7,12-Dimethylbenz[*a*]anthracene activates protein-tyrosine kinases Fyn and Lck in the HPB-ALL human T-cell line and increases tyrosine phosphorylation of phospholipase C- γ 1, formation of inositol 1,4,5-trisphosphate, and mobilization of intracellular calcium

(polycyclic aromatic hydrocarbons/immunotoxicity)

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ABSTRACT Previous studies have shown that the immunosuppressive and carcinogenic polycyclic aromatic hydrocarbon 7,12-dimethylbenz[*a*]anthracene (DMBA) impairs Ca^{2+} -dependent transmembrane signaling in human and murine lymphocytes. The purpose of the present studies was to analyze potential mechanisms of immunosuppression by DMBA and to examine effects on Ca^{2+} homeostasis and antigen-receptor signaling in human T cells. DMBA produced a rapid and sustained increase in Ca^{2+} levels in HPB-ALL cells by release of cytoplasmic Ca^{2+} . DMBA also inhibited anti-CD3/CD4 mobilization of Ca^{2+} in HPB-ALL cells, with half-maximal inhibition occurring at ≈ 4 hr. Thus, the kinetics for initial Ca^{2+} mobilization and inhibition of the anti-CD3/CD4 response differed. The rapid rise in intracellular Ca^{2+} induced by DMBA alone was accompanied by a rapid but transient increase in inositol 1,4,5-trisphosphate and tyrosine phosphorylation of phospholipase C- γ 1. The pattern of tyrosine phosphorylation induced by DMBA in HPB-ALL cells was remarkably similar to that induced by anti-CD3/CD4 activation. Thus, DMBA-induced phosphorylation may mimic antigen-receptor activation in T cells, which may lead to alterations in antigen responsiveness. The mechanism of DMBA-induced tyrosine phosphorylation of phospholipase C- γ 1 may have been due to an increase in protein-tyrosine kinase activity, since it was found that DMBA produced a >2 -fold increase in the activity of the T-cell receptor-associated Src-family kinases Fyn and Lck. The kinetics of activation of protein-tyrosine kinases demonstrated that Fyn activity was increased within 10 min of exposure to DMBA, whereas maximal Lck activation required 30 min. Thus, it is likely that the Fyn kinase or other protein-tyrosine kinases may be responsible for the early tyrosine phosphorylation of phospholipase C- γ 1, which results in inositol 1,4,5-trisphosphate release and mobilization of intracellular Ca^{2+} .

The compound 7,12-dimethylbenz[*a*]anthracene (DMBA), a synthetic methylated polycyclic aromatic hydrocarbon, is a potent carcinogen and immunosuppressive agent (1). DMBA has been shown to inhibit humoral immunity to thymus-dependent and thymus-independent antigens (2, 3), prevent activation of cytotoxic T lymphocytes and natural killer cells (4, 5), suppress *in vitro* responses to mitogens (6, 7), and prevent interleukin 2 production (4, 8). DMBA inhibits Ca^{2+} -dependent pathways of T- and B-cell activation. It has been demonstrated that DMBA interferes with phytohemaggluti-

nin-induced Ca^{2+} mobilization in the Jurkat human T-cell line and that this effect is accompanied by a rise in baseline Ca^{2+} in unstimulated cells (9). Murine spleen cells treated with DMBA *in vitro* or *in vivo* also demonstrated a decreased Ca^{2+} mobilization response to phytohemagglutinin (6, 7), and murine B cells were significantly inhibited in their Ca^{2+} mobilization response to anti-IgD antibodies, whereas Ca^{2+} -independent pathways of B cell activation appeared to be relatively unaffected by DMBA (10). Thus, DMBA appears to alter lymphocyte Ca^{2+} homeostasis, which may result in disruption of Ca^{2+} -dependent signaling in B and T cells.

