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SPECIFIC CHANGES IN THE SURFACE GLYCOPROTEIN PATTERN OF A HUMAN LEUKEMIC NULL CELL LINE NALL-1 ASSOCIATED WITH MORPHOLOGIC AND BIOLOGICAL ALTERATIONS INDUCED BY PHORBOL-ESTER

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Summary; 12-O-tetradecanoylphorbol-13-acetate, a highly active tumor-promoting agent, inhibits cell proliferation of human leukemic null cell line NALL-1 at a dose range of $10^{-9} - 10^{-7}$M.
Soon after the addition of 12-O-tetradecanoylphorbol-13-acetate to suspension culture, cells began to adhere to the substratum. Associated with the change in cell behavior, rate of DNA synthesis decreased rapidly but rate of RNA and protein synthesis remained essentially unchanged. After 48hr treatment with 12-O-tetradecanoylphorbol-13-acetate, adherent and growth arrested cells were all alive.
Changes in surface glycoproteins of these cells were analyzed by the neuraminidase/galactose oxidase or periodate/NaB[$^3H_4$] surface-labeling technique followed by polyacrylamide gel electrophoresis and fluorography. In 12-O-tetradecanoylphorbol-13-acetate treated cells, a glycoprotein with an apparent molecular weight of 145,000 was strongly labeled. The amount of HLA-DR antigen was also increased. These and other observations suggest that NALL-1 cells are induced by 12-O-tetradecanoylphorbol-13-acetate to differentiate into mature cells having some properties of B-cell blasts.

Introduction: It has been reported that some phorbol diesters, of which TPA is the most active, interfere in culture with the process of spontaneous or induced differentiation of Friend erythroleukemia cells and of several other cell systems(1-7). It has also been reported that in some lines of murine erythroleukemia cells TPA induces rather than inhibits differentiation (8). Recently, induction of terminal differentiation in human promyelocytic

Abbreviations: TPA, 12-O-tetradecanoylphorbol-13-acetate; FCS, fetal calf serum; KM, kanamycin; PBS$^-$, phosphate buffered saline, pH 7.4, is composed of 8.0 g NaCl, 0.2 g KCl, 0.2 g KH_2PO_4 and 1.15 g Na_2HPO_4 in one liter of distilled water; PMSF, phenyl methyl sulphonyl fluoride; SDS, sodium dodecyl sulfate.

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leukemia cells by TPA has been reported(9-10). We report here that TPA treatment induces differentiation of a human leukemia null cell line, NALL-1, into mature cells with several characteristics of human B-blasts.

Materials and Methods:

Cell line and cell culture: A human leukemia null cell line NALL-1(12) was grown in plastic tissue culture dishes in RPMI medium 1640(GIBCO. New York, N.Y.) supplemented with 20% FCS and 60 μg/ml of KM. TPA(Consolidated Midland Corp., Brewster, N.Y.) dissolved in acetone(10^{-4}M) was added to the cell suspension 1 day after seeding at the final concentration detailed in Results. Acetone had no detectable effect on the cell growth and cell behavior at this concentration. Cell growth was determined from a cell count after trypan blue-staining.

Protein synthesis: NALL-1 cells were labeled continuously with [^{3}H]-leucine. At indicated times, aliquots were withdrawn and washed twice with PBS$^-$. The amount of radioactive precursor incorporated was determined after 10% trichloroacetic acid precipitation onto glass filters.

DNA and RNA synthesis: DNA and RNA synthesis were evaluated by determining [^{3}H]-thymidine and [^{3}H]-uridine incorporation into trichloroacetic acid-insoluble fraction during 2 hr labeling pulses.

Cell surface labeling and polyacrylamide gel electrophoresis: Cells(3x 10^7) were surface-labeled with NaB[$^3H_4$] after treatment with neuraminidase (500 U/ml; Behring Institute, Marburg, West Germany) and galactose oxidase (140 U/mg protein; Sigma Chem. Co., St. Louis, Mo.)(13) or periodate(14). After washing, the cells were suspended in 200 μl of 1% Triton X 100 in PBS$^-$ containing 2 mM PMSF and 1.5 mM $MgCl_2$ and kept for 15 min at 4°C. Insoluble material was removed by centrifugation. Radioactivity was determined after 10% trichloroacetic acid precipitation onto glass filters. The same amount of radioactive materials was dissolved in sample buffer(15), heated to 100°C for 3 min, and applied to gel electrophoresis. Gel electrophoresis on 8 M urea-0.1% SDS-8% polyacrylamide gel was carried out according to the method of Mizushima and Yamada(15). The gels were fixed and treated for fluorography as described by Laskey et al.(16)(17).

