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Surface Characteristics of the U-937 Human Histiocytic Lymphoma Cell Line: Specific Changes During Inducible Morphologic and Functional Differentiation In Vitro

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A. Introduction

A number of human hematopoietic cell lines representative of the neoplastic cell clone in vivo have been established in vitro during the last decade (for recent reviews see Minowada et al. 1980a, Nilsson 1979). Lymphoid leukemia and lymphoma lines all seem to be arrested at a particular stage of lymphoid differentiation (Nilsson 1978). Attempts to induce differentiation with mitogens have generally been unsuccessful. Only in the promyelocytic leukemia cell line HL-60 has spontaneous terminal differentiation been documented (Collins et al. 1977; Gallagher et al. 1979). Recently, however, inducible differentiation in vitro has been demonstrated in three non-lymphoid hematopoietic cell lines using substances known to induce differentiation in animal cell systems. Thus, dimethyl-sulfoxide (DMSO) and 12-tetradecanoyl-phorbol-13-acetate (TPA) will differentiate HL-60 cells (Collins et al. 1978; Gahmberg et al. 1979; Huberman and Callaham 1979; Rovera et al. 1979), butyric acid or hemin will induce the erythroleukemic K-562 cells (Andersson et al. 1979; Rutherford et al. 1979), and TPA or supernatant from mixed lymphocyte cultures (MLC), the histiocytic lymphoma U-937 cells (Koren et al. 1979; Nilsson et al. 1980a).

The phenotypic features of the U-937 cells (Sundström and Nilsson 1976) suggest that they represent immature monocytoid cells. When induced to differentiate, such cells will undergo a stepwise morphologic and functional maturation similar to that described for differentiating normal monocytic cells (Koren et al. 1979; Nilsson et al. 1980a). It was therefore suggested that the U-937 cells, although neoplastic, may be used as a model in studies of various biologic aspects of cells in the normal monoblast-macrophage differentiation lineage (Nilsson et al., to be published a).

The present paper summarizes our studies so far of inducible differentiation in U-937 and deals mainly with surface changes during treatment with MLC supernatant of TPA but also demonstrates that the U-937 may be induced to display functional features of mature monocytic cells.

B. Materials and Methods

I. Cells

The U-937 cell line was established in 1975 from a patient with a "true" histiocytic lymphoma (Sundström and Nilsson 1976). The line is Epstein-Barr virus negative and its neoplastic nature is demonstrated by its aneuploidy (Zech et al. 1976) and tumorigenic potential in nude mice (Nilsson, unpublished work). The morphology, the cytochemical features, and the surface and functional characteristics of U-937 are summarized in Table 1. Taken together the phenotype of U-937 cells is unique, without resemblence to any other published hematopoietic cell line, and suggests that the U-937 cells represent monocytic cells arrested at a fairly immature stage in the monocyte differentiation lineage.

All experiments with U-937 cells were performed as follows: Cells were harvested from optimally growing stock cultures and were incubated under standard culture conditions at an initial cell concentration of 2×10^5 cells/ml. The medium was F-10 supplemented by 10% foetal calf serum from a selected batch (GIBCO) and antibiotics (penicillin 100 IU/ml, streptomycin 50 μg/ml, gentamycin 50 μg/ml and amphotericin B 1.25 mg/ml). The medium was not changed during the incubation periods of 24–96 h: TPA was purchased from Sigma (United States); MLC supernatant was harvested from six day cultures as described (Gidlund et al., to be publi-

Characteristics	Reference
Monocytoid morphology	Sundström and Nilsson 1976
Monocytoid-myeloid cytochemical profile	Nilsson et al. 1980a, Sundström and Nilsson 1977
Expression of Fc and C3 receptors, β_2-microglobulin, HLA, and Ia-like antigen	Huber et al. 1976; Nilsson et al., to be published
Absence of Helix Pomatia A agglutamin receptor	Nilsson et al., to be published
Monocyte-like surface glycoprotein pattern	Nilsson et al. 1980b
Capacity for lysozyme secretion	Ralph et al. 1976; Sundström and Nilsson 1976
Capacity (weak) for phagocytosis	Sundström and Nilsson 1976
Activity (weak) as effector cell in ADCC	Nilsson et al. 1980a
Tumorigenicity in nude mice	Nilsson, unpublished work
Aneuploid karyotype	Zech et al. 1976

Table 1. Selected properties of U-937

shed). Monocytes were idolated from fresh human buffy coats on a two step Percoll gradient according to Pertoft et al. (1980).

