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Bacterial Lipopolysaccharide Activates Protein Kinase C, but Not Intracellular Calcium Elevation, in Human Peripheral T Cells

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Abstract The increase of intracellular free calcium concentration ($[Ca^{2+}]_i$) and protein kinase C (PKC) activity are two major early mitogenic signals to initiate proliferation of human peripheral T cells. Bacterial lipopolysaccharide (LPS) is nonmitogenic in human T cells. However, in the presence of monocytes, LPS becomes mitogenic to proliferate T cells. The aim of this study was to define the incompetency of LPS on two mitogenic signals in human peripheral T cells. T cells were isolated from human peripheral blood. $[Ca^{2+}]_i$ and pH_i were determined by loading the cells with the fluorescent dyes, Fura-2 acetoxymethyl ester (Fura-2/AM) and 2′,7′-bis(2-carboxyethyl)-5-(and 6)carboxyfluorescein acetoxymethyl ester (BCECF/AM). PKC activity was determined by protein kinase assay and cell proliferation was estimated from the incorporation of [^{3}H]-thymidine. The results indicated that (1) LPS (10 μg/ml) stimulated PKC activity significantly within 5 min, reached a plateau at 30 min, and maintained that level for at least 2 h; and (2) LPS stimulated cytoplasmic alkalinization but did not affect the levels of $[Ca^{2+}]_i$ and [^{3}H]-thymidine incorporation into T cells. Moreover, the combination of calcium ionophore A23187 with LPS significantly stimulated [^{3}H]-thymidine incorporation into T cells. Thus, the results demonstrate that LPS failed to proliferate T cells, probably because of a lack of the machinery necessary to stimulate the mitogenic signal on $[Ca^{2+}]_i$ elevation. J. Cell. Biochem. 76:404–410, 2000.

Key words: lipopolysaccharide; Ca^{2+}; PKC; pH_i; proliferation; T cells

Bacterial lipopolysaccharide (LPS) derived from Gram-negative microorganisms has become a popular microbial activator in many studies. LPS stimulates the proliferation and antibody production in B cells [DeFranco et al., 1987]. However, LPS is known to be nonmitogenic on human T cells. Therefore, the effects of LPS on human T cell are less well known. LPS and its lipid A component are recognized as potent inducers of both human T-cell proliferation and Th1-like lymphokines [Mattern et al., 1994]. Meanwhile, the presence of viable monocytes is required to proliferate T cells by LPS. Recently, LPS-primed monocytes have been reported to stimulate the proliferation of T cells by costimulatory signals via CD28 [Mattern et al., 1998]. This evidence suggests that the stimulation of T-cell proliferation by LPS is dependent on signals induced by direct cell-to-cell contact between T cells and accessory monocytes, whereas, until now, there have been few reports investigating how LPS directly affects the mitogenic signals in human peripheral T cells.

Interleukin-2 (IL-2) receptor expression and T-cell proliferation are inhibited by the protein kinase C (PKC) inhibitor, H7 [Hengel et al., 1991]. Blocking the increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) by removal of extracellular calcium with chelators or calcium channel blockers is associated with an inhibition of IL-2 secretion and T-cell proliferation [Mills et al., 1985a,b; Gelfand et al., 1986]. Moreover, the stimulation of T-cell proliferation can be mimicked by combined stimulation with ionophores

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to elevate $[Ca^{2+}]_i$ and phorbol ester to activate PKC [Truneh et al., 1985; Clevers et al., 1985; Koyasu et al., 1987]. Therefore, two major mitogenic signals—sustained elevation in $[Ca^{2+}]_i$ and PKC activity—are recognized to drive T cells from differentiation toward proliferation [Berry and Nishizuka, 1990; Weiss and Littman, 1994]. The activity of Na^+/H^+ exchange is enhanced by PKC [Mills et al., 1985c; Gelfand et al., 1987; Ebanks et al., 1989]; this study uses the rise in pH_i (alkalinization) by enhancing the activity of Na^+/H^+ exchange as a physiological response produced by PKC activation in T cells.

In this study, responsiveness of T cells to elevation of $[Ca^{2+}]_i$, PKC activation, pH_i, and proliferation was evaluated by LPS. Mitogen, phytohemagglutinin (PHA), and the co-mitogen, phorbol 12-myristate 13-acetate (PMA), were used as controls of LPS. A 10-μg/ml dose of LPS was chosen to stimulate the proliferation and antibody production in B cells [DeFranco et al., 1987], whereas the concentration of PMA at 100 pM has been used frequently to study the signaling of T cells [Whelan et al., 1992]. We found LPS to stimulate PKC activity and subsequent intracellular alkalinization but not the elevation of $[Ca^{2+}]_i$ or proliferation of T cells.

