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Induction of plasmacytoid differentiation by phorbol ester in B-cell lymphoma cell lines bearing 8;14 translocations

(Burkitt lymphoma/c-*myc* expression/IgM expression/maturational arrest)

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ABSTRACT **At nanomolar concentrations, phorbol 12-myristate 13-acetate induced differentiation in a human Epstein–Barr virus-negative B-cell line, JD 38, derived from an undifferentiated lymphoma and containing an 8;14 translocation. The changes induced by phorbol 12-myristate 13-acetate were consistent with differentiation towards plasma cells and included (*i*) a marked increase (30-fold) in IgM secretion; (*ii*) a decrease in the nuclear/cytoplasmic ratio associated with the development of a single prominent nucleolus instead of multiple nucleoli; (*iii*) the development of parallel arrays of rough endoplasmic reticulum, eccentric nuclei, and marginated heterochromatin; (*iv*) a reduction in the expression of surface markers, including common acute lymphoblastic leukemia antigen, IgM, and C3 receptors. Essentially all cells showed plasmacytoid differentiation, although the degree varied. Rare cells (<1%) appeared to be terminally differentiated into plasma cells. The increase in secreted IgM was preceded by a small increase in μ-chain RNA, with an increase in the ratio of secreted to membrane form. A small increase in c-*myc* RNA was also detected with differentiation. This might reflect coordinate regulation of the transcription of immunoglobulin and the translocated c-*myc* gene. Thus, the maturational arrest of this lymphoma cell line can be overcome with phorbol 12-myristate 13-acetate, indicating that translocation of the c-*myc* gene does not permanently block the capacity for differentiation. Further, this gene continues to be expressed to at least the same level during cell maturation. Similar ultrastructural changes were induced by phorbol 12-myristate 13-acetate in four of seven additional lines studied.**

Considerable evidence has been compiled that supports the hypothesis that failure of differentiation with resultant accumulation of less differentiated cells may be of pathogenetic significance in the development of human hematopoietic malignancies, including Burkitt lymphoma (1, 2). Burkitt lymphomas and the closely related undifferentiated non-Burkitt lymphomas almost invariably contain a reciprocal chromosomal translocation involving the terminal portion of the long arm of chromosome 8 and one of the chromosomal locations of the immunoglobulin genes (3). c-*myc*, the cellular equivalent of the v-*myc* oncogene of the MC29 virus, has been shown to reside in this terminal (q24 → qter) region of chromosome 8 in man and, in some cases, has been shown directly to be translocated into the heavy chain region in cell lines that contain an 8;14 chromosomal translocation (4). It is probable that altered expression of c-*myc*, presumably brought about by the proximity of c-*myc* to an immunoglobulin gene as a consequence of the chromosomal translocation, is a critical component of the pathogenesis of Burkitt lymphoma. This is supported by the occurrence of analogous abnormalities in mouse plasmacytomas (5, 6). Because of the possibility that the genetic rearrangements in Burkitt lymphoma cells are responsible for the differentiation failure, it was of particular interest to determine whether the defect in differentiation was reversible and whether, in the event that differentiation could be induced, c-*myc* gene transcription would be altered compared to the uninduced cells.

Of the various chemical agents that have been shown to induce cellular differentiation *in vitro* (7, 8), one of the most potent is phorbol 12-myristate 13-acetate (PMA). Recent reports have provided evidence that PMA can induce differentiation of human leukemia cells, including those of acute myeloid leukemia (9), chronic lymphocytic leukemia (10), and acute lymphoblastic leukemia (11).

We have studied the effect of PMA in detail on a cell line (JD 38) derived from a malignant undifferentiated lymphoma of non-Burkitt type (modified Rappaport classification) that contains an 8;14 translocation. The JD 38 line was induced to undergo morphologic and functional changes consistent with differentiation towards a plasma cell. Changes in C_μ and c-*myc* mRNAs will be described and discussed. We have also studied other undifferentiated lymphoma-derived cell lines of both Burkitt and non-Burkitt type and have observed PMA-induced morphological changes similar to those seen in JD 38 cells.

