, while the rest of the treated cells stay in suspension, usually in aggregates. The staining characteristics of TPA-treated HEL cells, following Wright-Giemsa staining, are distinctly different from untreated control cells; following TPA treatment, the adherent cells lose most of their cytoplasmic basophilia, display a dramatic decrease in nuclear/cytoplasmic ratio, and their nuclear chromatin appears more leptochromatic with prominent single or multiple small nucleoli. Similar changes are observed in nonadherent TPA-treated cells. Cells with cytoplasmic vacuolization are seen more frequently than in control cells. Also, cells with more than one nucleus are far more frequent in TPA-treated cells than in control cells.

**Surface Antigens**

Surface antigenic profile was evaluated with a panel of monoclonal antibodies (Table 2). Some of these detect determinants that are erythroid-specific (i.e., glycoporphin) or determinants that are not erythroid-specific but have a characteristic expression in erythroid cells (i.e., blood group alloantigens such as ABH, iI) or determinants that are normally associated with nonerythroid cells (i.e., myeloid, monocytic, or platelet antigens).

Reactivity of HEL cells with glycoporphin and blood group alloantigens before and after TPA treatment is summarized in Table 2. Untreated HEL cells react positively with an anti-glycophorin-A antibody (kindly provided to us by Dr. L. Andersson), with a monoclonal anti-H antibody (or with a fluoresceinated Ulex Europeus-I lectin), and with anti-i antisera. They are also partially reactive with monoclonal antibody L-21 [reacting with blood group Lu\(^a\)Lu\(^b\) but negative with Lu(a-b-)] and with anti-Pk antibody. They are negative in anti-blood-group A, B, anti-I, anti-Le\(^e\), and antigloboside antibody (Table 2). Of the antibodies primarily expressed in nonerythroid cells (Table 2), positivity is observed in those with megakaryocyte/platelet specificity (PGP II\(^a\)/II\(^b\)) and in determinants positive for nonspecific esterase (butyrase) and acid phosphatase, and the majority are positive for PAS; they are negative for peroxidase, chloroacetate esterase, and alkaline phosphatase. TPA-treated HEL cells display the same cytotoxic characteristics overall as described for control cells. One notable exception is the Sudan black B stain; TPA-treated cells, especially those cells with large cytoplasm, show a significant positivity in the perinuclear region, whereas untreated cells are virtually negative.

**Scanning Electron Microscopy**

The typical appearance of control cells is shown in Fig. 3A. Most HEL cells are round with a villous outer surface and occasional blebs (Fig. 3A). Attached TPA cells, on the contrary, appear as large flattened cells with thin stretched cytoplasm and thinner villi (Fig. 3C). Unattached TPA cells differ from control cells by a higher proportion of cells with “blebby” surfaces.

**Cytochemical Observations**

Cytochemical findings in HEL cells have been previously described. In brief, these cells are virtually all
Fig. 3. Scanning electron microscopy of HEL cells before and 5 days following TPA addition (10^{-8} M). Control cells are smaller, with villous outer surfaces (A, on facing page, ×2,000), whereas nonadherent treated cells display numerous "blebs" (B, on facing page, ×1,800) and adherent treated cells appear as thin cytoplasmic stretches with small villi (C, ×660).

Table 2. HEL Cells: Surface Antigen Profile Before and 4 Days After TPA Treatment (10^{-8} M)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Reference</th>
<th>Control Cells</th>
<th>TPA Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-glycophorin-A</td>
<td>66</td>
<td>+</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Anti-H-antigen</td>
<td>67</td>
<td>+</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Ulex Europeus-1</td>
<td></td>
<td>+</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Anti-IgM</td>
<td>68</td>
<td>+</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Anti-Le^a (SSEA-1)</td>
<td>69</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-Le^b</td>
<td>70</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-globoside</td>
<td>Hakomori S et al., unpublished</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anti-Lu^a/Lu^b (L-21)</td>
<td>Marcus D et al., unpublished</td>
<td>+</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Anti-Pk</td>
<td>71</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10-75-3</td>
<td>72</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mac-120</td>
<td>73</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>My-1</td>
<td>74</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5F-1</td>
<td>75</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BB-2</td>
<td>Holly F et al., unpublished</td>
<td>±</td>
<td>+ (4)</td>
</tr>
<tr>
<td>Antitransferrin</td>
<td></td>
<td>+</td>
<td>+ (4)</td>
</tr>
</tbody>
</table>

shared by monocytes, platelets, and erythroblasts (10-75-3, 5F1).

