Response of Non-T, Non-B Acute Lymphocytic Leukemia Cells to Phorbol Ester

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ABSTRACT

Non-T, non-B acute lymphocytic leukemia cells were cultured in vitro with or without the tumor promotor 12-O-tetradecanoylphorbol-13-acetate (TPA), a potential modulator of differentiation. The eight cases studied were representative of non-T, non-B acute lymphocytic leukemia (ALL) cells and expressed amounts of Ia antigens varying from 0.9 x 10⁶ to 7.1 x 10⁶ molecules/cell; these levels were measured in a cellular radioimmunoassay with 21W4 monoclonal antibody directed at a monomorphic human Ia determinant. With all cases, TPA caused a significant increase in the level of Ia. Cultures with TPA expressed 4.3 times the amount of Ia found on fresh ALL cells, and a correlation was observed (r = 0.92) between the level of Ia following culture with TPA and that found on fresh ALL cells. A 25% increase in the modal volume of ALL cells was also caused by TPA. There was no detectable induction of surface or cytoplasmic immunoglobulin and no change in the expression of the common ALL antigen. Inhibition of [³H]thymidine incorporation and stimulation of 14C-leucine incorporation into RNA and protein were accompanied by an increase in protein synthesis induced by phorbol ester. Following culture with TPA, a substantial increase in the ability of the ALL cells to stimulate in a mixed-lymphocyte reaction was observed. These results suggest that ALL cells, like other cell types, are susceptible to the effects of TPA and respond by changes in cell volume, surface antigen expression, and mixed-lymphocyte reaction stimulating capacity.

INTRODUCTION

The majority of leukemia cells from patients with ALL are classified as non-T, non-B ALL because they do not form rosettes with sheep erythrocytes, do not react with monoclonal anti-T cell antibodies, and do not express surface immunoglobulin (11, 26). However, non-T, non-B ALL cells express Ia antigens and, in most cases, also bear CALLA (4, 13, 27). Several studies have indicated that 20 to 30% of cases referred to as non-T, non-B ALL have a pre-B cell phenotype, since they express cytoplasmic µ-chain (12, 30). B-cell markers identified with monoclonal antibodies and immunoglobulin gene rearrangements in non-T, non-B ALL have suggested that these leukemic cells are committed to the B-lineage (1, 14, 15, 22, 28).

The tumor promotor TPA can be an active modulator of differentiation of both myelogenous and lymphocytic leukemia cells (10, 23, 29). We have demonstrated recently with 10 cases of CLL that, among the changes induced by TPA, there were increases in Ia expression and in the ability of CLL cells to stimulate in a MLR (24). Such changes were accompanied by the induction of cytoplasmic µ-chain and, in one case studied, by IgM secretion (24, 25). Furthermore, the capacity of the CLL cells to respond to TPA appears to be correlated with their phenotypic markers. CLL cells with low amounts of surface immunoglobulin and relatively low levels of Ia responded well to TPA, while those cases with bright surface immunoglobulin and elevated amounts of Ia responded poorly to TPA (24).

In a recent study, we have demonstrated the heterogeneity of non-T, non-B ALL in terms of Ia expression. In an attempt to determine if the amount of Ia present on non-T, non-B ALL cells is related to the ability of these cells to respond to TPA, as seen with CLL cells, we measured the levels of Ia on ALL cells following culture with TPA. We also investigated the effects of TPA on the modal volumes of these cells and on their ability to stimulate in a MLR.

MATERIALS AND METHODS

Patients. Nine patients with newly diagnosed ALL who had not received previous chemotherapy were studied. Their ages, sex, and WBC are shown in Table 1. The diagnosis of ALL was based on the morphology of Wright-stained blast cells on bone marrow and peripheral blood smears and on cytochemical studies including myeloperoxidase, Sudan black B, periodic acid-Schiff, and nonspecific esterase stainings.

Cell Separation, Volume, and Marker Studies. Mononuclear cells from bone marrow or peripheral blood were obtained by Ficoll-Hypaque density gradient centrifugation and washed with PBS, pH 7.25. Cell size distribution was determined using a Model ZBI Coulter Counter linked to a Coulter Channelyzer and was calibrated with polystyrene microspheres of 10- and 19-μm diameter (Coulter Electronics, Hialeah, FL). The modal volume corresponding to the peak distribution was calculated and expressed in fl.