II. Cell Surface Studies

The Fc and C3 receptors were quantitated using rosette assays (Huber et al. 1976; Sjöberg and Inganäs 1979). Expression of Ia-like antigen (HLA-DR), HLA, and B_2-microglobulin was quantitated by a single cell Zeiss cytophotometer as described (Nilsson et al. 1974). Indirect immunofluorescence was performed using the following antisera: rabbit anti-HLA-DR (Klareskog et al. 1978) rabbit anti-HLA (gift from Dr. P. Peterson, Uppsala), rabbit anti-B_2-microglobulin (Dakopatts, Copenhagen), and fluoresceinisothiocyanate labeled swine anti-rabbit serum (Dakopatts, Copenhagen).

The expression of major surface glycoprotein was studied by the galactoseoxidase tritiated sodium borohydride surface labeling technique of Gahmberg and Hakamori (Gahmberg and Hakomori 1973). The methodology used to study the sensitivity to natural killer (NK) cells have been detailed in Gidlund et al. (Gidlund et al., to be published).

III. Functional Assays

The activity as effector cell in an antibody-dependent cellular cytotoxicity (ADCC) assay with antibody-coated chicken erythrocytes (CRBC) as targets was studied as described (Gidlund et al., to be published). The phagocytic activity of immunoglobulin coated latex particles was quantitated under phase contrast after incubation of the cell particle mixture for 30 min at +37°C. The capacity for lysozyme secretion was quantitated by a radioimmunoassay (Venge et al. 1979).

C. Results

Morphologic and functional changes may be induced in U-937 cells by addition of 10^{-12}–10^{-7} M TPA or 20%–30% of MLC supernatant to the culture medium (Nilsson et al. 1980a). Comparative studies on the effect of TPA and MLC supernatants showed that the induced changes in morphology, expression of nonspecific esterases, and activity as effector cells in ADCC were similar (Gidlund et al., to be published; Nilsson et al. 1980a). In the following the presentation will focus on our results with TPA as inducer, since this substance has the advantage over the MLC supernatant of being well defined.

I. Alterations of Growth Properties, Morphology, and Cytochemical Profile After Exposure to TPA

The optimal concentrations for the induction of the morphologic and functional changes listed in Table 2 was 1.6×10^{-9}–1.6×10^{-7} M. After addition of TPA increased cell clustering and the appearance of a small fraction of adherent cells (glass or plastic surfaces) was noted within 1 h. The adherent fraction increased with time to a maximum of 60%–80% during the first 24 h of incubation. After 24

Characteristic	Reference (if not this paper)
Morphologic maturation	Nilsson et al. 1980a
Increased content of nonspecific esterase (NASDAE, ANAE)	Nilsson et al. 1980a
Increased expression of Fc receptors	
Increased expression of Ia-like antigen, β_2-microglobulin, and HLA antigen	
Specific changes in the surface glycoprotein pattern	
Decreased sensitivity to natural killer (NK) cells	Gidlund et al, to be published
Increased activity as effector cell in ADCC	Gidlund et al., to be published
Increased phagocytic activity	
Increased secretion of lysozyme	
Concomitant inhibition of growth and DNA synthesis	

Table 2. Phenotypic changes in U-937 cells after exposure to TPA or MLC supernatant

h detachment of cells was noted, but 10%–20% of the cells remained on the surface during the observation time of up to 8 days. These cells spread out and acquired a macrophage-like morphology as described (Nilsson et al. 1980a) (Fig. 1). Also in free-floating clusters the individual cell size increased. Suface attachment of TPA-treated cells could be prevented by covering the petri dishes with agarose. Such cells clumped to form large aggregates.

Comparative studies on the various properties (Table 2) in U-937 of cells grown in petri dishes with and without an agarose bottom overlayer showed that the acquisition of macrophage-like features in U-937 cells after TPA treatment was not surface dependent. The cell clustering and increased adhesiveness induced by TPA seem to be reversible within 2 h. However, so far the reversibility of the simultaneous functional changes has not been studied.

