

INDUCTION OF HLA-DC/DS (LEU 10) ANTIGEN  
EXPRESSION BY HUMAN PRECURSOR B CELL LINES\*

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HLA-D region related antigens have been shown to be expressed on B cells, a minor population of T cells, activated T cells, monocytes, and hematopoietic precursor cells (reviewed in 1). The recently described HLA-linked DC/DS antigens (2, 3) have more limited cell distribution (4-6) and are expressed predominantly on B cells, activated T cells, and a minor population of monocytes. Although their expression is documented in cases of B cell chronic lymphocytic leukemia (CLL), they are infrequently found on cells of acute lymphoblastic leukemia (ALL) of non-T cell type (4).<sup>1</sup> Cell lines of ALL origin also fail to express these antigens (6). In addition, nonlymphoid leukemia cells as well as precursor cells of the erythroid and myeloid series have been shown to lack them (5, 6).

Phorbol esters have been found to exert a variety of effects on hematopoietic cells as well as on other tissue (7). In particular, the phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA) has been shown to induce CLL cells to differentiate (8) and to cause phenotypic changes in ALL cells as well as causing ALL cell lines to express certain B cell antigens (9, 10). In the present investigation, TPA is shown to induce three precursor B cell lines to express HLA-DC/DS antigens that are detected by the Leu 10 antibody. This is accompanied by a concomitant decrease in the expression of the common ALL antigen (CALLA) and the enzymatic activity of terminal deoxyribonucleotidyl transferase (TdT).

**Materials and Methods**

*Cells and Culture Conditions.* Human non-T ALL cell lines Reh and Nalm 12 (11) were kindly provided by Dr. P. Ralph of the Memorial Sloan-Kettering Cancer Center. Cell line Josh-7 was derived from a patient with X-linked agammaglobulinemia (12). It has little detectable Ig and is HLA-DR<sup>+</sup>, CALLA<sup>+</sup>, and TdT<sup>-</sup>. Cell lines were maintained in RPMI-1640 with 10% heat-inactivated fetal calf serum, glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (regular medium).

*TPA Cultures.* TPA (Sigma Chemical Co., St. Louis, MO) was stored at -20°C at 1 mg/ml in acetone. For induction experiments, cells were cultured in regular medium at 5 × 10<sup>5</sup> cells/ml. TPA was added to yield appropriate final concentrations. Control cultures received an equal amount of acetone without TPA. 10 ml of cells were cultured in 25-cm<sup>2</sup> flasks (Falcon Labware, Oxnard, CA) at 37°C in a humidified atmosphere of

\* Supported by grants AI-18321 and CA-34546 from the U. S. Public Health Service, grant IM-255 from the American Cancer Society, and by the Levine Laboratory Fund.

<sup>1</sup> Al-Katib, A., and B. Koziner. Leu 10 antigen distribution in human leukemic disorders as detected by a monoclonal antibody: correlation with HLA-DR expression. Manuscript submitted for publication.

5% CO<sub>2</sub>. Media were changed every 2 d. Cells were maintained for 6–8 d with viability >80% by trypan blue exclusion.

*Monoclonal Antibodies.* An IgG<sub>1</sub>(CW) mouse monoclonal antibody against HLA-DR was prepared in our laboratory. Monoclonal anti-HLA-DR(BD) and Leu 10 antibodies were obtained from Becton, Dickinson & Co., Mountain View, CA. Anti-CALLA antibody J5 was from Coulter Electronics, Inc., Hialeah, FL. Antibody ILR-1 directed against a HLA-SB antigen (13) was kindly provided by Dr. E. Yunis, Sydney Farber Cancer Center, Boston, MA. All these antibodies were used at saturated concentrations. Normal mouse IgG (Cappel Laboratories, Cochranville, PA) was used as a control at 100 µg/ml.

*Immunofluorescence.* Monoclonal antibody binding to cell surface was detected by flow cytometry with a FACS IV (B-D FACS systems, Becton, Dickinson & Co., Sunnyvale, CA).  $5 \times 10^5$  cells were incubated with 100 µl of supernatants at 4°C for 1 h. After two washings with phosphate-buffered saline, the cells were stained with 100 µl of fluorescein-conjugated F(ab')<sub>2</sub> fragments of affinity-purified goat anti-mouse Ig antibodies (Cappel Laboratories) for 1 h. After three washes, the cells were analyzed.