Marker studies were performed as described previously (24, 25). The percentage of T-cells was determined by spontaneous rosette formation with untreated sheep erythrocytes and with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes. Surface immunoglobulin and cytoplasmic immunoglobulin were assessed using fluorescein isothiocyanate-conjugated goat anti-human µ-chain (Meloy Laboratories, Springfield, VA). For the determination of cytoplasmic µ-chain, ALL cells were prepared by cytocentrifugation and fixed prior to staining. In the current study, the leukemic cell preparations were essentially free of cells forming rosettes with sheep erythrocytes and of surface immunoglobulin-positive cells; 8 of 9 cell preparations were negative for cytoplasmic µ-chain.

Patient K.E. was considered an example of a pre-B cell leukemia,
Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>WBC ($\times$ 10$^9$/liter)</th>
<th>Source</th>
<th>la* (%)</th>
<th>la level#</th>
<th>Volume (fl)</th>
<th>CALLA* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. O.</td>
<td>2</td>
<td>M</td>
<td>7.0</td>
<td>BM</td>
<td>89</td>
<td>0.9</td>
<td>172</td>
<td>ND</td>
</tr>
<tr>
<td>T. A.</td>
<td>6</td>
<td>M</td>
<td>8.9</td>
<td>BM</td>
<td>81</td>
<td>1.6</td>
<td>152</td>
<td>ND</td>
</tr>
<tr>
<td>M. C.</td>
<td>8</td>
<td>F</td>
<td>1.9</td>
<td>BM</td>
<td>90</td>
<td>2.6</td>
<td>206</td>
<td>ND</td>
</tr>
<tr>
<td>B. R.</td>
<td>4</td>
<td>F</td>
<td>47.0</td>
<td>PB</td>
<td>95</td>
<td>2.7</td>
<td>178</td>
<td>89</td>
</tr>
<tr>
<td>R. O.</td>
<td>7</td>
<td>F</td>
<td>296</td>
<td>PB</td>
<td>95</td>
<td>3.6</td>
<td>138</td>
<td>65</td>
</tr>
<tr>
<td>K. E.</td>
<td>5</td>
<td>M</td>
<td>44.0</td>
<td>BM</td>
<td>97</td>
<td>4.6</td>
<td>193</td>
<td>96</td>
</tr>
<tr>
<td>B. A.</td>
<td>6</td>
<td>F</td>
<td>64.8</td>
<td>PB</td>
<td>77</td>
<td>5.0</td>
<td>210</td>
<td>93</td>
</tr>
<tr>
<td>C. L.</td>
<td>8</td>
<td>M</td>
<td>5.4</td>
<td>BM</td>
<td>98</td>
<td>5.9</td>
<td>160</td>
<td>99</td>
</tr>
<tr>
<td>T. H.</td>
<td>3</td>
<td>M</td>
<td>66.3</td>
<td>BM</td>
<td>99</td>
<td>7.1</td>
<td>195</td>
<td>100</td>
</tr>
</tbody>
</table>

*The level of la is expressed in molecules of RAM-Fc $\times 10^5$ bound per cell, estimated under saturating conditions.

# BM, bone marrow; PB, peripheral blood; ND, not done.

because smears contained 8 to 10% of cells which stained for cytoplasmic $\mu$-chain.

The percentage of la-positive cells was determined by incubation of leukemic cells with monoclonal antibody 21-w4 or with the control P3/X63-Ag8 culture supernatant (2, 24). The percentage of CALLA-positive cells was determined using BA-3 monoclonal antibody (18) or control T15 ascites. Cells were then incubated with fluorescein isothiocyanate-labeled anti-mouse IgG (Meloy). In all immunofluorescence assays, 1 x 10$^6$ cells/ml, with or without TPA, in RPMI 1640 medium supplemented with penicillin, streptomycin, and 10% heat-inactivated fetal calf serum at 37$^\circ$C in a 5% CO$_2$ humidified incubator. Because of the small number of ALL cells available, duplicate cultures were set up for each case. After culture, cells were harvested and washed 3 times with PBS. Viability was assessed by trypan blue exclusion, and cell number was estimated with the Coulter Counter.