No drastic quantitative changes in positivity with the above antibodies are observed up to 5 days following TPA treatment, although the degree of reactivity with anti-glycophorin-A, with Ulex Europeus-1, and with anti-Pk appeared decreased in most of the preparations examined. Findings with monoclonal antibody L-21 are of interest, since they suggest the presence of two populations: one (the majority) strongly positive and one (the minority) negative (Fig. 4). Of the other antibodies tested (Table 2), the only remarkable change was with antibody BB-2 (reacting mainly with activated B cells), which is virtually negative before treatment, but becomes positive in 10% of the cells after TPA treatment (Fig. 5).

The presence of Fc receptors was tested by the cells' ability to form rosettes with sensitized red cells. Untreated and TPA-treated HEL cells form rosettes with IgG-coated ox red cells with high frequency
(Table 3), although the proportion of cells with complete rosettes is higher in treated cells (20% versus 13% in the untreated).

**Phagocytic Activity**

The phagocytic ability of TPA-treated HEL cells and control cells was evaluated by incubating them in the presence of 2μ latex beads or in the presence of sensitized ox or sheep red cells (EA, EAC cells). Whereas less than one-third of control cells have ingested latex beads (>3 beads/cell), over 70% of the treated cells have engulfed high numbers of latex beads (usually >10 beads/cell) after an overnight incubation at 37°C (Table 3 and Fig. 6, A and B). In the absence of serum, 5%-8% of TPA-treated HEL

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**Table 3. HEL Cells—Phagocytic Properties**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Fc Receptors (% EA Rosettes)</th>
<th>Phagocytosis</th>
<th>Immune</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Latex Beads</td>
<td>Cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[% (+) Cells]</td>
<td>[% (+) Cells]</td>
</tr>
<tr>
<td>HEL-Control</td>
<td>81 (13)‡</td>
<td>30</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>HEL + TPA§</td>
<td>76 (20)€</td>
<td>78</td>
<td>5-8</td>
</tr>
</tbody>
</table>

*Cells containing over 3 beads per cell.
†Cells with intracellular EA red cells.
‡Numbers in parentheses indicate percentage of complete, tight rosettes.
§10^{-6} M.
cells showed phagocytosis of EA cells (Table 3 and Fig. 6C), whereas virtually no phagocytosis was observed in control cells.

Presence of Acetyl-LDL Receptors

The binding and degradation of native and acetylated LDL by TPA-treated cells and controls was studied in two separate experiments. Detailed data from one experiment are presented in Table 4 and diagrammatically in Fig. 7 (data from two experiments). Untreated nonadherent HEL cells degraded about half as much acetyl as native LDL. By contrast, TPA-treated nonadherent cells degraded 30 times more acetyl than native LDL. In adherent cells, the ratio of acetyl/native LDL binding and degradation is about 5 (versus 30.8 in nonadherent cells), mainly because adherent cells degraded more native LDL than did nonadherent TPA-treated cells (Table 4). Untreated HEL cells, freshly isolated lymphocytes, and fibroblasts from culture displayed low ratios (Fig. 7), within the expected range for non-monocyte/macrophage cells.46 It is of interest that the values for TPA-treated cells are similar to the ones obtained from macrophages in culture46 and much higher than those for freshly isolated monocytes.