MATERIALS AND METHODS

Cells and Cell Culture. JD 38 is an Epstein–Barr virus (EBV)-negative cell line derived in our laboratory from tumor cells circulating in the peripheral blood in a 3-year-old male child with recurrent undifferentiated non-Burkitt lymphoma (12). The cell line contains an 8;14 chromosomal translocation (13) and expresses C3 receptors (14), common acute lymphoblastic leukemia antigen (CALLA), β_2-microglobulin, and HLA-DR antigens (unpublished data). JD 38 cells also express surface IgM/κ and secrete IgM/κ (15), as did the original neoplastic cells, but do not exhibit plasmacytoid features. The other cell lines used in this study have been described elsewhere (15). The cell lines were maintained as suspension cultures as described (15).

Cells obtained on the 4th day after subculture were resuspended in fresh medium at a concentration of 5×10^5 cells per ml and incubated at 37°C in 5% CO_2 in air for 1–6 days in the presence of a broad range of PMA (P-L Biochemicals) concentrations (0.1 μM → 0.01 nM) or no PMA.

Quantitation of Ig Secretion and Cytoplasmic Ig Assay. Culture supernatants were assayed for IgM by an ELISA using an alkaline phosphatase-coupled antibody to human IgM (Sigma). Cytoplasmic IgM was assessed with a rhodamine-

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; EBV, Epstein–Barr virus; kb, kilobase(s); CALLA, common acute lymphoblastic leukemia antigen.

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coupled $F(ab)_2$ anti-human IgM (μ specific) that was allowed to react against cells fixed on slides in a 50:50 mixture of methanol/acetone.

Transmission Electron Microscopy. Cells for transmission electron microscopy were prepared from suspension and adherent cells. Nonadherent cells suspended in complete medium were fixed at 20°C with an equal volume of 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.4) for 3–8 hr. Adherent cells (present in PMA-treated cultures only) were covered with 5–10 ml of complete medium, followed by an equal volume of the same fixative as above. Following fixation, the cells were removed with a scraper and pelleted. Both adherent and nonadherent cell pellets were then embedded in epoxy resin, cured, sectioned at 80–100 mm, and stained with lead citrate and uranyl acetate.

Cell Surface Antigens. C3 receptors were investigated by assaying the ability of cells to form rosettes with erythrocyte–antibody–complement (EAC_m) complexes made as described (14).

Other cell surface antigens were detected with monoclonal antibodies, using indirect immunofluorescence and flow cytometry as described (16).

Monoclonal antibodies employed were Leu-1, Leu-5, Leu-10, and anti-HLA-DR (Becton Dickinson); BA-1, BA-2, and BA-3 (Hybritech, San Diego, CA); OKT-10 (Ortho Diagnostics); anti-β_2-microglobulin (kindly provided by A. K. Ng). The fluorescein isothiocyanate-conjugated $F(ab)_2$ component of goat anti-mouse IgG (heavy and light chain specific) (TAGO, Burlingame, CA) served as the second antibody. Fluorescein isothiocyanate-conjugated $F(ab)_2$ preparations of goat anti-human heavy and light immunoglobulin chains were used to detect surface immunoglobulin by direct fluorescent staining.

Enzymatic Assays for Esterases. Cytocentrifuge preparations of PMA-treated cells and control cells were stained with α-naphthyl acetate esterase and naphthol AS-D chloroacetate esterase by using a Sigma kit, according to the manufacturer's instructions.

Immunoglobulin and c-*myc* DNA and RNA Analysis. High molecular weight DNA was extracted from cell line JD 38 both before and following 4 days of PMA exposure. This DNA was digested to completion with the appropriate restriction endonuclease (*Bam*HI or *Eco*RI), size separated by electrophoresis on agarose gels, transferred to nitrocellulose paper, and hybridized with genomic probes of the human joining heavy region (J_H), constant μ region (C_μ), and c-*myc* (17, 18).