*TdT assay.* Enzymatic detection of TdT was carried out as described previously (14).

*Iodination, Immunoprecipitation, and Two-dimensional Gel Electrophoresis.* Cell surface iodination and immunoprecipitation were performed as described previously (15). Immunoprecipitated material was analyzed on a two-dimensional gel system, modified from the technique of O'Farrell et al. (16). First-dimensional resolution was performed on an isoelectric focusing (IEF) slab gel with pH 3.5–10 ampholines. The slabs were then sliced into strips for subsequent second-dimensional resolution with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Marker proteins of known molecular weight were co-electrophoresed on each SDS-PAGE gel. Gels were then stained, dried on filter paper under vacuum, and autoradiographed on Kodak X-Omat R film.

## Results

*TPA Induction of HLA-DC/DS Antigen and Disappearance of CALLA by Immunofluorescence.* Three cell lines (Josh 7, Reh, and Nalm 12) were selected for this investigation. By immunofluorescence, Josh 7 and Reh did not make any Ig whereas Nalm 12 made only cytoplasmic µ chains. These cell lines represent human precursor and pre-B cell lines (11, 12). Immunofluorescence studies as shown in Fig. 1A indicated that they expressed HLA-DR antigens (>85% positive) as detected by two monoclonal anti-HLA-DR (BD and CW) reagents. Although not shown, antibody ILR-1, reactive with SB antigens (13), stained these cell lines in a similar manner. All three cell lines expressed CALLA (>80% positive) as detected by antibody J5. In contrast, very few cells stained with the antibody Leu 10, which reacts with HLA-DC/DS molecules linked to HLA-DR 1–6 but not with those linked to HLA-DR7 (F. Brodsky, Becton, Dickinson & Co., Mountain View, CA, personal communication and 17).

The expression of HLA-DR, HLA-DC/DS, and CALLA antigens were studied further with the addition of TPA to the cultures. Preliminary experiments indicated that  $1.6 \times 10^{-9}$  M was optimal for induction studies. This concentration was used for all subsequent experiments. TPA at this concentration induced marked morphological changes in all three cell lines. Analysis with [<sup>3</sup>H]thymidine incorporation indicated that DNA synthesis was markedly inhibited in these cultures. Care has also been taken to change media every other day to maintain >80% viability in all cultures studied.

TPA was found to induce HLA-DC/DS antigen expression in all three cell lines (Fig. 1B). An increase in Leu 10<sup>+</sup> cells in TPA-treated Josh 7 cultures was evident after 96 h of incubation. After 6 d, >80% of cells in TPA-treated cultures stained positive for the antibody Leu 10. In addition, there were changes in

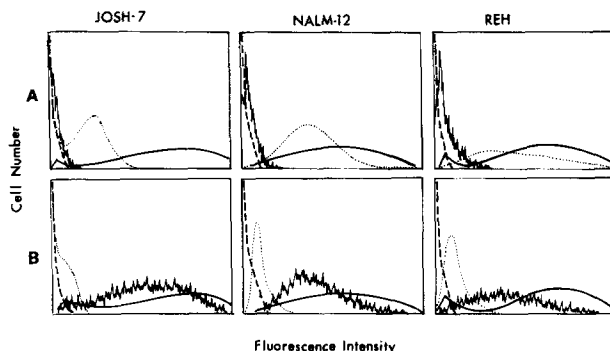


FIGURE 1. Indirect immunofluorescence analysis by flow cytometry. (A) Day 6, control cultures; (B) day 6, TPA-treated cultures; (---) control using mouse Ig; (jagged line) HLA-DC/DS detected by monoclonal antibody Leu 10; (···) CALLA detected by monoclonal antibody J5; and (—) HLA-DR detected by monoclonal antibody anti-HLA-DR (CW).

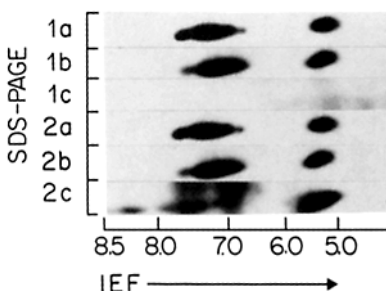


FIGURE 2. Two-dimensional gel electrophoresis of iodinated molecules precipitated by (a) monoclonal antibody anti-HLA-DR (CW), (b) monoclonal antibody anti-HLA-DR (BD), and (c) monoclonal antibody Leu 10. 1a, 1b, and 1c were from control Josh 7 cells and 2a, 2b, and 2c were from TPA-treated Josh 7 cells.