MATERIALS AND METHODS

Chemicals

Fura-2/AM, BCECF/AM, nigericin, and valinomycin were purchased from Molecular Probes (Eugene, OR). PHA, RPMI 1640 medium (RPMI), Hank's balanced salt solution (HBSS), and fetal calf serum (FCS) were obtained from Gibco (Grand Island, NY), whereas LPS from *Escherichia coli,* PMA, A23187, bovine serum albumin (BSA), and ficoll/hypaque were obtained from Sigma Chemical Co. (St. Louis, MO). P-81 phosphocellulose paper was supplied by Whatman (Maidstone, UK), myelin basic protein (MBP_{4-14}) was purchased from Research Biochemicals International (Natick, MA), and [^{3}H]-thymidine from New England Nuclear (Boston, MA).

Separation of T Cells

Heparinized peripheral blood samples were obtained from male volunteers. The blood mononuclear cells (MNCs) were isolated by the Ficoll-Hypaque gradient-density method. A total of 15 ml of MNC suspension was put into a 100 × 15-mm plastic Petri dish and incubated in a humidified, 37°C, 5% CO_2 incubator for 50 min. The adherent cells were harvested by a rubber policeman and washed. The whole process was repeated three times. The nonadherent cells were separated by E-rosettes technique and flow cytometry (Coulter EPICS C, Hialeah, FL). The T cells forming E-rosettes were pelleted on the bottom and separated with cold distilled water. To verify the effectiveness of the separation procedure, T cells were incubated for 30 min at 4°C with phycoerythrin-labeled monoclonal antibodies to CD_3 (Ortho Pharmaceuticals, Raritan, NJ). T cells bound to monoclonal antibodies were sorted with a fluorescence-activated cell sorter (Coulter EPICS C). Using this sorting technique, the T-cell suspension was almost 100% CD_3-positive cells [Lin and Lo, 1991].

Measurement of $[Ca^{2+}]_i$

T cells (2×10^7 cells/ml) were loaded with Fura-2/AM (5 μM) in medium RPMI 1640 with 10% FCS (v/v) for 30 min at 25°C, washed free of extracellular Fura-2/AM with RPMI 1640 three times, and resuspended (4×10^8 cells/ml) in RPMI 1640 with 10% FCS. To determine $[Ca^{2+}]_i$, portions of cell suspension (2×10^6 cells) were washed twice, resuspended in 2.5 ml of loading buffer (152 mM NaCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 5 mM KCl, 10 mM glucose, 10 mM HEPES, pH 7.4) and placed in a plastic cuvette at 37°C in a dual-wavelength spectrofluorometer (Spex Industries, model CM1T11I, Edison, NJ). Fluoresecence emission was measured at excitation wavelengths of 340 nm and 380 nm, with emission at 505 nm. $[Ca^{2+}]_i$ was determined by monitoring the Fura-2 fluorescence-ratio signal. Intracellular Ca^{2+} concentration was calculated by Spex DM3000 software [Grynkiewicz et al., 1985].

Measurement of pH_i

T-cell suspensions (2×10^7 cells/ml) were incubated with BCECF/AM (3 μM) in HBSS and supplemented with 5 mM glucose and 0.2% BSA at 37°C for 30 min. Cells were then washed three times with Na^+ HBSS and resuspended in HBSS supplemented with 5 mM glucose and 0.2% BSA. For pH_i measurements, portions of cell suspension (1×10^6 cells) were washed twice with Na^+ HBSS, resuspended in 2.5 ml of

the same solution, transferred to a plastic cuvette at 37°C, and allowed to stabilize for 15 min before stimulation. BCECF fluorescence was measured with the spectrofluorometer and calculated from the ratio of emission at 525 nm for excitations at 435 nm and 500 nm. A mixture of 1×10^6 cells and 3 μM nigericin was added to the solutions at pH values of 2–10. Valinomycin (3 μM) was then added and allowed to react for 5 min, and the fluorescence signals were calibrated. The pH of K^+ HBSS was measured to the nearest 0.001 unit with a pH meter (Radiometer Copenhagen, model PHM 93). The calibration values were fitted as a standard sigmoid curve from which pH_i values were calculated.