The mechanism by which DMBA alters antigen receptor-coupled signal-transduction pathways in lymphocytes has not been elucidated. We hypothesized that DMBA may stimulate Ca^{2+} mobilization via phosphatidylinositol hydrolysis and the release of inositol 1,4,5-trisphosphate (IP_3) resulting from activation of phospholipase C- γ 1 (PLC- γ 1). To test this hypothesis, we utilized the HPB-ALL human T-cell line as an *in vitro* model, since it appears to be similar to normal T cells in its antigen-receptor activation (11). We show here that DMBA transiently activates the protein-tyrosine kinases Fyn and Lck, increases tyrosine phosphorylation of PLC- γ 1, and increases formation of IP_3 , which may be responsible for the rapid increase in intracellular Ca^{2+} produced in human T cells by DMBA.

MATERIALS AND METHODS

***In Vitro* Model of Human T-Cell Activation.** The use of the HPB-ALL human T-cell line for T-cell activation studies has been previously reported (11, 12). HPB-ALL cells were grown in suspension culture in RPMI-1640 (Whittaker) supplemented with 10% fetal bovine serum (HyClone), penicillin and streptomycin, and 100 mM glutamine. Cells harvested during logarithmic growth were used for all experiments, under conditions where cell viability was $>95\%$.

Monoclonal Antibodies and Reagents. Anti-CD3 (G19-4, BALB/c, IgG1) and anti-CD4 (G17-2, BALB/c, IgG1) monoclonal antibodies were obtained from murine hybridomas grown in tissue culture. These antibodies were conjugated with biotin by using *N*-hydroxysuccinimidobiotin (Sigma) as described by Goding (13). Affinity-purified anti-phosphoty-

Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; IP_3 , inositol 1,4,5-trisphosphate; TCR, T-cell antigen receptor; PLC- γ 1, phospholipase C- γ 1; DMSO, dimethyl sulfoxide.

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rosine antibodies used for immunoblots were prepared as described (14, 15). Anti-PLC- γ 1 antibodies were prepared in rabbits, purified, and used in Western blots as described (16). DMBA was purchased from Sigma (>95% purity), and fresh solutions were prepared in anhydrous tissue culture-grade dimethyl sulfoxide (DMSO, Sigma) before each use. The final concentration of DMSO in all DMBA experiments was 0.1%. This concentration of DMSO was found to be without effect on any of the parameters measured.

Lymphocyte Activation and Flow Cytometry Analysis of Ca^{2+} Mobilization. HPB-ALL cells were activated at 37°C by incubation for 5 min with biotin-conjugated anti-CD3/anti-CD4 antibodies (1 $\mu\text{g}/\text{ml}$), followed by crosslinking with streptavidin (2 $\mu\text{g}/\text{ml}$, Sigma), as described (11). Intracellular levels of free cytosolic Ca^{2+} were examined by flow cytometry with published methods (9, 17). Briefly, HPB-ALL cells were suspended at 10^6 per ml for indo-1 loading and exposure to DMBA. Indo-1 acetoxymethyl ester (Calbiochem) was loaded into cells at a concentration of 1 μM for 45 min at 37°C before analysis of the violet/blue mean-channel fluorescence

FIG. 1. (Upper) Kinetics of DMBA-induced increases in intracellular Ca^{2+} in HPB-ALL cells, expressed as the mean channel violet/blue fluorescence ratio over time, measured by flow cytometry. Data are shown for four separate determinations using the ratio calculated for cells approximately every second. Cells were analyzed by flow cytometry at ≈ 300 cells per second. Indo-1-loaded samples were analyzed for 30 sec, and DMSO vehicle ($\bullet$) or 3, 10, or 30 μM DMBA ($\times$, $\blacktriangle$, and ∇ , respectively) was then added to the cells and analysis was continued for ≈ 100 sec. (Lower) Kinetics of DMBA inhibition of anti-CD3/CD4 Ca^{2+} mobilization. HPB-ALL cells were treated with 10 μM DMBA for various times and were analyzed for Ca^{2+} mobilization following crosslinking with anti-CD3/CD4. The mean channel fluorescence ratio change from baseline (DMBA alone) was determined for triplicate HPB-ALL samples at each time point. Error bars show $\pm$ SD.