Cell Cultures. ALL cells were cultured for 1 to 3 days at a concentration of 1 x 10$^6$ cells/ml, with or without TPA, in RPMI 1640 medium supplemented with penicillin, streptomycin, and 10% heat-inactivated fetal calf serum at 37$^\circ$C in a 5% CO$_2$ humidified incubator. Because of the small number of ALL cells available, duplicate cultures were set up for each case. After culture, cells were harvested and washed 3 times with PBS. Viability was assessed by trypan blue exclusion, and cell number was estimated with the Coulter Counter.

TPA (P-L Biochemicals, Milwaukee, Wl) was dissolved in acetone at a 1.6 x 10$^{-4}$ M concentration and stored at -20$^\circ$C. It was diluted in culture medium at the time of the experiment. TPA was used at a final concentration of 1.6 x 10$^{-8}$ M since, in dose-response experiments, this concentration was optimal for the stimulation of la expression. This concentration was also found to be optimal for the induction of differentiation in non-T, non-B ALL tumor cells (21).

Quantitation of la and CALLA Antigens. The amount of la and CALLA expressed on the surface of ALL cells was quantitated in a cellular radioimmunoassay with saturating concentrations of monoclonal antibody and of RAM-Fc as described previously (2, 24, 25). Monoclonal antibody 21-w4, which recognizes a monomorphic determinant of la and which binds to la-like molecules on the surface of all leukemic and normal human cells tested so far, irrespective of their HLA-DR allotype (2, 19), was used in the la quantitation assay. This antibody cross-reacts with murine la.7 specificity, present on the $\alpha$-chain of the I-E molecule and homologous to the $\alpha$-chain of HLA-DR molecule (9). Culture supernatant of the P3/X63-Ag8 myeloma parental line was used as a control in the la assay. The amount of CALLA antigen (data shown in Chart 1) was quantitated using BA-3 monoclonal antibody (18) kindly provided by Dr. T. LeBien (University of Minnesota, Minneapolis, MN). Ascites of the T15 tumor was used as a control in the CALLA assay.

The latter cells were mononuclear cells obtained by Ficoll-Hypaque density gradient centrifugation of peripheral blood from healthy donors. MLRs were set up in triplicate and were incubated for 6 days. One $\mu$Ci of [3H]thymidine (6.7 Ci/mmol) was added to the cultures. After 16 hr, cells were transferred onto fiberglass discs with an automated harvester and were dried, and the radioactivity present was measured and expressed as cpm radioactivity of RAM-Fc ranged from 400 to 700 cpm/ng protein in the la assay and 700 to 1500 cpm/ng protein in the CALLA assay. This specific radioactivity was calculated in every experiment, and ng RAM-Fc bound were converted to molecules RAM-Fc bound per cell assuming all cells were labeled (24, 25); 100 ng of RAM-Fc bound per 5 x 10$^6$ cells corresponds to 1 x 10$^5$ molecules/cell. Saturation levels were achieved in every case, and the level of la was estimated from the plateau of the saturation binding curves. It has been demonstrated previously that saturation of la-accessible sites on the chosen number of target cells was achieved with a final concentration of 21-w4 culture supernatant of 12% and a BA-3 ascites of 0.12%.5