Cytoplasmic RNA was extracted at serial time points of 0, 3, 6, 9, and 24 hr and 4 days from equal numbers of JD 38 cells cultured both in the presence and absence of PMA (19). Serial dilutions of the cytoplasmic RNA from a given number of cells were dotted on nitrocellulose using a 96-well suction manifold. In addition, total cellular RNA was extracted by utilizing a guanidine thiocyanate procedure from JD 38 cells both in the presence and absence of PMA at times of 0 and 24 hr and 4 days. Ten micrograms of the total RNA was electrophoresed through formaldehyde gels and transferred to nitrocellulose for analysis with specific DNA probes (20). Poly(A) RNA was prepared from ≈2 mg of the total cellular RNA by selection over an oligo(dt)-cellulose column (21). Serial dilutions of this poly(A) RNA were dot blotted onto nitrocellulose. All nitrocellulose filters were baked and hybridized to either the C_μ, membrane exon (μ), or c-*myc* probe (22). Serial autoradiograms within the linear dose–response range of the film were developed and the intensity of hybridization was assessed by densitometric scanning with a DU-8 spectrophotometer (Beckman). In addition, the exact intensity of hybridization of c-*myc* to the poly(A) RNA dot blots was determined by scintillation spectroscopy of each hybridized RNA sample.

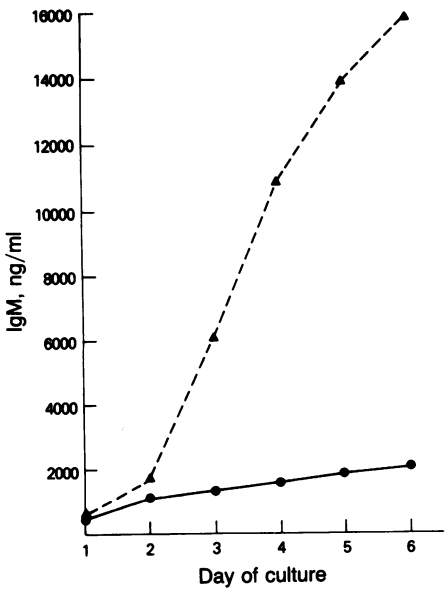

FIG. 1. IgM secretion (assayed by an ELISA technique) in control (●; no PMA) versus PMA-treated (▲; 10 mM PMA) JD 38 cells.

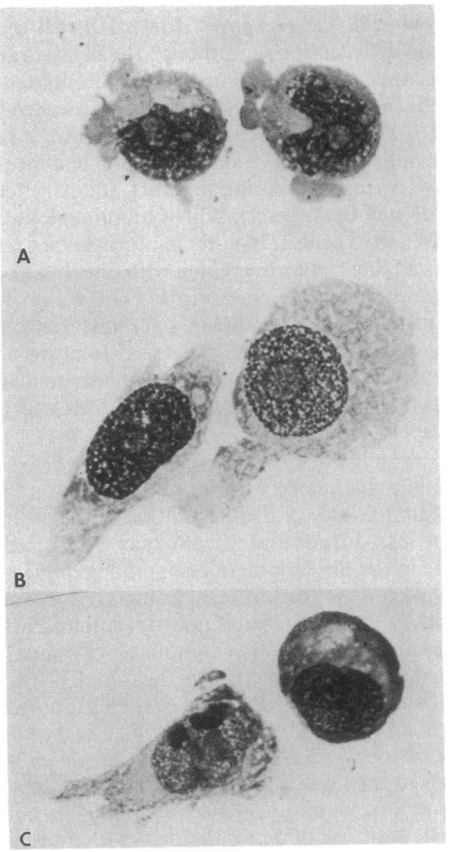

FIG. 2. Cytospin preparations of cultured JD 38 cells. (*A*) Control (untreated) cells; (*B* and *C*) treated with PMA (50 nM) for 4 days. In the absence of PMA, JD 38 cells are undifferentiated with a high nuclear/cytoplasmic ratio, multiple nucleoli, and cytoplasmic basophilia with occasional lipid droplets. After PMA treatment, the cells undergo striking morphological alteration with a decreased nuclear/cytoplasmic ratio and a single prominent nucleolus (*B*). Rare (<1%) cells closely resemble plasma cells (*C*) with condensed nuclear chromatin, an eccentric nucleus with perinuclear "hof" (Golgi apparatus), and intense peripheral cytoplasmic basophilia. (≈×420.)