ratio (an indicator of intracellular Ca^{2+}) via multiparameter flow cytometry with a Coulter 753 flow cytometer. Biotinylated anti-CD3/CD4 antibodies were added to the cells under conditions that did not mobilize Ca^{2+} before crosslinking. After addition of streptavidin, intracellular Ca^{2+} rapidly and markedly increased. The CD3/CD4-induced violet/blue ratio change was determined by subtracting the mean fluorescence ratio of baseline Ca^{2+} from the mean fluorescence obtained after stimulation of the cells. In some experiments, DMBA was directly added to indo-1-loaded cells to measure the kinetics of Ca^{2+} mobilization due to DMBA exposure alone.

IP_3 Analysis by Radioreceptor Assay. IP_3 levels were measured by a bovine cerebellum radioreceptor assay (18). DMBA-treated cells (5×10^6) were stimulated with anti-CD3/CD4 and crosslinked with streptavidin. The reaction was stopped by addition of an equal volume of 16% (wt/vol) trichloroacetic acid. The mixture was incubated on ice for 15 min, samples were centrifuged ($2000 \times g$, 5 min) at 4°C, and the pellets were dissolved in 1 ml of 0.15 M NaOH for protein measurements. The supernatants were extracted with water-saturated ether to remove the trichloroacetic acid. The amount of IP_3 present in the extract was determined by using a standard curve based upon a competitive binding reaction with bovine cerebellar membranes and [^3H] IP_3 (DuPont/NEN; 15–30 Ci/mmol; 1 Ci = 37 GBq).

Immunoprecipitation. Whole cell lysates of DMBA-treated HPB-ALL cells were immunoprecipitated by a modification of the method of Kanner *et al.* (19). Immunoprecipitation with anti-PLC- γ 1 antibodies at an antibody/protein ratio of 1:50 was accomplished by incubating HPB-ALL cell lysates for 2 hr in an ice/water bath. Immune complexes were recovered

FIG. 2. (Upper) Dose-dependent changes in intracellular Ca^{2+} produced by a 4-hr treatment with 3–30 μM DMBA in resting HPB-ALL cells (filled bars) and after anti-CD3/CD4 stimulation (open bars). (Lower) Lack of effect of 10 mM EDTA on DMBA-induced Ca^{2+} mobilization in resting HPB-ALL cells or after anti-CD3/CD4 activation. All values are the replicates for three samples (mean $\pm$ SD). Asterisks denote values that are significantly different from controls ($P < 0.05$).

by incubation with protein A-Sepharose CL-4B (Pharmacia) for 1 hr at 4°C with rotation. The Sepharose beads were washed four times with RIPA lysis buffer (150 mM NaCl/50 mM Tris Cl, pH 7.5/1% Nonidet P-40/0.25% sodium deoxycholate) supplemented with phosphatase and protease inhibitors (Na_3VO_4 , 1 mM; phenylmethylsulfonyl fluoride, 1 mM; aprotinin, 0.5%; leupeptin, 50 $\mu\text{g}/\text{ml}$; α_2 -macroglobulin, 10 $\mu\text{g}/\text{ml}$) and twice with TN buffer (150 mM NaCl/50 mM Tris Cl, pH 7.5). Samples were eluted from the beads by boiling with 2× SDS sample buffer.