CALLA expression. In the case of Josh 7, >80% of control cells were CALLA<sup>+</sup> and only 15% of the TPA-treated cells were stained. Similar results were obtained in Reh and Nalm 12. TdT enzymatic activity was also determined in Reh and Nalm 12. After TPA treatment, TdT was not detectable in Nalm 12 (from 0.43 nmol/10<sup>8</sup> cells to 0) and a >90% reduction (from 1.40 nmol/10<sup>8</sup> to 0.11 nmol/10<sup>8</sup>) of enzyme activity was observed in Reh. In contrast, there were no appreciable changes in HLA-DR and HLA-SB antigen expression in the TPA-treated cultures of all cell lines. There were also no changes in the Ig expression patterns and no induction of expression of sheep erythrocyte receptors.

*Further Evidence for HLA-DC/DS Induction.* Two additional experiments were carried out to document TPA induction of HLA-DC/DS expression in precursor B cell lines. 30 × 10<sup>6</sup> cells from day 6 Josh 7 control and TPA-treated cultures were iodinated by the lactoperoxidase method. After solubilization, immunoprecipitates with two monoclonal antibodies against HLA-DR antigens (BD and CW) and monoclonal antibody Leu 10 were obtained and analyzed by two-dimensional gel electrophoresis (IEF, SDS-PAGE). As shown in Fig. 2, both anti-HLA-DR antibodies precipitated a 29,34 kD bimolecular complex from the control and TPA-treated cultures (Fig. 2 1a, 1b, 2a, and 2b). In contrast, antibody Leu 10 did not precipitate any discernable components from the control culture (Fig. 2

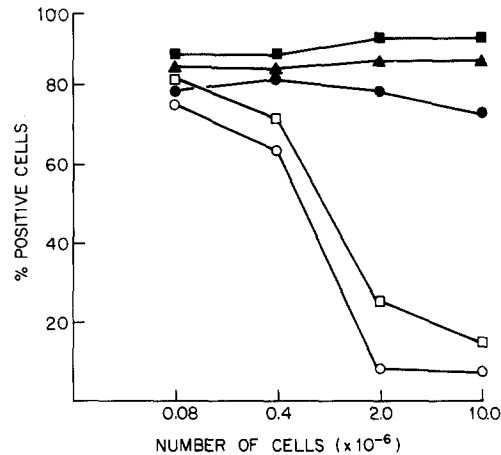


FIGURE 3. Absorption of monoclonal antibody Leu 10 with cell lysates. Monoclonal antibody Leu 10 was absorbed with varying amount of cells from different cell lines and the supernatants were used to stain TPA-treated Josh 7 cells. Control cells from Josh 7 (■), Reh (●), and molt 4 (▲). TPA-treated cells from Josh 7 (□) and Reh (○).

1c) as well as from untreated cultures (not shown). However, a distinct molecular complex with a similar molecular weight to that of the HLA-DR antigen and with a much wider isoelectric point range for both the  $\alpha$  and  $\beta$  chains (as shown in Fig. 2 2c) was observed in TPA-treated Josh 7 cells.

Absorption experiments were carried out to determine the amount of HLA-DC/DS antigen expressed by TPA-treated cells. For control experiments, Josh 7 and Reh, as well as Molt 4, a T cell line that did not express Leu 10 antigen and which was not inducible, were used. TPA-treated Josh 7 and Reh cells were pelleted and disrupted by three freeze and thaw cycles. Antibody Leu 10 was used at a concentration two-fold above the end point. After absorptions, the antibody was used to stain day 6 TPA-treated Josh 7 cells. As shown in Fig. 3, TPA-treated Josh 7 and Reh cells were efficient in removing the Leu 10 reactivity. Untreated Reh cells were able to remove some Leu 10 antibody at high cell concentrations. Both Molt 4 and untreated Josh 7 cells failed to remove any Leu 10 activity. Quantitatively, TPA-treated Josh 7 cells as well as TPA-treated Reh cells were at least 25-fold more efficient in removing Leu 10 antibody activity than their respective untreated cells, indicating a significant induction of Leu 10 antigen synthesis and expression after TPA treatment.