MLR. ALL cells cultured with or without TPA were harvested; washed 3 times with PBS; resuspended in RPMI 1640 medium supplemented with penicillin, streptomycin, and 20% heat-inactivated human AB serum; and irradiated with 2500 rads. ALL cells were tested as stimulators at concentrations ranging from 0.5 x 10$^6$ to 10 x 10$^6$ cells/ml in flat-bottomed wells, in a 200-µl volume containing 5 x 10$^4$ responder cells. The latter cells were mononuclear cells obtained by Ficoll-Hypaque gradient centrifugation of peripheral blood from healthy donors. MLRs set up in triplicate were incubated for 6 days. One $\mu$Ci of [3H]thymidine (6.7 Ci/mmol) was added to the cultures. After 16 hr, cells were transferred onto fiberglass discs with an automated harvester and were dried, and the radioactivity present was measured and expressed as cpm radioactivity of RAM-Fc ranged from 400 to 700 cpm/ng protein in the la assay and 700 to 1500 cpm/ng protein in the CALLA assay. This specific radioactivity was calculated in every experiment, and ng RAM-Fc bound were converted to molecules RAM-Fc bound per cell assuming all cells were labeled (24, 25); 100 ng of RAM-Fc bound per 5 x 10$^6$ cells corresponds to 1 x 10$^5$ molecules/cell. Saturation levels were achieved in every case, and the level of la was estimated from the plateau of the saturation binding curves. It has been demonstrated previously that saturation of la-accessible sites on the chosen number of target cells was achieved with a final concentration of 21-w4 culture supernatant of 12% and a BA-3 ascites of 0.12%.5

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RESULTS

Characteristics of Leukemic Cells. Nine patients diagnosed as non-T, non-B ALL were studied. Their peripheral WBC counts, ranging from 1.9 x 10^9/liter to 296 x 10^9/liter, and the characteristics of their leukemic cells are shown in Table 1. The mononuclear cell populations obtained from bone marrow or peripheral blood contained more than 90% leukemic cells as determined by Wright-Giemsa staining. The mean percentage of la+ and CALLA+ cells, assessed by immunofluorescence with monoclonal antibodies, were 90% (Table 1). The amount of la found on the leukemic cells varied from 0.9 x 10^9 to 7.1 x 10^9 molecules of RAM-Fc bound/cell with a mean of 3.8 ± 2.0 x 10^9 molecules/cell. This value is not significantly different from that calculated in a recent study for a group of 37 similar patients, i.e., 4.2 ± 2.0 x 10^9 molecules/cell. Thus, in terms of la expression, the patients studied here are representative of non-T, non-B ALL.

The levels of la are expressed in molecules of RAM-Fc bound/cell and not in molecules of 21w4 antibody bound/cell. However, in cases where quantitation experiments were done with purified 125I-labeled 21w4-igg, the RAM-Fc/21w4 ratio was shown to be one. Also, as mentioned earlier, 21w4 antibody probably reacts with the a-chain of HLA-DR molecules. The latter is not polymorphic, and it is likely that the epitope recognized by 21w4 antibody probably reacts with the a-chain of HLA-DR molecules. The latter is not polymorphic, and it is likely that the epitope recognized by 21w4 antibody probably reacts with the a-chain of HLA-DR molecules. Therefore, we are confident that the cellular radioimmunoassay used reflects the quantitative expression of la (HLA-DR-like) on human cells.

The modal volumes of ALL cells are also illustrated in Table 1. The range observed was from 138 to 210 fl with a mean of 178 ± 25 fl. In the previous study, a range of 138 to 302 fl was observed for 34 cases with a mean of 189 fl. These values are similar to those observed previously for CLL cells (163 ± 31 fl) (24) and for normal peripheral blood lymphocytes (205 ± 10 fl) (6).

Effects of TPA on Cell Markers and Volume of ALL Cells. The ability of the tumor promoter TPA to modulate the differentiation of non-T, non-B ALL was investigated. The presence of cytoplasmic µ-chain was not induced in any of the cases studied. In Case K. E., in which 8 to 10% of the cells were positive for cytoplasmic µ-chain, there was little change in staining intensity following culture with TPA. Surface Immunoglobulin was negative in all cases following culture with TPA.

The percentage of cells staining for la did not change significantly following culture with or without TPA. Table 2 shows that ALL cells cultured with and without TPA contained 94 ± 5 and 86 ± 10% of la+ cells, respectively, compared to 90 ± 8% prior to culture. Similarly, the percentage of CALLA+ cells was unchanged following culture with or without TPA in the 2 cases studies (C. L. and K. E.).