Anti-Phosphotyrosine Western Blots. Western blotting with anti-phosphotyrosine antibodies was performed on cell lysates obtained from resting cells, cells treated with DMBA, or cells stimulated with anti-CD3/CD4. After treatment, cells were spun at $300 \times g$ for 5 min, the supernatant was removed, and the cell pellet was treated with RIPA buffer on ice for 10 min to obtain whole cell lysates used for immunoprecipitation. These lysates as well as specific immunoprecipitates were electrophoresed in an SDS/10.5% polyacrylamide gel and then transferred to nitrocellulose (Schleicher & Schuell) or Immobilon (Millipore). The filter was blocked by incubation with blocking buffer containing 6% bovine serum albumin and then incubated with anti-phosphotyrosine antibodies overnight. After washing to remove unbound antibodies, the filter was incubated with ^{125}I -labeled protein A (ICN, 30 $\mu\text{Ci}/\mu\text{g}$), washed, and exposed to Kodak XAR film at -70°C for 72 hr with an intensifying screen.

Immune-Complex Protein Kinase Assays. The Lck and Fyn protein-tyrosine kinases were analyzed by a modification of the method of Eiseman and Bolen (20), and cell lysates were prepared as recently described (15). Cell lysates were thawed in a room-temperature water bath and immunoprecipitated with antiserum for the specific kinase of interest, Lck or Fyn (antisera were generously provided by Joseph Bolen, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ). The antisera were used at a dilution of 1:500 and immune complexes were collected by incubation with protein A-Sepharose (Pharmacia). After four washes in lysis buffer, the protein A-Sepharose beads were washed once in kinase buffer and resuspended in the same buffer. Kinases were characterized for autophosphorylation and phosphorylation of enolase. Kinase reactions were initiated by the addition of acid-denatured rabbit muscle enolase (Sigma), 25 μCi of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (5000 Ci/mmol), and unlabeled ATP (5 μM). Reactions were terminated by boiling for 5 min in SDS sample buffer. Samples were electrophoresed in an SDS/9.5% polyacrylamide gel and labeled proteins were detected by auto-

FIG. 3. Intracellular levels of IP_3 (pmol/mg of protein) following exposure of HPB-ALL cells to DMSO vehicle or 1, 3, 10, or 30 μM DMBA. Values are the mean $\pm$ SD of four separate determinations. Asterisks indicate values that are significantly different ($P < 0.05$).

FIG. 4. Membrane and cytosolic protein-tyrosine phosphorylation obtained from lysates of HPB-ALL cells following an *in vitro* exposure to 10 μM DMBA. Shown is an anti-phosphotyrosine immunoblot of RIPA lysates of control HPB-ALL cells that were not stimulated with anti-CD3/CD4 antibodies (–), cells stimulated with anti-CD3/CD4 as a positive control (+), and cells treated with 10 μM DMBA alone for the times indicated.

radiography. The incorporation of ^{32}P label into proteins was quantitated by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA).

RESULTS

DMBA Increases Intracellular Ca^{2+} and Inhibits Anti-CD3/CD4 Ca^{2+} Mobilization in HPB-ALL Cells in the Absence and Presence of EDTA. DMBA produced a rapid and dose-dependent increase in intracellular Ca^{2+} in HPB-ALL cells, as indicated by the increase in the indo-1 violet/blue fluorescence ratio change measured by flow cytometry (Fig. 1 *Upper*). Intracellular Ca^{2+} increased within seconds of exposure to DMBA and reached a maximum within minutes of exposure, depending upon the dose of DMBA examined. To investigate the kinetics for DMBA inhibition of anti-CD3/CD4 Ca^{2+} mobilization, cells were exposed to 10 μM DMBA for various lengths of time and were then loaded with indo-1 for analysis of Ca^{2+} mobilization induced by crosslinking anti-CD3 and anti-CD4. Experiments were performed to ensure that DMBA did not alter the loading of HPB-ALL cells with indo-1. Since DMBA produced a rise in Ca^{2+} levels in nonstimulated cells, the data are shown as the change in the mean channel ratio for violet/blue fluorescence for cells prior to exposure and ≈ 3 min after stimulation with anti-CD3/CD4 (Fig. 1 *Lower*). The results show that DMBA produced a half-maximal inhibition of anti-CD3/CD4 Ca^{2+} mobilization after a 4-hr exposure to DMBA.