Glycoprotein Profile

The surface glycoprotein profile of HEL cells undergoes a characteristic change after TPA treatment. A strongly labeled glycoprotein of approximately 85K appears in TPA-treated cells (Fig. 8). Enhancement or induction of a similar glycoprotein has been found previously in TPA-treated U937 cells (monocytoid cell line), and such a glycoprotein also appears to be present in untreated monocytes.16

Globin Synthesis

Labeling of untreated HEL cells with anti-γ-chain monoclonal antibodies reveals from 0.1% to 10% γ-chain-positive cells. When HEL cells are treated with TPA, the number of positive cells at 3 days following TPA addition is either the same or less than in uninduced control cells. The proportion of positive cells decreases further when studied 6 days after TPA addition.

We have previously shown that hemin augments the synthesis of γ chains. In hemin-treated cells 50%-90% of the viable cells are γ-chain-positive. When hemin is added concurrently with TPA, there is no induction of γ-globin synthesis, as assessed by the number of γ-chain-positive cells. Inhibition of globin induction by hemin persisted even when cells were in contact with TPA only for 24 hr. The same findings were obtained when K562 cells were treated with TPA and hemin (Fig. 9, A and B). In contrast to hemin-induced K562 cells, which showed >90% positive cells with anti-γ-chain antibodies, hemin added to TPA-treated cells, either concurrently with TPA or 1 day after the addition of TPA, failed to induce globin chain synthesis (Fig. 9B). In fact, TPA-treated K562 cells showed fewer γ-chain-positive cells (11% positive) than control untreated K562 cells (~25% positive). TPA-treated HEL or K562 cells could not be induced to synthesize globin if inducers other than hemin, such as butyric acid or δ-aminolevulinic acid, were used (data not shown).

It is of interest that when hemin was added simultaneously with TPA, and as long as it was present in the culture, it largely inhibited the TPA-induced adherence. None of the other inducers elicited this adherence inhibition.

DISCUSSION

Several lines of evidence point to two important facets concerning the cellular effects of phorbol diesters. First, it appears that their primary target of action is the cell membrane. The TPA-induced cellular effects are attributable to events following the TPA binding to specific cellular receptors and/or to the interference with the function of other cellular receptors.30-35,44 Second, it is becoming increasingly clear that the diverse effects elicited by phorbol diester treatment of various cell types are dependent on the
distinct characteristics of the target cells. In line with this notion is the observation that cells of myelomonocytic origin respond to TPA by a phenotypic change towards the monocyte-macrophage,\textsuperscript{11-14,35-41} whereas cells with lymphoid characteristics show a different response, which leads to their further differentiation along the B-cell or T-cell pathway.\textsuperscript{16,17} In view of these observations, it is of interest that the human erythroleukemia line (HEL) changes to a macrophage-like phenotype after TPA treatment. Of further interest is

<table>
<thead>
<tr>
<th>Table 4. Binding and Degradation of LDL</th>
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<tbody>
<tr>
<td>Cells</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Degradation (ng/10(^6) cells/24 hr)</td>
</tr>
<tr>
<td>Nonadherent HEL</td>
</tr>
<tr>
<td>Nonadherent HEL</td>
</tr>
<tr>
<td>Adherent HEL</td>
</tr>
<tr>
<td>Binding (ng/mg protein/24 hr)</td>
</tr>
<tr>
<td>Adherent HEL</td>
</tr>
</tbody>
</table>

\textsuperscript{*}10\(^{-6}\) M, 5 days in culture.
TPA INDUCES MACROPHAGE SHIFT IN HEL CELLS

Fig. 7. Degradation of 125I-labeled acetyl and native LDL in TPA-treated and untreated HEL cells. The values are expressed as ratios of acetyl/native LDL and represent the mean of two experiments. (Detailed data from one experiment are presented in Table 4.)

the magnitude of this response compared to myelomonocytic cells.