An increase in the volume of ALL cells was observed following culture with TPA. The average ratio calculated for the modal volume after culture with TPA to the modal volume of fresh ALL cells is 1.25 (from 0.96 to 1.54), whereas the ratio for the modal volume after culture without TPA to the modal volume of fresh ALL cells was 1.01, ranging from 0.78 to 1.11 (Table 2). These results imply a mean increase of 25% in ALL modal volume induced by culture with TPA. The level of statistical significance between these 2 ratios has a p value of <0.05.

Effects of TPA on la Expression. The level of la found on the surface of ALL cells following culture with or without TPA was estimated as described above for fresh ALL cells. Saturation binding levels were established for every sample, prior to and following culture with or without TPA. Results, summarized in Chart 2, indicate that, with all cases studied, TPA induced an increase in la expression (Chart 2, ■ compared to □). In all cases, the levels of la in the TPA+ cultures (Chart 2, ■) were higher than those in the TPA− cultures (Chart 2, □). However, in 4 cases (M. C., B. R., R. O. and K. E.), culturing the cells without TPA also resulted in an increase in la level. Similar observations have been made for CLL cells (24, 25), and the increase in la was ascribed to the presence of mitogenic factors in fetal calf serum (6).

When the la levels observed after culture with or without TPA are compared to those found on fresh ALL cells, a ratio of 4.25

<table>
<thead>
<tr>
<th>Patient</th>
<th>% of la+ cells</th>
<th>Ratios of volumes to fresh ALL</th>
<th>Ratios of la levels to fresh ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPA+</td>
<td>TPA−</td>
<td>TPA+</td>
</tr>
<tr>
<td>G. O.</td>
<td>79</td>
<td>86</td>
<td>1.33</td>
</tr>
<tr>
<td>T. A.</td>
<td>78</td>
<td>90</td>
<td>0.25</td>
</tr>
<tr>
<td>M. C.</td>
<td>84</td>
<td>97</td>
<td>1.84</td>
</tr>
<tr>
<td>B. R.</td>
<td>71</td>
<td>91</td>
<td>2.77</td>
</tr>
<tr>
<td>R. O.</td>
<td>96</td>
<td>98</td>
<td>3.19</td>
</tr>
<tr>
<td>K. E.</td>
<td>99</td>
<td>97</td>
<td>1.89</td>
</tr>
<tr>
<td>B. A.</td>
<td>86</td>
<td>91</td>
<td>1.00</td>
</tr>
<tr>
<td>C. L.</td>
<td>98</td>
<td>99</td>
<td>1.14</td>
</tr>
<tr>
<td>Mean</td>
<td>86 ± 10 &amp;</td>
<td>94 ± 5 &amp;</td>
<td>1.68 ± 0.96 &amp;</td>
</tr>
</tbody>
</table>

* Mean ± S.D.

* & Mean ± S.D.
± 1.11 is observed for TPA+ culture/fresh ALL, whereas a ratio of 1.68 ± 0.96 is obtained for TPA− culture/fresh ALL (Table 2). These ratios are significantly different with a probability value of <0.01. A linear regression analysis was performed between the level of la observed on leukemic cells following culture with TPA and that measured on the corresponding fresh ALL cells (Chart 3). A correlation factor of 0.92 was calculated. This analysis suggests that there is, on average, a 4-fold increase in the la level induced by TPA in the non-T, non-B ALL cells studied.

Increase in la but not in CALLA Expression Induced by TPA. With leukemic cells from 5 patients, the levels of CALLA antigens were measured following culture with and without TPA. Chart 1 illustrates the results obtained with Cases C. L. and K. E. No increase in the level of CALLA was observed in either case, although a significant increase in la was induced by TPA. In the 3 other cases studied (data not shown), no changes in CALLA levels were observed while changes in la similar to those reported in Chart 2 were seen. Notice that CALLA levels, 1 to 2 × 10^5 molecules/cell (Chart 1), are much lower than la levels. In a study of 13 cases assessed simultaneously for la and CALLA, an average level of CALLA of 1.1 × 10^5 molecules of RAM-Fc bound/cell (from 0.34 × 10^5 to 2.2 × 10^5) and an average level of la of 4.1 × 10^5 molecules of RAM-Fc bound/cell (from 0.6 to 8.7 × 10^5) were estimated.