Protein Kinase C Assay

Cells were washed twice with 200 μl buffer solution (137 mM NaCl, 5.4 mM KCl, 0.3 mM sodium phosphate, 0.4 mM potassium phosphate, 1 mM calcium chloride, 1 mg/ml glucose, 20 mM Hepes, pH 7.2, 30°C). To permeabilize the cells and initiate the protein kinase assay, the medium was replaced with 40 μl buffer solution containing 50 μg/ml digitonin, 10 mM $MgCl_2$, 25 mM β-glycerophosphate, 100 μM [γ-^{32}P]-ATP, and 300 μM myelin basic protein fragment ($MBP_{4\text{-}14}$). Kinase reactions were allowed to proceed for 10 min at 30°C and then terminated with 10 μl of 25% (w/v) trichloroacetic acid. Aliquots (45 μl) of the reaction mixtures were spotted on 2 × 2-cm strips of Whatman P-81 phosphocellulose paper and further reactions were terminated by immersion of strips into ice-cold 75 mM phosphoric acid. The strips were then washed four times with 75 mM phosphoric acid for 10 min and dried at 80°C. Radioactivity in the phosphorylated protein was measured by a liquid scintillation counter (Wallac 1409, Pharmacia, Finland) and quantified as described elsewhere [Roskoski, 1983]. The protein content in T cells was determined by Lowry's assay [Lowry et al., 1951].

Proliferation Studies

T cells (2×10^6 cells/ml) were plated in triplicate in a 96-well flat bottom plate (Corning, NY) and stimulated separately with PHA, LPS, or PMA. After 72 h, [^{3}H]-thymidine, (spec act 1 μCi/mM, New England Nuclear, Boston, MA) was added to the wells, incubated for 18 h, and harvested with a multiwell cell harvester (Dynatech, Automash 2000, Billing Shourst, UK). Radioactivity incorporated into DNA was measured by a liquid scintillation counter (Wallac 1409).

Statistical Analysis

Data concerning $[Ca^{2+}]_i$ and pH_i were analyzed by Student's paired *t*-test, with the significance level at $P < 0.05$. Data concerning PKC activity and proliferation were analyzed by Student's unpaired *t*-test. The values were expressed as mean ± standard error of the mean (SEM).

RESULTS

Effect of LPS on Protein Kinase C Activity and Intracellular pH in Human Peripheral T Cells

The effects of LPS (10 μg/ml) on PKC activity are shown in Figure 1. The stimulation of LPS for 5–120 min resulted in a significant ($P < 0.01$) increase in PKC activity in T cells. Administration of PHA (10 μg/ml) or PMA (100 pM) for 5–120 min also significantly increased PKC activity as compared with unstimulated cells. The time required for PKC peak activity was 15 min by PMA and 30 min by LPS or PHA. The activity of PKC was elevated and maintained at a plateau for at least 2 h after stimulation with LPS, PHA, or PMA (Fig. 1). The effects of LPS (10 μg/ml) on pH_i are shown in Figure 2. The intracellular pH began to rise 5 min after administration of LPS into T cells, whereas no effects were observed in medium control (Fig. 2). At the end of 30-min measurement, pH_i had changed from the basal level of 7.231 ± 0.078 (n = 19) to 7.356 ± 0.016 (n = 6, $P < 0.01$) with LPS; to 7.371 ± 0.034 (n = 7, $P < 0.01$) with PHA, and to 7.388 ± 0.031 (n = 6, $P < 0.01$) with PMA.

Effects of LPS on Intracellular Ca^{2+} Changes

T cells had been used to demonstrate the dose-response relationship between $[Ca^{2+}]_i$ elevation and stimulation caused by PHA before the experiments (Fig. 3). Administration of PHA (10 μg/ml) resulted in an increase in $[Ca^{2+}]_i$ starting within 1 min from the resting level of 98.2 ± 12.2 nM. $[Ca^{2+}]_i$ reached a plateau of 168.4 ± 21.9 nM (n = 15, $P < 0.05$) at 3 min, continuing at ≥10 min (Fig. 3). However, stimulation of cells with LPS (10 μg/ml) or PMA (100 pM) did not produce detectable changes in $[Ca^{2+}]_i$ (Fig. 4).