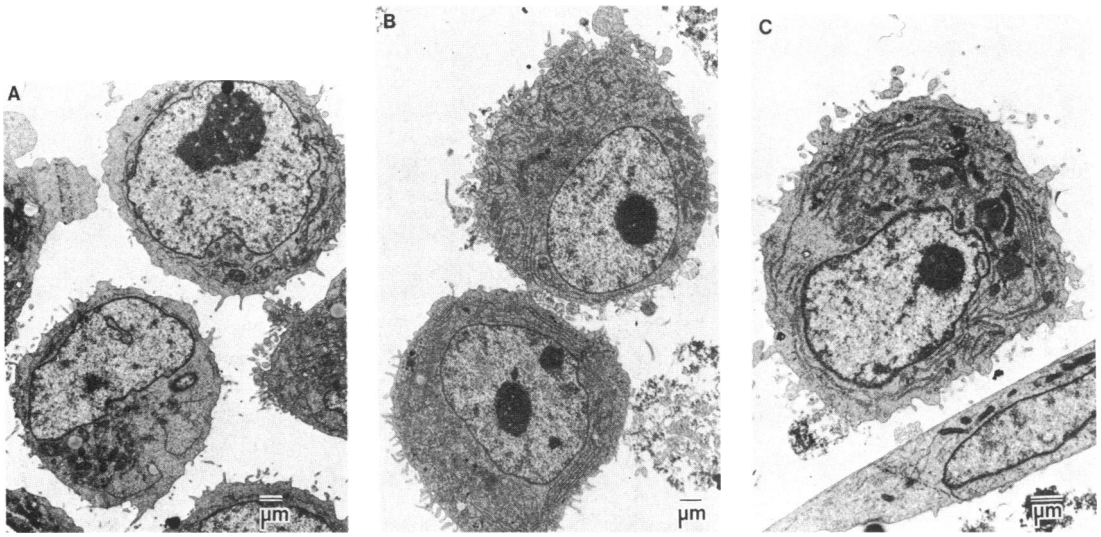

FIG. 3. Ultrastructural changes associated with PMA-induced plasmacytoid maturation of JD 38 cells. (*A*) Cells cultured for 3 days in the absence of PMA. (*B*) Nonadherent cells exposed to 50 nM PMA for 3 days. (*C*) Adherent cells exposed to 50 mM PMA for 3 days. Observed ultrastructural changes are described in the text.

RESULTS

Changes in Adherence After Treatment with PMA. JD 38 cells normally grow in suspension culture with no visible clumping. When exposed to PMA (5 nM and 0.1 μM), numerous cell clumps were apparent and a fraction of the cells (≈40%) became surface (plastic) adherent by 18 hr. The adherent cells demonstrated an increase in staining for α-naphthyl acetate esterase. There was a negative effect on cell growth with increasing PMA concentrations above 50 nM and an increase in the number of dead cells as measured by trypan blue.

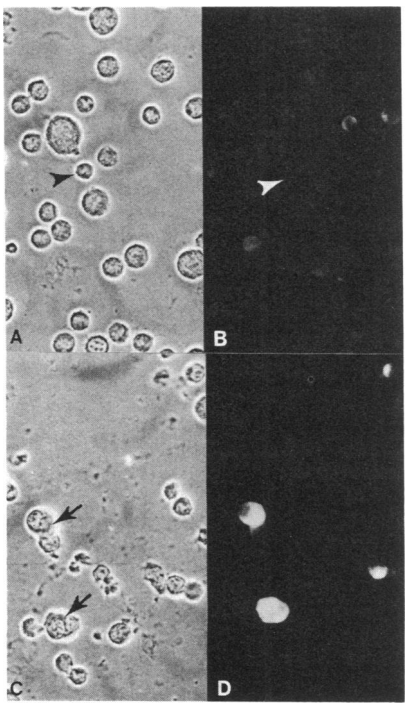

FIG. 4. Phase-contrast (left) and immunofluorescence (right) photomicrographs of JD 38 cells. (*A* and *B*) Control (untreated) cells; (*C* and *D*) cells treated with 50 nM PMA for 4 days. Untreated JD 38 cells express only small amounts of cytoplasmic IgM. Many cells seen in the same field by phase-contrast (*A*) contain little or no detectable IgM by fluorescence (*B*) (arrowhead). In contrast, PMA-treated cells occasionally demonstrate intense IgM-specific cytoplasmic fluorescence (*D*) associated with plasma cell morphology by phase-contrast (*C*) (arrows). (≈×192.)