### Discussion

Three methods, i.e., immunofluorescence, biochemical identification, and absorption, have been used to show that TPA can induce precursor and pre-B cell lines to express the HLA-DC/DS antigens. This expression is accompanied by the disappearance of CALLA and a marked reduction of TdT. These changes indicate more mature phenotypic expression. LeBien and coworkers (9) have shown that TPA induced Reh to express BA-2 antigen (p24) with a concomitant decrease in TdT expression. In four common ALL cell lines, Nadler et al. (10) reported that TPA induced the expression of B1 antigen. In their study, the expression of CALLA was not significantly changed. This difference may be due

to the shorter time course in their study. Nevertheless, their results suggest that most non-T ALL cell lines can be induced to mature in vitro.

The monoclonal antibody Leu 10 precipitated a 29,34 kD bimolecular complex with different  $\alpha$  and  $\beta$  chains from those of HLA-DR antigens. The antibody stains B cells from donors with HLA-DR 1-6 and B cell lines transformed by Epstein-Barr virus. In addition, few bone marrow cells (a much lower number than that of HLA-DR-positive cells) have been found to express this antigen. The studies carried out by F. Brodsky (personal communication) at the Becton Dickinson Monoclonal Center, Inc. have led to the conclusion that the Leu 10 antigenic determinant is expressed on the HLA-DC/DS molecules linked to HLA-DR 1-6. This antibody has broader reactivity than the antibody Genox 3.53, which detects a polymorphic determinant on DC/DS molecules linked to DR1, DR2, and DR6 (17). The complexity of the HLA-D region antigenic specificities is beyond the scope of this report. It suffices to state that both Leu 10 and Genox 3.53 antibodies give similar cell staining patterns in reactive individuals. It is of interest to note that the DC/DS determinant detected by Genox 3.53 is not expressed on AML blasts and precursor cells of the erythroid and myeloid series (4, 5). In addition, Genox 3.53 fails to stain TdT<sup>+</sup> bone marrow cells and only 20% of the pre-B cells are positive (6). It appears that DC/DS expression is limited to more mature stages of B cell differentiation. In the present study, the failure of control Josh 7 cell lysate to absorb Leu 10 antibody and the efficient removal of this antibody by TPA-treated Josh 7 lysate indicate that newly synthesized DC/DS molecules are induced. Thus, it appears that there is a stage of human B cell differentiation with phenotypes TdT<sup>-</sup>, CALLA<sup>-</sup>, Ig<sup>-</sup>, DR<sup>+</sup>, and DC/DS<sup>+</sup>. The expression of HLA-DC/DS antigen precedes the expression of membrane Ig but follows the expression of HLA-DR antigen. This is perhaps relevant to the role of these molecules in cell interaction in subsequent B cell development.

### Summary

The expression of HLA-DC/DS antigen detected by the monoclonal antibody Leu 10 was studied in three human precursor and pre-B cell lines (Josh 7, Reh, and Nalm 12). Flow cytometric analysis showed that none of these cell lines stained for the HLA-DC/DS antigen. In the presence of  $1.6 \times 10^{-9}$  M of 12-*O*-tetradecanoylporbol-13-acetate (TPA), expression of this antigen was detected. The expression was completed after 168 h of incubation. Iodination of cell surface, immunoprecipitation by Leu 10 antibody, and two-dimensional gel analysis revealed that TPA-treated Josh 7 cells synthesized and expressed a 29,34 kD bimolecular complex with both  $\alpha$  and  $\beta$  chains different from those of HLA-DR antigen. Quantitative absorption experiments with cell lysates indicated a >25-fold increase in HLA-DC/DS antigen in TPA-treated cells.

With the induction of HLA-DC/DS antigen expression, there are concomitant decreases in the expression of the common acute lymphoblastic leukemia antigen (CALLA) and the enzymatic activity of terminal deoxynucleotidyl transferase. No appreciable changes in HLA-DR and Ig expression were observed. There was also no change in HLA-SB expression as detected by antibody ILR-1. However, DNA synthesis was markedly inhibited by TPA treatment. These results indicate that precursor and pre-B cell lines can be induced to mature in

vitro. They also suggest that the expression of HLA-DC/DS antigen which precedes the expression of membrane Ig and follows the HLA-DR expression is relevant to human B cell development and cell interaction.

*Received for publication 8 September 1983.*

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