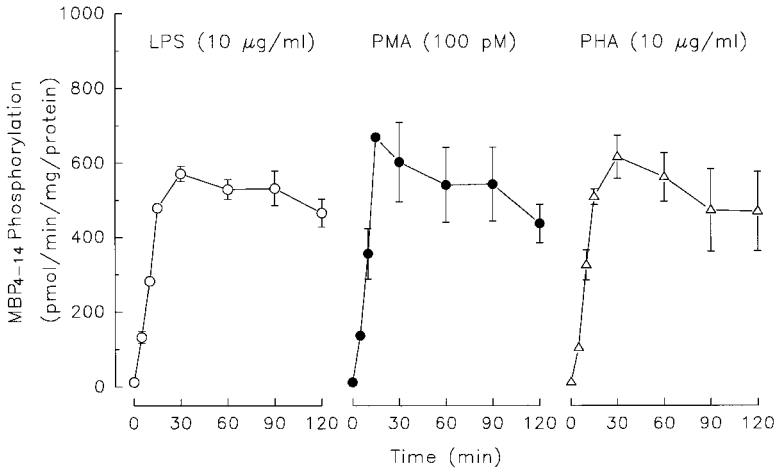

Fig. 1. Time course of protein kinase C activities in T cells after stimulation with 10 μg/ml lipopolysaccharide (LPS) (**left**), 100 pM phorbol myristate acetate (PMA) (**center**), and 10 μg/ml phytohemagglutinin (PHA) (**right**). The phosphorylation of myelin basic protein fragment ($MBP_{4–14}$) was assayed with cell-free extracts from each group of stimulated cells. The activities of protein kinase C increased significantly ($P < 0.01$) immediately after administration of LPS, PMA, or PHA. Each value represents mean ± SEM, n = 3.

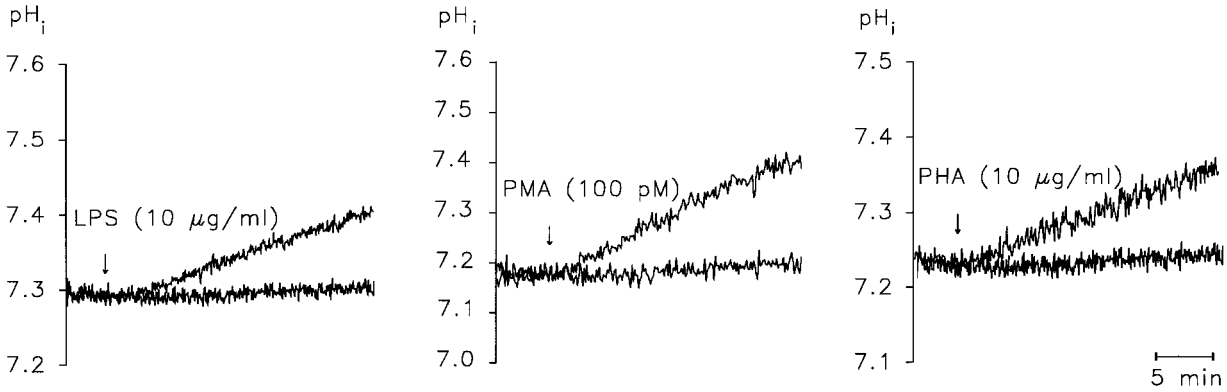

Fig. 2. Effects of lipopolysaccharide (LPS), phorbol myristate acetate (PMA), or phytohemagglutinin (PHA) on intracellular pH in human peripheral T cells. BCECF-loaded cells (1×10^6 cells/ml) were suspended in Na^+ Hank's solution with 10 μg/ml LPS (**left**), 100 pM PMA (**center**), or 10 μg/ml PHA (**right**) against vehicles (medium for LPS and PHA, and dimethylsulfoxide [DMSO] for PMA). Arrows indicate the addition of the ligands. Tracings are from one representative of six similar experiments.

Effects of LPS on T-Cell Proliferation

As shown in Figure 5, different concentrations of PHA (0.1, 1, and 10 μg/ml) led to a significant ($P < 0.01$) increased in the degree of proliferation by [^{3}H]-thymidine incorporation into T cells; By contrast, in the controls, LPS (10 μg/ml) and co-mitogen PMA (100 pM) failed to stimulate the uptake of [^{3}H]-thymidine, hence are unable to proliferate T cells. However, the combination of the calcium ionophore, A23187 (0.05 μM), with LPS led to significant ($P < 0.01$) stimulation in the uptake of [^{3}H]-thymidine into T cells after incubation with or without LPS.