The growth of U-937 cells was partially inhibited by TPA as evidenced by growth curves and a decreased uptake of ^{3}H-thymidine (Forsbeck, to be published). The cytochemical profile, as tested by the panel of cytochemical stains described by Sundström and Nilsson (1977) changed within 24 h after treatment of TPA. The expression of the nonspecific esterases naphtol AS-D esterase (NASDAE) and acid α-naphtyl-acetate esterase

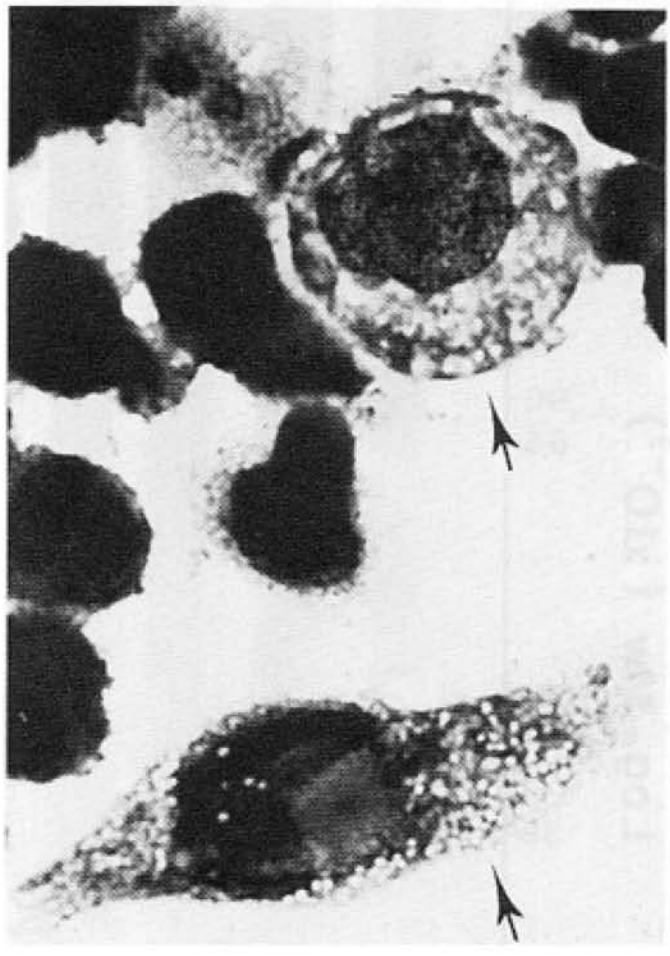

Fig. 1. U-937 cells exposed to 1.6×10^{-9} M TPA for 4 days in plastic petri dishes. Note the two macrophage-like cells *(arrows)* which contrast to the remaining, essentially morphologically unaltered cells

increased, with the most notable change found for NASDAE. The latter staining reaction could also be inhibited by NaF, a feature considered to be specific for the NASDAE of monocytic cells.

II. Surface Changes Induced by TPA

The composition of the major surface glycoproteins (SGP) of U-937 cells underwent characteristics changes after incubation with TPA or MLC supernatant (Fig. 2). The SGP of U-937 cells was characteristically different from that of other human hematopoietic cell lines (Nilsson et al. 1977). The most prominently labeled SGP had an apparent mol. w. of 160,000–145,000 daltons (160–145K). Less strongly labeled bands had apparent mol. wts. of 210K, 200K, 190K and 90K. After TPA treatment the following major changes in the SGP were found: the 200K band disappeared and the 145K-160K and the 90K bands were more strongly labeled. The "new" bands of apparent mol. wts. of 180K, 140K and 85K respectively, appeared. The MLC supernatant treated U-937 cells underwent essentially similar SGP changes with TPA treatment. Taken together the changes induced by TPA or MLC supernatant in the SGP in the U-937 cells made them resemble mature blood monocytes more closely than before.

The change in the expression of Fc receptors after TPA treatment varied from experiment to experiment, although culture conditions were strictly standardized. In most experiments, however, the percentage of rosetting cells increased from around 25% to 50%. In contrast the frequency of C3 receptor positive cells remained unchanged.