IgM Induction by PMA. JD 38 cells normally produce IgM with no detectable secretion of IgG or IgA (15). In the presence of PMA (optimal concentration, 10 nM), there was a marked increase in the secretion of IgM (Fig. 1), up to a maximum of 30-fold on the 4th day of subculturing.

Morphological Changes Induced After PMA Treatment. *Light microscopy.* A marked difference between control and PMA-treated cells was apparent in Romanowski-stained cell smears. PMA-treated cells were larger, due to a decrease in the nuclear/cytoplasmic ratio and developed a single large nucleolus. Occasionally, cells closely resembled mature plasma cells (Fig. 2).

Transmission electron microscopy. Transmission electron microscopy, on the 3rd day after subculture, revealed unequivocal differences between PMA-treated and control JD 38 cells. Untreated cells exhibited a lymphoblastic appearance with little rough endoplasmic reticulum or Golgi development. The cytoplasm was filled with free polyribosomes, and nuclei were generally irregular, centrally placed, and contained little peripheral heterochromatin (Fig. 3*A*). In contrast, the PMA-treated cells manifested a striking plasmacytoid appearance, which was present in both floating and adherent cells (Fig. 3 *B* and *C*). Eccentric nuclei with prominent nucleoli, a regular border, and marginated heterochromatin were frequently seen; cytoplasmic maturation was evidenced by the appearance of numerous parallel arrays of rough endoplasmic reticulum, occasionally so pronounced that the cells resembled malignant plasma cells. Free ribosomes were reduced in number.

We have studied seven additional undifferentiated lymphoma cell lines of both Burkitt and non-Burkitt origin and also EBV and non-EBV associated. All contain 8;14 translocations. Four of these lines showed ultrastructural changes similar to those observed in JD 38, although to varying degrees. The most marked changes—essentially identical to those observed in JD 38—were seen in an African Burkitt lymphoma line, Namalwa (EBV positive), and an American Burkitt lymphoma line, CA 46 (EBV negative) (15). Three lines, although showing structural changes when cultured with PMA, did not evidence plasmacytoid differentiation.

Cytoplasmic Ig. Staining of PMA-treated cells with rhodamine-coupled anti-IgM showed a slight general increase in cytoplasmic fluorescence compared to control cells (Fig. 4*B*) and occasional (<1%) brilliantly stained cells indistinguish-