DISCUSSION

In the present study, we found that the activity of PKC in human peripheral T cells increased and was maintained for at least 2 h after stimulation by LPS (Fig. 1). The results of activation of PKC by PHA and phorbol ester are consistent with observations in permeabilized T cells [Alexander et al., 1990]. Prolonged activation of PKC is required for T-cell proliferation [Berry et al., 1990]. Activation of PKC by LPS has been reported to increase nitric oxide synthetase activity and to reduce contractility in cardiac cells [McKenna et al., 1995]. It is well known that the activation of PKC is required

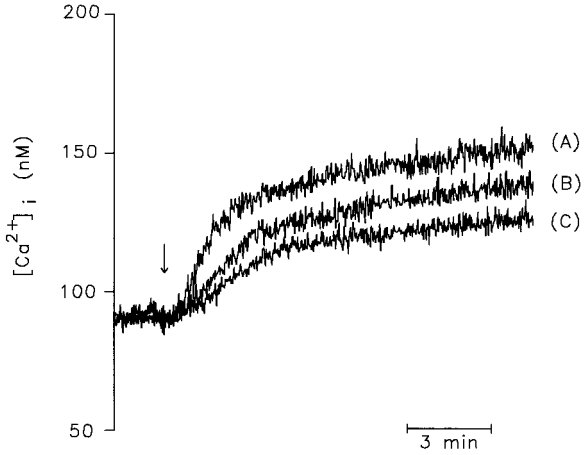

Fig. 3. Dose-response curve of phytohemagglutinin (PHA)-induced $[Ca^{2+}]_i$ changes in Fura-2-loaded human T cells. Cells were stimulated with (**A**) 20 μg/ml PHA, (**B**) 10 μg/ml PHA, or (**C**) 5 μg/ml PHA. Arrow indicates the time of addition of the stimulants. Tracings are obtained from one representative of more than 15 experiments.

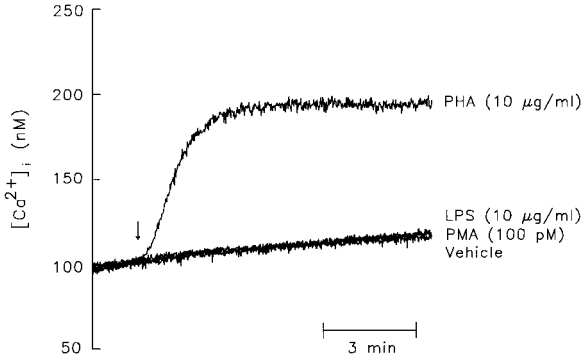

Fig. 4. Effect of phytohemagglutinin (PHA), lipopolysaccharide (LPS), and phorbol myristate acetate (PMA) on intracellular calcium in T cells. Fura-2-loaded T cells (2×10^6 cells/ml) were suspended in loading buffer at 37°C. The arrow indicates the time of addition of the ligands. Tracings are obtained from one representative of more than 15 experiments.

not only for T-cell proliferation, but for IL-2 receptor expression as well [Hengel et al., 1991].

The increase in pH_i in human peripheral T cells by LPS and by the controls, PMA, and PHA can be observed in Figure 2. Comparison of the time course of PKC activation in Figure 1 and alkalinization in Figure 2 showed that alkalinization begins after the activation of PKC in T cells. These results further confirm that the intracellular alkalinization in T cells can be used as a downstream physiological indicator for PKC activation. Thus, the present result of alkalinization in T cells by LPS is a new finding. Both the activation of amiloride-sensitive Na^+/H^+ exchange and the increased levels of pH_i have been demonstrated after the activation of T-cell surface receptors on T cells by lectin or antibodies [Mills et al., 1985c, 1986]. Two shifts in the intracellular alkalinization have been observed in T cells after stimulation with mitogens [Gerson et al., 1982]. The first shift in pH_i within minutes after addition of mitogen or phorbol ester has been considered early biochemical events in quiescent T cells or thymocytes [Grinstein et al., 1985, 1988; Gukovskaya et al., 1990; Astashkin et al., 1993]. The second shift coincides with enhancing thymidine incorporation into T cells as late responses in mitosis after the stimulation of mitogen [Gerson et al., 1982]. The production of diacylglycerol (DAG) after hydrolysis of phosphatidylinositol activates PKC, which in turn enhances the activity of Na^+/H^+ exchange to increase the pH_i in T cells [Gelfand et al., 1987]. Although LPS is nonmitogenic to T cells, the present findings involving an increase of PKC activity and intracellular alkalinization by LPS indicate that LPS can induce T cells to generate some biochemical signals. Whether the IL-2 receptor can be expressed by LPS needs further study.

