EFFECT OF 12-\(O\)-TETRADECANOYLPHORBOL-13-ACETATE ON EXPRESSION OF CELL SURFACE ANTIGENS IN TWO SUBCLONES OF HUMAN LEUKEMIA K562 CELLS

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Two subclones (K562-L and -H) were previously isolated from K562 human leukemic cells according to hemoglobin production: K562-L was expressed in less than 5% and K562-H in more than 90% of dianisidine positive cells. 12-\(O\)-Tetradecanoylphorbol-13-acetate (TPA) suppressed the expression of the erythrocytic (glycophorin A) and myelocytic (CD11b) antigens in K562-L, but increased the expression of these antigens in K562-H. TPA increased the megakaryocytic (CD61) antigens in both cells. These findings suggest that there are distinct TPA responsible factors in K562-L and -H on the expression of the erythrocytic and myelocytic antigens.

**KEYWORDS** K562 cell line; 12-\(O\)-tetradecanoylphorbol-13-acetate (TPA); glycophorin A; CD11b; CD61

Various factors cause multipotent hematopoietic stem cells to grow, differentiate and mature in their respective blood cells.\(^1\)\(^2\) Granulocytes and monocytes express CD11b, megakaryocytes express CD61, and erythrocytes express hemoglobin and glycophorin A.

K562 human erythroleukemia cells were established from a chronic myelogenous leukemia patient and behaved like multipotent hematopoietic stem cells.\(^3\) Erythrocytic, monocytic, granulocytic and megakaryocytic cell surface antigens could be induced on K562 cells by various factors.\(^4\) Hemin,\(^5\) sodium butyrate\(^5\) and 1-\(\beta\)-d-arabinofuranosylecytosine (ara-C)\(^6\) could induce erythroid differentiation. 12-\(O\)-Tetradecanoylphorbol-13-acetate (TPA) is well known as a modulator of protein kinase C (PKC) and induces monocytic and megakaryocytic differentiation of K562 cells.\(^7\) PKC plays a critical role in many cellular responses,\(^8\)-\(^10\) for example, proliferation of T lymphocyte and production of interleukin 2. PKC is activated by diacylglycerol, which is produced by hydrolysis of inositol phospholipid, and its activation is required Ca\(^{2+}\).\(^11\)

We previously investigated two subclones of K562 (K562-P): K562-L, which has low hemoglobin-producing activity and high expression of cell surface CD and glycophorin A antigens, and K562-H, which has high hemoglobin-producing activity and low expression of cell surface CD and glycophorin A antigens.\(^12\) Herein, we examine the effect of TPA on the expression of erythrocytic, myelocytic, and megakaryocytic cell surface antigens in K562-L and -H and discuss the change of PKC activity by TPA.

**MATERIALS AND METHODS**

K562 cells were maintained on RPMI-1640 medium with 10% FBS in 5% CO\(_2\) in humidified air. The
cells were cultured with TPA (Sigma Chemical Company, St. Louis, MO) or sphingosine (Serdaery Research Laboratories, Inc., Ontario, Canada) for 72 h. The cells were washed and, after the addition of anti CD11b or CD61 (Cosmo Bio Co. Ltd., Tokyo) monoclonal antibodies, were incubated for 30 min at 4°C. The cells were washed and, after the addition of anti mouse Ig antibody (conjugated with fluorescein isothiocyanate, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA), were incubated for 30 min at 4°C. TPA- and sphingosine-treated cells were also incubated with anti glycoporphin A (conjugated with fluorescein isothiocyanate, Immunotech S.A., France) monoclonal antibody. These cells were washed and analyzed by FACScan (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA).

**RESULTS AND DISCUSSION**

As shown in Fig. 1, TPA increased the CD61 expression in K562–P, –L and –H, but the increase was especially marked in K562–H. However, TPA decreased the expression of glycoporphin A and CD11b in K562–L (Figs. 2 and 3). The expression of glycoporphin A and CD11b in K562–P was also slightly decreased by TPA. On the other hand, TPA increased the expression of glycoporphin A and CD11b in K562–H. These findings indicate that TPA has different effects on the expression of glycoporphin A and CD11b in K562–L and –H. Because only the expression of CD61 in K562–L was increased by TPA and the expression of CD11b and glycoporphin A in K562–L was decreased by TPA, megakaryocytic differentiation may be induced in K562–L. However, the cell lineage affected by TPA in K562–H was not determined because CD61, CD11b and glycoporphin A expression in K562–H was increased.

Watanabe et al. demonstrated that ara-C could induce erythroid differentiations, such as heme synthesis, accumulation of globin mRNA and expression of glycoporphin A in K562 cells, and that TPA inhibited the induction of erythroid differentiation by ara-C. 13) Sutherland et al. 4) also reported that TPA decreased the expression of glycoporphin A on K562 cells, although it increased the expression of the monocytic antigen.
Their findings are in agreement with our finding that TPA decreased the expression of glycophorin A on K562–L (Fig. 1. B). However phorbol dibutyrate and thymidine–hypoxanthine increased the expression of erythrocytic and megakaryocytic antigens, which is in agreement with the finding that TPA induced the expression of both antigens in K562–H (Figs. 1. C and 2. C).

![Fig. 3. Expression of CD61 on K562 Cells after TPA Treatment](image)

TPA, which down-regulates PKC, and sphingosine, which inhibits PKC, suppressed the maturation of murine erythroleukemia cells committed to erythroid differentiation. To determine whether the different effects of TPA on the expression of glycophorin A and CD11b between K562–L and –H was dependent on the activity of PKC, we examined the effects of sphingosine on the expression of glycophorin A, CD11b and CD61. Sphingosine did not have the same effect as TPA on the expression of glycophorin A, CD11b and CD61 antigens (data not shown). This suggests that PKC was not related to the effect of TPA in K562–L or –H, although we did not measure the phosphorylation activity of PKC in detail. Many reports identified several isozymes of PKC and showed its different properties. We expect that these isozymes, which may show different responses to TPA and sphingosine, are expressed differently on K562–L and –H.

In this report, we suggest that K562–L and –H have distinct TPA responsible factors, which cause the difference in the effect of TPA on the expression of glycophorin A and CD11b, respectively.

REFERENCES


(Received July 28, 1993)