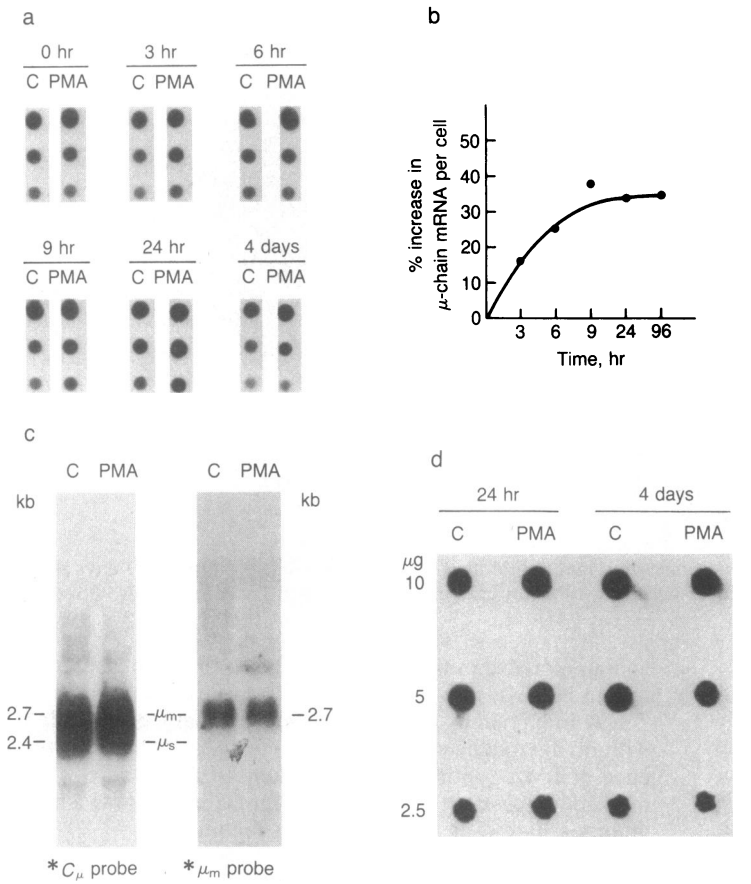

FIG. 5. (*a*) Representative examples of serially diluted cytoplasmic RNA dot blots that were hybridized with the C_μ probe. Assays were performed in triplicate in the presence and absence (C) of PMA. (*b*) Graphic demonstration of the increment in μ-chain mRNA in PMA-treated cells compared to untreated cells. (*c*) Blot analysis of JD 38 RNA at 24 hr in the presence and absence (C) of PMA, probed with C_μ and μ_m probes. (*d*) Dot blot comparison of poly(A)-selected RNA from JD 38 in the presence or absence (C) of PMA at 24 hr and 4 days of culture. The small increase in c-*myc* RNA, detected by actual counting of radioactivity in the dots is not perceptible by eye. The total cmp increased from 1274 to 1580 with PMA at 24 hr (23%) and from 1514 to 1823 at 48 hr (20%). These changes are small but clearly show that c-*myc* is not reduced by culture of JD 38 in PMA.

able from plasma cells (Fig. 4*D*).

Effect of PMA on Surface Markers. A kinetic study over 4 days in culture, using a broad range of concentrations of PMA (0.1 μM → 0.1 nM), revealed consistently decreased expression of CALLA (detected by BA-3) and surface IgM compared to cells similarly cultured without PMA. Maximal changes were observed on day 3, at which time a >50% reduction in the proportion of cells reacting with anti-μ and anti-κ and an ≈30% reduction in reactivity with BA-3 was observed at the highest PMA concentrations. Borderline decreases in OKT-10 and β_2-microglobulin was seen, but no changes were detected with the other monoclonal antibodies employed.

PMA caused a significant decrease in EAC_m rosette-forming cells—from 60% in untreated JD 38 cells to 7% in PMA-treated cells—after 48 hr in culture. Maximal reduction in rosette formation occurred at a concentration of 0.1 μM.

Rearrangement of Immunoglobulin and c-*myc* Genes in JD 38. Southern analysis of *Eco*RI- or *Bam*HI-digested JD 38 DNA probed with a J_H or C_μ region revealed that both heavy chain genes were rearranged (data not shown). One of these rearrangements represents the effectively recombined $V_H/D_H/J_H$ region responsible for the production of IgM that has been shown to be located on the normal, nontranslated chromosome 14 (23). The other rearrangement is an aberrant rearrangement of the immunoglobulin gene allele that is associated with the translocation of chromosome 8. In JD 38 the translocation of c-*myc* to the 14q+ chromosome has resulted in a detectable rearrangement around the c-*myc* gene that is observed in both *Eco*RI and *Bam*HI digests (24). There were no differences in the configuration of immunoglobulin or c-*myc* genes following PMA, which could account for the changes observed in IgM secretion or plasmacytoid differentiation.

Analysis of Immunoglobulin and c-*myc* RNA. To investigate the potential mechanism responsible for the enhanced IgM secretion seen by JD 38 following PMA stimulation, a serial time course of cytoplasmic μ-chain RNA was assessed (Fig. 5 *a* and *b*). The specific μ-chain RNA within the same numbers of cells, cultured in the presence or absence of PMA, was determined in triplicate at time points 0, 3, 6, 9, and 24 hr and 4 days. An increment in μ-chain RNA was detectable as early as 3 hr after PMA and plateaued at an ≈35% increase by 24 hr (Fig. 5 *a* and *b*). Electrophoresis and blot analysis of total cellular RNA after 24 hr of culture in the presence or absence of PMA also revealed an increase in μ-chain RNA with PMA. Hybridization with the μ membrane (μ_m) exon probe revealed no significant change in the amount of μ-chain RNA after PMA stimulation (Fig. 5*c*). Therefore, the increment in μ-chain RNA noted following PMA stimulation appears to be predominantly of the 2.4-kilobase (kb) μ-secreted (μ_s) species. The expression of c-*myc* in JD 38 has been shown to be slightly increased (≈2-fold) when compared to B-cell lines without translocations (25). If the product of the translocated c-*myc* is intimately involved in maintaining the transformed phenotype in this cell, then the induction of differentiation with PMA might conceivably be associated with a reduction in the level of c-*myc* RNA. We therefore studied c-*myc* RNA expression in JD 38 by quantitative dot blot analysis of poly(A)-selected RNA. A small but reproducible increment of 20% was seen in c-*myc* RNA at 24 hr and 4 days in the presence of PMA. Thus, rather than a reduction in c-*myc* expression, c-*myc* RNA actually increased slightly with differentiation concomitant with the increase in μ-chain RNA. These findings suggest that the expression of the translocated c-*myc* may actually be coordinately regulated with Ig gene expression.