The amount of Ia-like antigen increased

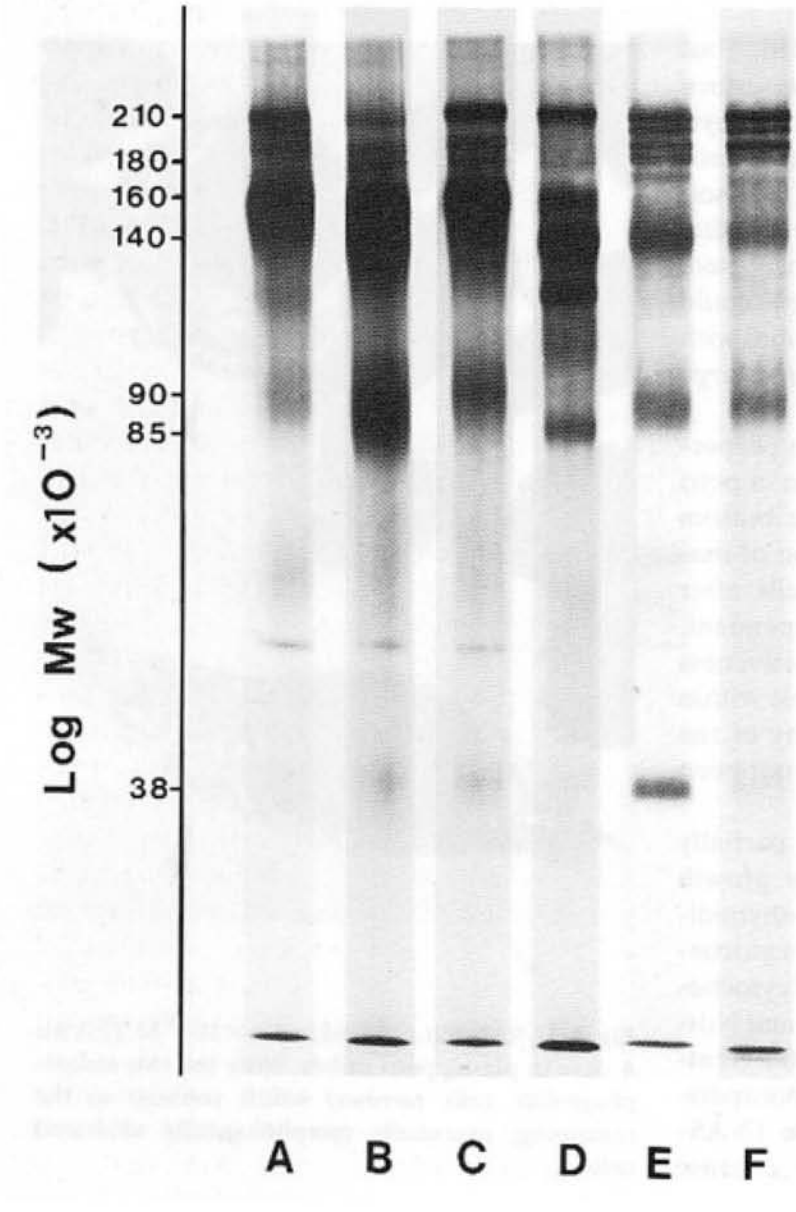

Fig. 2. Fluorography patterns of labeled surface glycoprotein obtained by SDS slab gel electrophoresis. Gel contration 7.5%. **A,** Untreated U-937 cells; **B,** TPA-treated U-937 cells; **C,** MLC-supernatant treated U-937; **D,** Untreated blood monocytes; **E,** TPA-treated blood monocytes; and **F,** MLC-supernatant treated blood monocytes. Molecular weights *(Mw)* calculated on basis of the following markers: myosin (200 KD), phosphorylase a (100 KD), albumin (66 KD), and ovalbumin (43 KD)

after TPA-induction (Table 3). The mean increase was twofold by day 2 and threefold by day 4. In a fraction (15%) of the cells, however, the change was 4–10-fold on day 2.

Table 3. Expression of Ia-like antigen on the surface of U-937 cells

	Day 2		
	No.	Mean fluorescence intensity (rel. units)	S.D.
Control	30	3.75	2.24
TPA-treatment	31	7.31	3.98
	Day 4		
	No.	Mean fluorescence intensity (rel. units)	S.D.
Control	30	3.74	1.65
TPA-treatment	32	10.50	6.33

The amount of HLA antigen and B_2-microglobulin also increased in four of five experiments. However, as for the expression of Fc receptors considerable variability was found in the different experiments (maximal increase on day 4 for HLA-antigen was two-fold and for B_2-microglobulin, five-fold).

The fact that TPA and MLC supernatant-induced differentiation of U-937 cells is associated with surface changes is also reflected by the dose-time dependent decrease in the sensitivity to human natural killer (NK) cells as has been reported (Gidlund et al., to be published).