Indirect immunofluorescence test: 0.1 ml of 1-2x10^6/ml cells were incubated with 10 μl of anti HLA-DR rabbit serum(a gift from Dr. M. Katagiri, Asahigawa medical college, Asahigawa, Japan) at 37°C for 30 min. Then they were washed in PBS$^-$ and incubated with 10 μl of fluorescein-conjugated goat anti rabbit IgG(Miles-Yeda Ltd., Rehovot, Israel) at 37°C for 30 min. Fluorescence microscopic observations were carried out with an Olympus fluorescence microscope model BH-RFL with a HBO 100W/2 light source. Photographs were taken with Kodak Tri-X film with an exposure time of 20 sec.

Radioisotopes: [^{3}H]-leucine(137 Ci/mmol), [^{3}H]-thymidine(40 Ci/mmol), [^{3}H]-uridine(28 Ci/mmol) and NaB[$^3H_4$](5 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, England.

Results and Discussion:

Effect of TPA on cell morphology and cell growth:

Whereas NALL-1 cells grew as single cell suspension (Fig.1A), as soon as 4 hr after exposure to TPA(10^{-9}-10^{-7}M), cells began to adhere to the substratum. After 24 hr, more than 90% of the cells were attached to the substratum(Fig. 1B). This adhesive behavior of TPA-treated cells was observed even in a

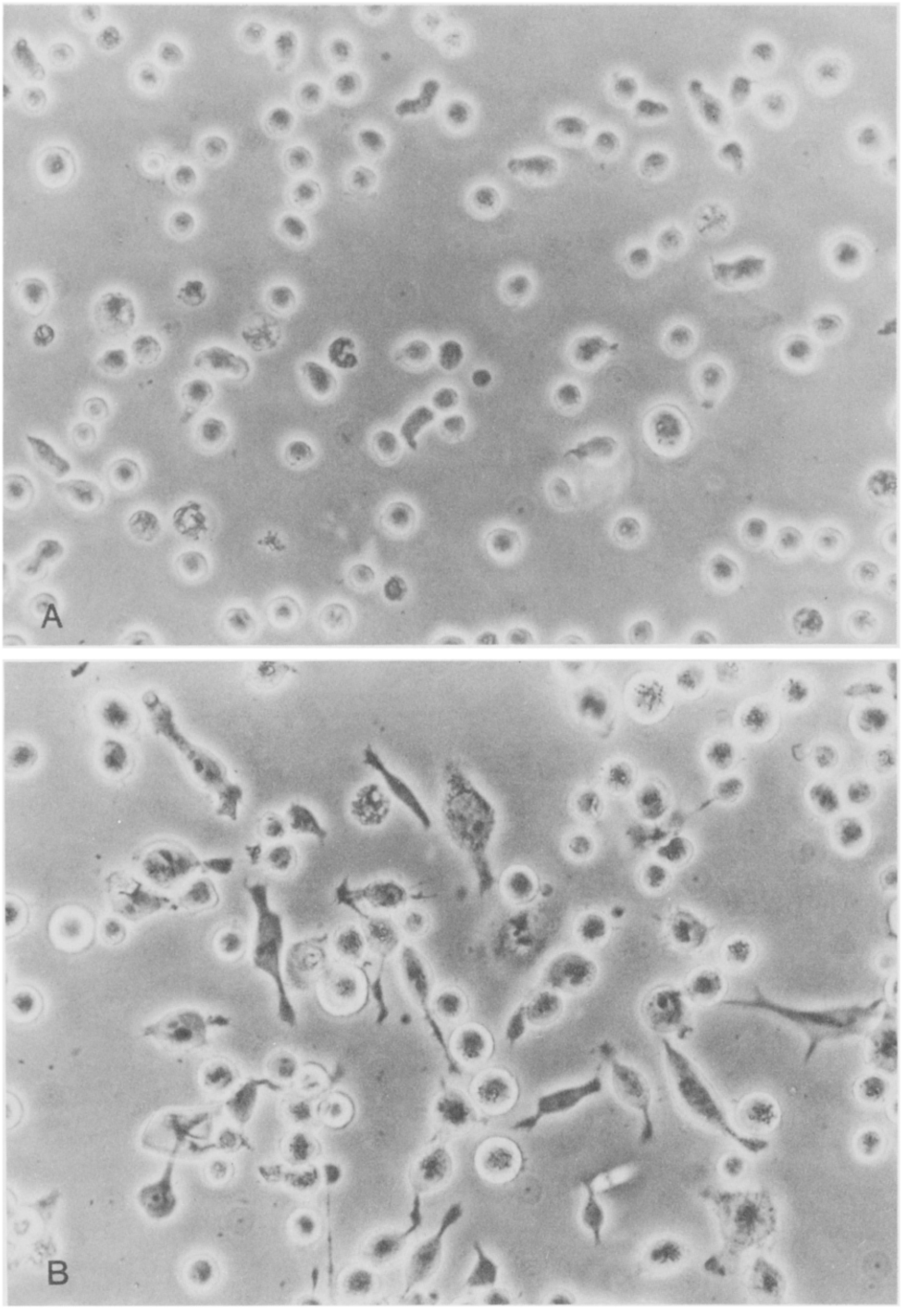
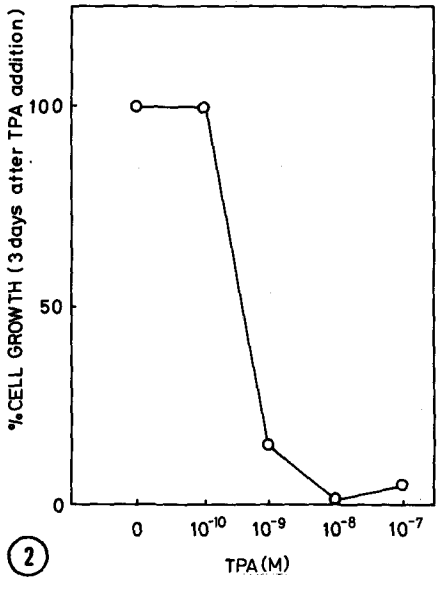