A 4-hr exposure to DMBA produced a dose-dependent increase in intracellular Ca^{2+} (Fig. 2 *Upper*). In addition, at 4 hr, there was a good correlation between the ability of

FIG. 5. Tyrosine phosphorylation and expression of PLC- γ 1 in HPB-ALL cells exposed *in vitro* to 10 μM DMBA for 0.5–60 min. (*Upper*) Anti-phosphotyrosine immunoblot of anti-PLC- γ 1 immunoprecipitates obtained from unstimulated HPB-ALL cells (–) and anti-CD3/CD4 stimulated cells (+) as a positive control. DMBA-treated cells were not stimulated with anti-CD3/CD4. (*Lower*) Positive control for PLC- γ 1 protein identity following blotting of anti-CD3/CD4-stimulated (+) and unstimulated (–) cell lysates with anti-PLC- γ 1 and development with ^{125}I -labeled protein A.

FIG. 6. Autoradiographs of SDS/polyacrylamide gels, demonstrating protein kinase activity following DMBA exposure. Fyn (Upper) and Lck (Lower) immunoprecipitates were obtained from HPB-ALL cells treated with 10 μ M DMBA for 10 or 30 min. Samples were run in quadruplicate and are compared with control cell extracts exposed to the DMSO vehicle. Kinase activity was determined for autophosphorylation as well as phosphorylation of enolase (enol).

DMBA to produce an increase in intracellular Ca^{2+} (filled bars) and to inhibit the mobilization of Ca^{2+} produced by anti-CD3/CD4 treatment (open bars). The simultaneous treatment of HPB-ALL cells with 10 mM EDTA (or EGTA in other experiments) did not influence the ability of DMBA to produce a rise in intracellular Ca^{2+} or change the anti-CD3/CD4 response (Fig. 2 Lower). These results strongly suggest that the rise in Ca^{2+} produced by DMBA is due to stimulation of Ca^{2+} release from storage pools.

DMBA Stimulates IP_3 Formation in HPB-ALL Cells. Since IP_3 is known to trigger Ca^{2+} release in many cells (21), we measured intracellular IP_3 by using a bovine cerebellum radioreceptor assay. DMBA was found to stimulate hydrolysis of phosphatidylinositol bisphosphate as measured by a transient increase in levels of IP_3 in HPB-ALL cells (Fig. 3). IP_3 formation was stimulated in a dose- and time-dependent manner by DMBA. Maximal elevation of IP_3 was seen 1 min after exposure to 10 or 30 μ M DMBA, whereas the 1 and 3 μ M exposures peaked at around 15 min. IP_3 levels returned to near baseline 30 min after exposure to all doses of DMBA.

DMBA-Induced Tyrosine Phosphorylation of Proteins. Anti-phosphotyrosine Western blotting showed that 10 μ M DMBA produced tyrosine phosphorylation of at least six proteins

detected in whole cell lysates (Fig. 4). The pattern of DMBA-induced tyrosine phosphorylation was remarkably similar to that obtained when cells were stimulated with anti-CD3/CD4 antibodies. The amount of tyrosine phosphorylation varied with the length of the exposure to DMBA, and the kinetics of phosphorylation differed somewhat for the major proteins.

DMBA Stimulates Phosphorylation of PLC- γ 1 in HPB-ALL Cells. Based upon the finding that IP_3 increases rapidly after DMBA treatment, we hypothesized that DMBA might activate PLC- γ 1 by tyrosine phosphorylation. We found that PLC- γ 1 is transiently phosphorylated on tyrosine, as detected by anti-phosphotyrosine Western immunoblotting of PLC- γ 1 precipitates obtained from HPB-ALL cells following DMBA treatment (Fig. 5). In this experiment, PLC- γ 1 phosphorylation was apparent at 0.5 min and then declined to background levels by 30 min. These results are similar to those obtained for the 140- to 150-kDa band demonstrated in Fig. 4.