Chart 1 also illustrates that adding phorbol instead of phorbol ester to the cultures (Chart 1, D) caused a minimum increase in la and no change in CALLA. The increase in la induced by the phorbol ester TPA is, thus, specific for that compound, since culture with phorbol in medium plus fetal calf serum, or culture with medium plus fetal calf serum alone, caused only a minimum increase in la relative to that induced by the tumor promoter.

Effects of TPA on DNA, RNA, and Protein Synthesis. The 2 cases described in Chart 1 were also assessed for their ability to incorporate [3H]thymidine, [3H]uridine, and a mixture of [14C]valine, threonine, leucine during a 16-hr pulse which followed the 48-hr culture period with or without TPA. Fresh ALL cells were also pulsed for 16 hr. Chart 4 demonstrates that culturing ALL cells from Patient C. L. either in medium plus fetal calf serum alone, or with 1.6 × 10^-9 M phorbol, increased significantly the incorporation of [3H]thymidine. An increase is also observed for ALL cells of Patient K. E. when cultured in medium plus fetal calf serum alone. This increase in DNA synthesis is likely to be due to the presence of mitogenic factors in the fetal calf serum. TPA induced a large decrease in [3H]thymidine incorporation in both cases; furthermore, it blocked the ability of fetal calf serum to stimulate DNA synthesis.
Results observed with [3H]uridine incorporation are also shown in Chart 4. Culturing ALL cells from Patient C. L. in either medium plus fetal calf serum alone, 1.6 x 10^-9 M phorbol, or 1.6 x 10^-9 M TPA caused a 2-fold increase in [3H]uridine incorporation. Culturing ALL cells from Patient K. E. with TPA caused a 3-fold increase in [3H]uridine uptake, whereas culturing the cells in medium plus fetal calf serum alone had no effect. It would be necessary to analyze several more cases to verify if TPA consistently causes an increase in RNA synthesis or not.

However, in the 2 cases studied, TPA caused a 3- to 4-fold increase in the incorporation of [14C]labeled amino acids (Chart 4). Culturing the cells with medium plus fetal calf serum alone, or in the presence of phorbol, had no effect on protein synthesis. These results suggest that the increase in la level might be occurring at the same time as an increase in protein synthesis; however, it is likely that the augmentation in the rate of protein synthesis is restricted to certain proteins, as suggested by the observation that the level of CALLA antigen is unchanged in the 5 cases studied.

Effects of TPA on MLR Stimulatory Capacity of Common ALL. Following culture in the presence or absence of TPA, ALL cells were tested for their ability to stimulate in a MLR. The values observed in the control group reflect the variation in the capacity of ALL cells to stimulate in the MLR. Cells from Patients G. O., M. C., and B. R. were poor stimulators, whereas cells from Patients T. A., R. O., and T. H. were relatively good stimulators (Table 3). TPA was able to increase by 63- and 38-fold, respectively, the MLR-stimulatory capacity of 2 of the ALL cases classified as poor stimulators, i.e., M. C. and B. R. With G. O., a 7-fold increase in MLR-stimulatory capacity was seen with one responder. TPA was also effective in potentiating the ability of the ALL cells already classified as good stimulators of the MLR. Increase of 2.3, 3.0, and 8.2 were observed for Cases T. A., T. H., and R. O., respectively.