Fig.1. Morphological changes after TPA treatment of NALL-1 human leukemic null cell line. (A) Not treated; (B) Cells treated with 10^{-8}M of TPA for 48 hr. Cells in tissue culture dishes were photographed directly by a phase contrast microscope. Magnification, (x 400).

bacterial culture dish. Adherent cells could be detached by vigorous shaking. All cells attached to the substratum remained alive for 3 days. TPA treatment inhibited cell growth in a dose-dependent manner. At a concentration range from 10^{-9} to 10^{-7}M, no cell growth was observed(Fig.2).

Pattern of DNA, RNA and protein synthesis after TPA treatment: The amounts of [^{3}H]-thymidine, [^{3}H]-uridine and [^{3}H]-leucine incorporated into the acid-insoluble fraction were determined at various times after addition of TPA (10^{-8}M). A sharp decrease in the amount of [^{3}H]-thymidine incorporated into



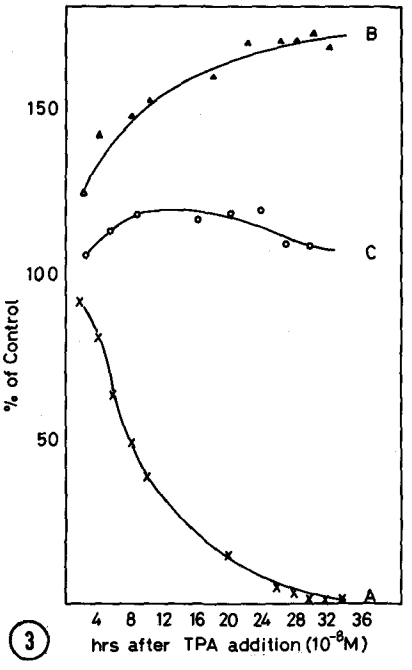


Fig.2. Effect of TPA on cell growth. Cells were seeded at 5×10^5/ml one day before the addition of TPA. Cell number of not-treated culture at day 0 and day 3, was 7.4×10^5 and 1.8×10^6/ml respectively. % cell growth $= \frac{\text{cell number(day3-TPA)} - \text{cell number(day0-Not treated)}}{\text{cell number(day3-Not treated)} - \text{cell number(day0-Not treated)}} \times 100$