Increases in Protein-Tyrosine Kinase Activity Following Exposure of HPB-ALL Cells to DMBA. Immune-complex protein kinase assays were performed to determine whether DMBA might increase tyrosine phosphorylation of PLC- γ 1 by increasing protein-tyrosine kinase activity. Since Fyn and Lck are known to play an important role in human T-cell activation, these two protein-tyrosine kinases were analyzed for autophosphorylation and their ability to phosphorylate acid-denatured enolase. These studies showed that 10 μ M DMBA produced a time-dependent increase in the activity of both Fyn and Lck (Fig. 6 and Table 1). Exposure of resting HPB-ALL cells to DMBA for 10 min resulted in a statistically significant increase in Fyn activity relative to control vehicle-exposed cells (i.e., cells treated with 0.1% DMSO), with a 2.1-fold increase in autophosphorylation of Fyn and a 2-fold increase in the phosphorylation of enolase by the immunoprecipitated Fyn kinase. When HPB-ALL cells were exposed to DMBA for 30 min prior to assay, a significant 2.2-fold increase in autophosphorylation of Fyn and a corresponding 3.2-fold increase in enolase phosphorylation by this kinase were seen. For the Lck kinase, a 1.7- to 1.8-fold increase in autophosphorylation was seen, as well as a 1.8-fold increase in enolase phosphorylation by the immunoprecipitated Lck kinase, following 30 min of DMBA exposure.

DISCUSSION

Several classes of known or suspected environmental carcinogens have been shown to suppress humoral and cell-mediated immunity in animals, and perhaps in humans (1). Previous studies by one of our laboratories have shown that DMBA, a methylated polycyclic aromatic hydrocarbon, interferes with Ca^{2+} homeostasis and cell activation produced by phytohemagglutinin in the Jurkat human T-cell line (9). Lymphoid cells obtained from mice treated *in vivo* or *in vitro* with DMBA are inhibited in their Ca^{2+} -dependent signaling

Table 1. PhosphorImager analysis of Lck and Fyn kinase activity following exposure of HPB-ALL cells to 10 μ M DMBA

Time, * min	Sample	Lck		Fyn	
		Enolase	Auto	Enolase	Auto
10	Control	41.9 $\pm$ 3.7	13.2 $\pm$ 0.7	6.9 $\pm$ 2.0	2.6 $\pm$ 0.7
	DMBA	45.8 $\pm$ 5.6	13.7 $\pm$ 3.2	13.7 $\pm$ 2.0 [†]	5.4 $\pm$ 0.4 [†]
30	Control	26.4 $\pm$ 5.5	10.7 $\pm$ 0.9	4.0 $\pm$ 0.7	3.5 $\pm$ 0.6
	DMBA	44.0 $\pm$ 6.9 [†]	16.8 $\pm$ 2.6 [†]	12.9 $\pm$ 3.8 [†]	7.6 $\pm$ 2.0 [†]
60	Control	17.8 $\pm$ 5.9	10.1 $\pm$ 5.7	4.4 $\pm$ 0.7	5.3 $\pm$ 1.6
	DMBA	22.3 $\pm$ 5.8	12.9 $\pm$ 2.9	5.0 $\pm$ 2.3	6.1 $\pm$ 2.1
120	Control	11.2 $\pm$ 1.9	6.8 $\pm$ 1.8	3.0 $\pm$ 0.8	5.0 $\pm$ 1.2
	DMBA	16.7 $\pm$ 1.6	10.9 $\pm$ 2.5	3.9 $\pm$ 1.2	5.2 $\pm$ 1.4

Results are from scanning of gels shown in Fig. 6 as well as later time points; values are expressed as the mean PhosphorImager units of four replicate determinations $\pm$ SD.

*Duration of exposure of HPB-ALL cells to 10 μ M DMBA.