III. Functional Changes Induced by TPA

Three functions (activity as effector cell in ADCC, secretion of lysozyme, and phagocytic activity) assumed to be typical for macrophages were studied. As is detailed by Gidlund et al. (to be published) U-937 acquired the capacity to kill antibody-coated CRBC. Even uninduced U-937 cells have a weak activity (<4% lysis) in ADCC against CRBC but only at a comparatively high effector to target cell ratio (Nilsson et al. 1980a). After exposure to TPA or MC supernatant the effectivity of the U-937 cells as killers increased as evidenced by a pronounced lysis (10%–20%) even at a very low (2:1) effector to target cell ratio. The activity of TPA-induced U-937 cells as ADCC effectors was both time and dose dependent, being maximal after 4 days treatment with 1.6×10^{-8}–1.6×10^{-7} M TPA.

The secretion of lysozyme has been studied onyl in a limited number of experiments using MLC supernatant of 1.6×10^{-9} M TPA treatment for 24 h. However, the results demonstrated a clear increase (three-fold) in the rate of lysozyme secretion.

The capacity for phagocytosis was regularly found in TPA-treated cultures but not in the control cultures. The fraction of phagocytic cells in TPA-treated cultures ranged from 10%–20%.

D. Discussion

When exposed to TPA or MLC supernatant the histiocytic lymphoma U-937 cells undergo multiple changes of their phenotype (Table 2). Taken together the alterations are those expected to occur when immature monocytic cell differentiate. It is not yet clear whether the induced differentiation is restricted only to a fraction of the cells or whether all cells repond to the inductive signals of TPA or MLC supernatant. The morphologic studies and the quantitative analyses of the expression of Ia-like antigen would favor the former possibility, while studies on the ADCC activity of various fractions of TPA stimulated cells obtained a 1 g gradient separation procedures (Gidlund, personal communication) would indicate that all cells may differentiate. If the latter suggestion is true, the finding of only 10%–20% strongly Ia-like antigen positive cells and a fraction (10%–20%) of surface adherent macrophage-like cells could be explained by the fact that the induced U-937 cells traverse different lengths along the presumed monocyte differentiation pathway. Be that as it may, the U-937 seems to be a satisfactory clonal model for studies of various aspects of the monocyte differentiation lineage in vitro.

The results from the cytoplasmic assays for esterases and expression of surface Fc and C3 receptors and Ia-like, HLA and B_2-microglobulin molecules have been variable for unknown reasons. We interpret this as due to a marked sensitivity of the differentiating U-937 system to factors in the culture and assay systems which we have been unable to

control. This variability for U-937, when induced to differentiate, has been noted before by Koren et al. (1979) using MLC supernatants to induce differentiation and concomitant increase in Fc receptor expression and activity in ADCC. A similar variability has been found also in differentiation experiments with the K-562 and HL-60 lines (Andersson, personal cummunication; Nilsson, unpublished work).

The functional assays alway gave reproducible results and leave room for no other interpretation than that the U-937 cells, after exposure to TPA or MLC supernatant, acquire the presumed typical functional features of mature monocytesmacrophages (increased capacity for phagocytosis, lysozyme secretion, and activity as effector cell in ADCC). We therefore suggest that the surface changes simultaneously recorded are associated with a change in the stage of monocytic differentiation of U-937 cells and therefore useful markers in studies of monoblast-macrophage differentiation. Of particular interest seems to be the possibility of using some of the major SGP, as detected by tritiated sodum borohydride labeling, as differentiation markers. The major SGP hallmarks for mature monocytic cells seems to be the 180K, 140K, and 85K SGP. All these are strongly labeled both in blood monocytes and TPA stimulated U-937 cells. Specific changes in the SGP compositions have previously been found in the HL-60 (Gahmberg et al. 1979) and in the K-562 cell lines (Nilsson et al. 1980a) during induced differentiation and thus further strenthens the usefulness of certain SGP as differentiation markers.

It is not yet clear whether "terimal" differentiation occurs at all in the U-937. Neither is the concomitant change in growth and DNA synthesis fully analyzed. Our studies so far indicate that at least partial inhibition of growth and DNA synthesis accompany TPA treatment and the induced phenotypic alterations. It is thus possible that TPA may induce a cell cycle block similar to what has been described by Rovera et al. (1979) for HL-60 cells.

D. Concluding Remarks

We conclude that the human histiocytic lymphoma cell line U-937 may be induced by TPA or MLC supernatant to undergo morphologic and functional differentiation in vitro which resembles the stepwise maturation events described for normal monocytic cells. The cell line may, therefore, be useful as a model for controlled in vitro studies of various aspects of monocytic differentiation. The observed specific changes in the composition of the major SGP suggest that such GP might be useful differentiation markers.

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