DISCUSSION

Undifferentiated lymphomas have been shown to express surface IgM (rarely other immunoglobulin classes) and therefore to be the malignant counterparts of cells within the

B-lymphocyte lineage (2). The uniform "blast-like" cytological appearance of these tumors raises the possibility that an essential aspect of their pathogenesis is differentiation failure, and, indeed, in several other neoplasms, there is evidence that this is the case (2, 11, 26).

In the present report we present evidence that cell lines derived from undifferentiated lymphomas, including Burkitt lymphoma, can be induced to undergo plasmacytoid differentiation by PMA. Our evidence is based upon both structural changes in the cells, as demonstrated by light and electron microscopy, and functional changes—namely, an increase in IgM secretion. In JD 38, a reduction in the expression of cell surface antigens, including CALLA, OKT-10, and surface IgM, accompanied these changes, although at present we cannot conclude that this is entirely due to differentiation. Alterations in the cell cycle might also account for such changes. Nearly all JD 38 cells cultured with PMA had a plasmacytoid appearance by light and electron microscopy, and some were essentially indistinguishable from malignant plasma cells as seen in multiple myeloma. The expression of cytoplasmic IgM was entirely consistent with these findings. Although a high proportion of the cells also became adherent to a plastic surface in the presence of PMA, we do not know the significance of this phenomenon. The presence of nonspecific esterases in B cells has been reported previously, and the increase seen in PMA-treated JD 38 cells is also consistent with differentiation into more mature B cells (27).

The demonstrated increase in IgM secretion in PMA-treated JD 38 cells led to a study of qualitative and quantitative changes in μ-chain mRNA species induced by PMA. The increases in μ-chain RNA, predominantly of the 2.4-kb μ_s species, clearly preceded the increased secretion of IgM. This change is consistent with our interpretation that plasmacytoid differentiation was induced in JD 38 by PMA since plasmacytomas selectively produce more μ_s than μ_m mRNA (28). The precise mechanisms accounting for this accumulation of μ-chain RNA are not certain, as pulse-chase kinetic experiments were not performed here. However, the very early increase in μ-chain RNA after addition of PMA to the cultures suggests that an actual increase in transcription is at least partially responsible. As the secretion rate of IgM increased roughly 30-fold and the increment in μ-chain RNA was only 35%, it is likely that a major portion of the increased secretion of IgM is mediated by post-transcriptional events, especially translational control. In this regard, it is pertinent that in our studies in this and similar cell lines, the induction of IgM secretion was always associated with a marked increase in rough endoplasmic reticulum as visualized by electron microscopy. Rough endoplasmic reticulum is known to be associated with the production of protein for export from the cell.

An uncoupling of growth and differentiation has been noted in a variety of malignancies. One aspect of this is a maturational arrest that maintains the neoplastic cells at a certain stage of development. Treatment with a phorbol ester overcame the relative arrest in several of our undifferentiated lymphoma cell lines and induced maturation to a more actively secreting, plasmacytoid B cell. If the c-*myc* cellular oncogene product was responsible for maintaining the arrested stage of development, a decrease in c-*myc* RNA might be anticipated during the induction of differentiation, as has been observed with the HL-60 cell line when differentiation is induced—e.g., with dimethyl sulfoxide (29). In fact, a small increase in c-*myc* RNA levels was noted in JD 38 following differentiation, suggesting that increased expression of c-*myc* is not responsible for the maturational arrest of these tumor cells. It is known that in several cell lines studied to date the c-*myc* gene has been introduced by translocation into the heavy chain gene region on the 14q+ chromosome (18, 23). It is possible that mechanisms that augment transcription of the effectively rearranged Ig gene may also positively influence the aberrantly rearranged Ig gene and its neighboring c-*myc* gene. The small, but coordinate, increase in c-*myc* RNA that was observed with the increment in μ-chain RNA following PMA treatment would be consistent with this hypothesis. Whether or not altered regulation of c-*myc* gene expression is essential to the pathogenesis of malignant lymphomas possessing an 8q−(q24) chromosome, the chromosomal rearrangements do not apparently block differentiation permanently, since we have been able to overcome the maturational arrest with PMA.