HEL cells constitutively express globin and some surface determinants, either specific or characteristic for erythroid cells (i.e., glycophorin, H-antigen), as well as surface antigens shared by monocytes and cells of the thrombopoietic series (Table 2). Granulocyte-specific antigens have not been detected in these cells (C. Civin, personal communication). In contrast, some of the platelet/megakaryocyte-specific surface antigens are displayed in abundance in these cells. It is also of interest that the HEL cells display Fc receptors, and they are positive for nonspecific esterase (butyrase). Although the latter two properties are characteristic of monocytes, they have been found in granulocytic cells at early stages or in cells of thrombopoietic or erythroid series. They may not, therefore, be considered, a priori, as definitive indicators of a monocytic phenotype in the HEL cells. The drastic phenotypic change induced by TPA can be considered as the most persuasive evidence that the HEL cells indeed possess an innate monocyte-macrophage phenotype. The fact that TPA can unmask or enhance this latent phenotype reinforces previous notions about its discriminating effects on cells of myeloid origin (conversion to macrophage-like cells) as opposed to cells of lymphoid origin (further differentiation towards the T or B pathway of differentiation).

The TPA-induced morphological changes in HEL cells are accompanied by some biochemical changes as well. In concert with the cessation of cell proliferation, the cells become noninducible for globin synthesis. Such an effect can be either secondary to inhibition of cell proliferation and DNA synthesis, or it can be interpreted as an expected consequence because of the unidirectional further differentiation towards the macrophage phenotype. Against the latter possibility are the results with K562 cells or the Friend cells, which do not show the same degree of macrophage-like phenotypic changes, but do show the same effects on cell proliferation and the inhibition of globin induction. Another possibility, which cannot be excluded by our data, is that the inhibition of inducible globin synthesis is due not only to cessation of DNA synthesis, but also to membrane changes introduced by TPA that interfere with inducers acting primarily on the cell surface. In this context it was of interest that in the simultaneous presence of hemin and TPA, adherence is virtually inhibited; however, the cells remain noninducible. This observation may indicate that adherence per se is independent of the other effects concerning DNA synthesis and the inhibition of induction. This notion is reinforced by the observation that nonadherent TPA-treated cells show the same inhibition of induction as adherent cells and by the demonstration that adherence, DNA synthesis, and differentiation are separable events in HL-60 cells, as well as the observations on the adherence induction by TPA in a specific clone of murine erythroleukemia cells. Other globin inducers (i.e., sodium butyrate, δ-ALA) did not seem to interfere with TPA-induced adherence, but, like hemin, they were ineffective as globin inducers in the presence of TPA. This abrogation of globin induction is observed also at the level of nuclear transcription (M. Groudine et al., unpublished data).

Among the positive biochemical consequences of TPA treatment of HEL cells, the most impressive was...
the demonstration of significant increase in the high affinity binding and degradation of acetylated LDL. Activity of this magnitude has been described for cultured macrophages, whereas freshly isolated monocytes were less active. Therefore, the increased binding and degradation of acetylated LDL induced by TPA treatment is interpreted as indicating that the morphologically transformed cells also have acquired another of the specialized functions of macrophages, and they express it at a level that is comparable to normal macrophages. This aspect has not been studied before in TPA-treated myelomonocytic cells and, therefore, comparisons cannot be made.

The study of the surface glycoprotein profile of TPA-treated HEL cells showed the marked enhancement of an 85K glycoprotein, which is barely detectable in control cells. The function of this glycoprotein is unclear; however, it is intriguing to speculate that this glycoprotein may be associated with the development of acetyl-LDL receptors in these cells or with the induction of transglutaminase activity associated with terminal differentiation of macrophages.

Most of the surface antigenic determinants present in HEL cells before TPA treatment were present after TPA treatment. However, presence of these proteins by immunofluorescence does not necessarily indicate continuous synthesis, and it is more dependent on the
turnover of any given protein, after synthesis is halted. Synthetic studies for some of the surface antigens (i.e., glycoporphin or H) may be of interest to identify those determinants whose synthesis is turned off, as globin synthesis is turned off. Two surface determinants were enhanced after TPA treatment, but the significance of this change is presently unclear.

ACKNOWLEDGMENT

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