Fig.3. Effect of TPA on DNA, RNA and protein synthesis. Experimental condition is as described in legend to Fig.2. Extent of DNA, RNA and protein synthesis was determined as described in Materials and Methods. % of control $= \frac{\text{Acid insoluble radioactivity(TPA-time t)}/10^6\text{cells}}{\text{Acid insoluble radioactivity(Not treated-time t)}/10^6\text{cells}} \times 100$
(A) — x — DNA; (B) — Δ — RNA; (C) — ○ — protein.

DNA was observed as early as 2 hr after TPA addition(Fig.3-A). In contrast, RNA and protein synthesis were increased slightly(Fig.3-B and C). These results indicate that after TPA treatment, NALL-1 cells lose proliferative capacity rapidly but still are able to synthesize RNA and protein efficiently.

Surface sialoglycoprotein patterns of cells labeled by periodate/NaB[$^3H_4$] method: The surface sialoglycoprotein profile of untreated cells is shown in Fig.4 lane-1. The most strongly labeled sialoglycoprotein had an apparent molecular weight of 145,000. After 40 hr in the presence of 10^{-8}M TPA, the pattern was dramatically changed with a reduction of GP 145K. Instead there appeared a strongly labeled GP 115K(Fig.4 lane-2).

Surface glycoprotein patterns of neuraminidase/galactose oxidase NaB[$^3H_4$] labeled cells: As can be seen in Fig.4 lanes 3 and 4, the changes in surface glycoprotein profile were remarkable. In TPA treated cells, new glycoprotein with an apparent molecular weight of 145,000 was strongly labeled and GP 120K and 80K increased significantly. Moreover, 41.5K and 34K glycoproteins were visualized. These two glycoprotein bands appeared to correspond to two sub-

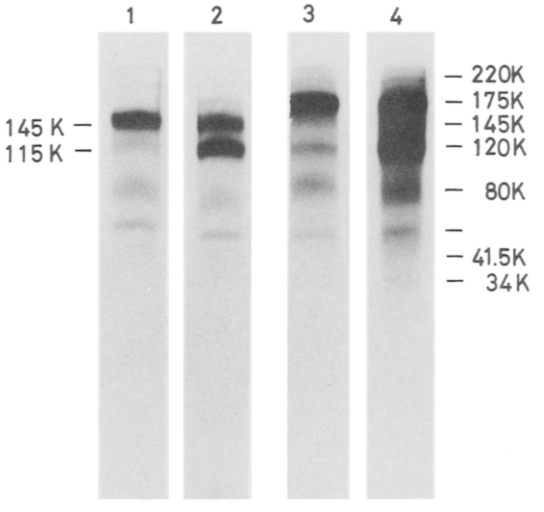


Fig.4. Fluorography patterns of surface glycoproteins labeled after periodate or neuraminidase/galactose oxidase treatment of cells. Periodate: (lane-1) Not treated; (lane-2) TPA treated. Neuraminidase/galactose oxidase: (lane-3) Not treated; (lane-4) Cells were treated with 10^{-8}M of TPA for 40 hr.