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1. Sachs, L. (1978) *Nature (London)* **274,** 535–539.
2. Magrath, I. T. (1981) *J. Natl. Cancer Inst.* **67,** 501–514.
3. Bernheim, A., Berger, R. & Lenoir, E. (1981) *Cancer Genet. Cytogenet.* **3,** 307–315.
4. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
5. Klein, G. (1981) *Nature (London)* **294,** 313–318.
6. Ohno, S., Babonits, M., Wiener, F., Spira, J., Klein, G. & Potter, M. (1979) *Cell* **18,** 1001–1007.
7. Breitman, T. R., Selonick, S. E. & Collins, S. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2936–2940.
8. Collins, S. J., Ruscetti, F. W., Gallagher, R. E. & Gallo, R. C. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2458–2462.
9. Koeffler, H. P., Bar-Eli, M. & Territo, M. (1980) *J. Clin. Invest.* **66,** 1101–1108.
10. Okamura, J., Gelfand, E. W. & Letarte, M. (1982) *Blood* **60,** 1082–1088.
11. Cossman, J., Neckers, L. M., Arnold, A. & Korsmeyer, S. J. (1982) *N. Engl. J. Med.* **307,** 1251–1254.
12. Benjamin, D., Magrath, I. T., Douglass, E. C. & Corash, L. M. (1983) *Blood* **61,** 1017–1019.
13. Douglass, E. C., Magrath, I. T., Lee, E. C. & Whang-Peng, J. (1980) *Blood* **55,** 148–155.
14. Benjamin, D., Magrath, I. T. & Freeman, C. B. (1983) *J. Natl. Cancer Inst.* **71,** 897–902.
15. Benjamin, D., Magrath, I. T., Maguire, R., Janus, C., Todd, H. D. & Parsons, R. G. (1982) *J. Immunol.* **129,** 1336–1342.
16. Schroff, R. W., Foon, K. A., Billing, R. J. & Fahey, J. L. (1982) *Blood* **59,** 207–215.
17. Korsmeyer, S. J., Arnold, A., Bakhshi, A., Ravetch, J. C., Siebenlist, U., Hieter, P. A., Sharrow, S. O., LeBien, T. W., Kersey, J. H., Poplack, D. G., Leder, P. A. & Waldmann, T. A. (1983) *J. Clin. Invest.* **71,** 301–313.
18. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7837–7841.
19. White, B. A. & Bancroft, F. C. (1982) *J. Biol. Chem.* **257,** 8569–8572.
20. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
21. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
22. Ravetch, J. C., Siebenlist, U., Korsmeyer, S. J., Waldmann, T. A. & Leder, P. (1981) *Cell* **27,** 583–591.
23. Nishikura, K., ar-Rushdi, A., Erickson, J., Watt, R., Rovera, G. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4822–4826.
24. Dalla-Favera, R., Martinotti, S. & Gallo, R. C. (1983) *Science* **219,** 963–967.
25. Maguire, R. T., Robins, T. S., Thorgeirsson, S. S. & Heilman, C. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1947–1950.
26. Lau, B., Jager, G., Thiel, E., Pachmann, K., Rodt, H., Huhn, D. & Dormer, P. (1980) *Leukemia Res.* **4,** 561–569.
27. Halper, J. P., Tolidjian, B. & Knowles, M. D. (1982) *Cell. Immunol.* **72,** 367–374.
28. Early, P., Rogers, J., Davis, M., Calmane, K., Bond, M., Wall, R. & Hood, L. (1980) *Cell* **20,** 313–319.
29. Westin, E. W., Wong-Staal, F., Gelman, E. P., Dalla-Favera, R., Lautenberger, J. P., Eva, A., Reddy, E. P., Tronick, S. R., Aaronson, S. A. & Gallo, R. C. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2490–2494.