### DISCUSSION

With the phorbol ester TPA as an active modulator of differentiation (10, 23–25, 29), it was of interest to study its ability to induce changes in non-T, non-B ALL cells arrested at early stages of differentiation. In the present study, we have found, in the 8 cases of non-T, non-B ALL studied, a 4-fold increase in la expression induced by TPA. The mechanism by which TPA causes such an effect is unclear. The increase in the level of la cannot be attributed to an increase in cell size; a 25% increase in cell volume cannot account for a 400% increase in antigen expression at the cell surface. There is a net increase in la density calculated in molecules/unit of surface area, and Chart 4 suggests that the augmentation in the la level might be due to an increase in the amount of la synthesized. In 2 cases studied for amino acid incorporation, a 3- to 4-fold increase in the rate of protein synthesis was demonstrated. However, the latter might be restricted to certain proteins, since no changes in CALLA levels were observed (Chart 1). In mouse epidermis, TPA induced the synthesis of specific proteins in a temporal sequence which reflected the program of differentiation of those cells (5, 16). Our results suggest that TPA induces an increase in la synthesis, although we have not demonstrated it directly by pulse chase experiments and immunoprecipitation. Other factors could be responsible for higher la levels. Unmasking of antigenic sites following treatment with TPA could be secondary to membrane perturbations induced by the drug. Alternatively, conformational changes in la molecules, following TPA treatment, could increase the avidity of the 21w4 antibody for accessible epitopes. Since the role of la in cells at early stages of lymphocyte differentiation is unknown, it is difficult to assess the functional significance of an increase in the la level. La glycoproteins are implicated in interactions between macrophages and T- and B-cells (3). However, a more general role of la in cellular interactions and/or in regulatory steps involved in differentiation cannot be excluded.

Nadler et al. (21) have recently reported that TPA could induce the expression of B1 antigen and of cytoplasmic μ-chain in several cases of non-T, non-B ALL, but they did not detect any changes in la. LeBien et al. (17) have reported changes in BA-2 antigen in 3 of 7 ALL cases, while no changes were observed in the percentage of cells labeled with BA-1, BA-3, or anti-la antibodies following culture with TPA. If one only considers the percentage of la’ cells, no changes in la can be detected in our study as well; 94 ± 5 and 86 ± 10% of cells were la’ following culture with or without TPA (Table 2). However, when la levels were estimated quantitatively using a cellular radioimmunoassay under saturating conditions, changes in la levels of the order of 4-fold were observed.

We did not detect the production of cytoplasmic μ-chain following culture with TPA. Similarly, LeBien et al. (17) could not induce the production of cytoplasmic μ-chain in a non-T, non-B ALL cell line following culture with TPA for periods of time of up to 96 hr. Cossman et al. (7) have demonstrated recently in one case of ALL the induction of cytoplasmic and surface μ- and κ-
chains following TPA treatment. Both mu- and kappa-genes were shown to be rearranged in these leukemic cells prior to TPA treatment, although no cytoplasmic mu-chain production could be detected. The authors concluded that TPA affected the transcription or translation of the effectively recombined genes (17). Thus, it is possible for a non-T, non-B ALL cell to carry functionally rearranged mu- and kappa-genes without producing detectable levels of cytoplasmic mu-chain. Since the majority of non-T, non-B ALLs appear to have rearranged immunoglobulin genes and to be genetically committed to the B-lineage, the lack of cytoplasmic mu-chain production following TPA treatment could be explained by nonfunctional mu-chain gene rearrangements in the cases that we studied. In contrast, the leukemias studied by Nadler et al. (21) and by Cosman et al. (7) would represent cells with efficiently rearranged mu-chain genes which can be triggered by TPA to produce cytoplasmic mu-chain. Alternatively, the fluorescence microscopy detection assay might not be sufficiently sensitive to permit the visualization of very low levels of cytoplasmic mu-chain present in the ALL cases studied here, before and/or after stimulation with TPA.

The ability of non-T, non-B ALL cells to stimulate in an MLR was variable and was not correlated with the level of la present on the cells (Tables 1 and 3). Following incubation with TPA, a substantial increase was seen in the ability of ALL cells to stimulate the MLR (24). Alternatively, the effect on the MLR stimulation might be due to a nonspecific interaction of lipophilic TPA with the plasma membrane.

It is interesting to speculate that the increase in la induced by TPA in non-T, non-B ALL is associated with a signal for differentiation. Since it is believed that most of these cells are B-cell precursors, it is surprising that TPA can induce the expression in these poorly differentiated cells of la levels reaching those found in mature B-cells and activated T-cells (19). This would imply that a high level of la does not necessarily correlate with a functionally mature B-cell or an activated T-cell but that it can be induced with the proper stimulus in other cells, as seen here, with poorly differentiated non-T, non-B ALL cells.

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Response of Non-T, Non-B Acute Lymphocytic Leukemia Cells to Phorbol Ester

Jun Okamura, Erwin W. Gelfand and Michelle Letarte

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