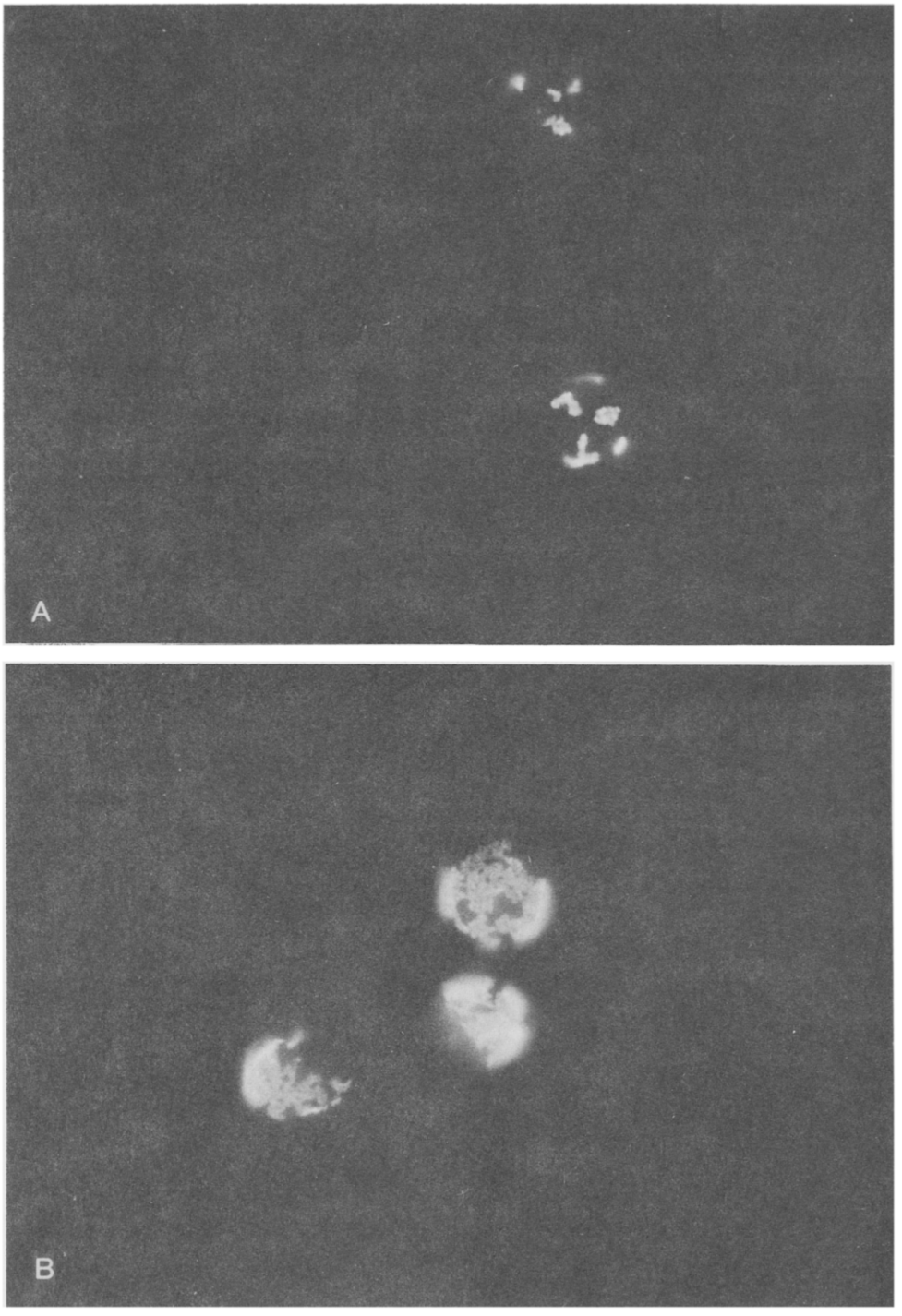


Fig.5. Expression of HLA-DR antigen on cell surface. (A) Not treated; (B) Cells were treated with 10^{-8}M of TPA for 48 hr. Magnification, (x400).

units of HLA-DR antigen. To confirm the increase in the expression of HLA-DR antigen, surface immunofluorescent study was carried out using a rabbit antiserum specific for HLA-DR antigen(18). On the surface of untreated NALL-1 cells, HLA-DR antigen was detected as a few patch-like stains(Fig.5A). Conversely, on the cells treated with TPA (10^{-8}M) for 48 hr, entire regions of the cell surface were brilliantly stained(Fig.5B).

Further experiments are necessary to conclude that TPA induces a terminal differentiation of NALL-1 cells. However, stimulation of HLA-DR antigen synthesis, inability of cells that are alive after TPA treatment to resume cell proliferation upon subculture, appearance of surface IgM (μ chain) and change in electrophoretic mobility of TPA treated cells to that of B-cell blasts (unpublished observation) suggest that NALL-1 cells were induced by TPA to differentiate to a terminally differentiated state that has some characteristic properties of B-cell blasts.

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