[†]Significantly different from control ($P < 0.05$).

pathways (6, 7), whereas Ca^{2+} -independent pathways of cell activation are unaffected (10). In agreement with other investigators (22), our results show that DMBA and other polycyclic aromatic hydrocarbons can produce immunosuppression in the absence of bioactivation produced by cytochrome P-450 or other enzymes (23). It is important to examine the mechanisms of altered Ca^{2+} homeostasis produced by polycyclic aromatic hydrocarbons such as DMBA, because immunotoxicity is one of the most sensitive indicators of exposure of animals, and perhaps humans, to this ubiquitous class of environmental pollutants. Recent studies also suggest that polycyclic aromatic hydrocarbons may produce lymphocytotoxicity and thymic atrophy via Ca^{2+} -dependent apoptotic pathways (24).

Binding of antigen to the T-cell receptor (TCR)/CD3 complex results in a series of early phosphorylation events that lead to T-cell activation (25, 26), presumably by activating CD4- or CD8-associated tyrosine kinases (27, 28). CD4 and CD8 are known to associate with the protein-tyrosine kinase p56^{Lck} (29), while another tyrosine kinase, p59^{Fyn} , is believed to interact with TCR/CD3 (30–32). TCR activation of the phosphatidylinositol-specific PLC pathway appears to be dependent upon protein-tyrosine kinase activation, followed by tyrosine phosphorylation and activation of PLC- γ 1 (33–36). The Src family of kinases, particularly Lck and Fyn, are most likely associated with activation of the phosphatidylinositol PLC pathway by increasing PLC- γ 1 tyrosine phosphorylation. Recently a non-Src protein-tyrosine kinase, ZAP-70 has been described that may also be important in CD3/CD4 signaling (37). The role of protein-tyrosine kinase activation in Ca^{2+} mobilization in lymphocytes has been further demonstrated by the ability of genistein and herbimycin, two inhibitors of protein-tyrosine kinases, to block TCR/CD3-mediated Ca^{2+} mobilization in human T cells (38, 39).

Exposure of resting HPB-ALL cells to DMBA produced a rapid and transient increase in tyrosine phosphorylation of PLC- γ 1 as detected by Western blots. Furthermore, phosphorylation of tyrosine on PLC- γ 1 was accompanied by a rise in IP_3 and mobilization of intracellular Ca^{2+} . IP_3 levels returned to baseline ≈ 30 min after exposure to DMBA, during which time the amount of tyrosine phosphorylation of PLC- γ 1 also declined to background levels. The mechanism by which DMBA increases tyrosine phosphorylation of PLC- γ 1 may be due to protein-tyrosine kinase activation. We found in these studies that DMBA transiently increased the activity of Fyn and Lck >2 -fold. The kinetics of kinase activation were somewhat different, with Fyn being activated within 10 min of exposure and Lck being activated at ≈ 30 min.

The kinetics of Ca^{2+} elevation in HPB-ALL cells indicated that DMBA mobilized Ca^{2+} within seconds of exposure and maintained elevated Ca^{2+} levels for several hours. Maximal inhibition of anti-CD3/CD4-induced Ca^{2+} mobilization occurred after several hours of exposure to DMBA. These results are similar to those previously reported for DMBA-induced elevation of Ca^{2+} and inhibition of phytohemagglutinin-induced Ca^{2+} mobilization in the Jurkat human T-cell line (9). It is unclear at this time how DMBA can maintain elevated levels of Ca^{2+} long after PLC- γ 1 phosphorylation and IP_3 levels have apparently returned to baseline. Since it is difficult to experimentally separate the early Ca^{2+} -mobilizing effects of DMBA from distal events that may maintain Ca^{2+} mobilization or lead to inhibition of anti-CD3/CD4 cell activation, we can conclude only that the early rise in Ca^{2+} induced by DMBA may, in part, be responsible for altered Ca^{2+} homeostasis and changes in signaling in human T cells.

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