The increase of $[Ca^{2+}]_i$ in T cells compared with PHA controls is shown in Figure 3. However, in the same cell condition, there were no detectable changes in $[Ca^{2+}]_i$ by either LPS or PMA (Fig. 4). When T cells are activated by T-cell receptor-specific monoclonal antibodies [Clayton et al., 1992] or mitogenic lectin [Gelfand et al., 1984, 1988; Hess et al., 1993], the early mitogenic signal of $[Ca^{2+}]_i$ elevation is observed. Since $[Ca^{2+}]_i$ elevation is essential for T-cell proliferation, and LPS failed to excite $[Ca^{2+}]_i$ elevation (Fig. 4), showing the inability of LPS to proliferate T cells (Fig. 5). The calcium ionophore A23187, which was used to elevate $[Ca^{2+}]_i$, is reported not to stimulate human T-cell proliferation [Chopra et al., 1987]. However, as shown in Figure 5, significant stimulation of T-cell proliferation was observed by the combination of A23187 with LPS. These results suggest that LPS failed to proliferate T cells because of its failure in stimulating $[Ca^{2+}]_i$ elevation. In addition, LPS was also demonstrated to be nonmitogenic in T cells. The failure of proliferating T cells by LPS also excludes the possibility of the existence of viable B cells or monocytes in isolated T cells because, in the presence of B cells or monocytes, incorporation

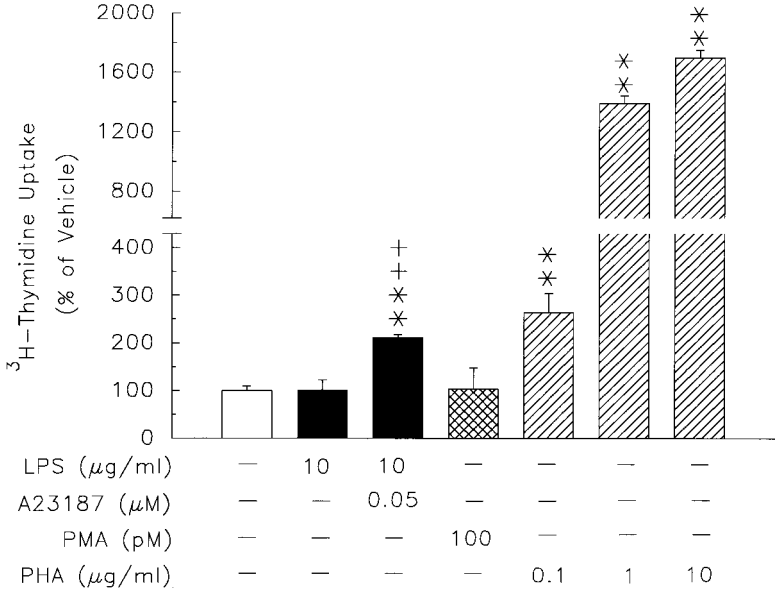

Fig. 5. Effects of lipopolysaccharide (LPS) (10 μg/ml), LPS plus A23187 (0.05 μM), phorbol myristate acetate (PMA) (100 pM), and phytohemagglutinin (PHA) (0.1, 1, and 10 μg/ml) on the incorporation of [^{3}H]-thymidine into T cells. The incorporation is presented as percentage of vehicle control. Data are expressed as the mean ± SEM, n = 3, $^{**}P <$ 0.01 compared with vehicle control, and $\dagger\dagger P < 0.01$ compared with the LPS group.

of [^{3}H]-thymidine might be increased [DeFranco et al., 1987; Mattern et al., 1994].

Studies of LPS effects on B cells suggest that Na^+/H^+ exchange can be stimulated by both elevation of cytosolic calcium and activation of PKC [Rosoff and Cantley, 1985]. However, in the present study, elevation in $[Ca^{2+}]_i$ was not shown to accompany PKC activation and alkalinization in T cells by LPS. The failure of elevation in $[Ca^{2+}]_i$ indicates that the origin of DAG in activation of PKC by LPS is probably not attributable to the hydrolysis of phosphatidylinositol. Therefore, the Na^+/H^+ exchange could be activated independently from changes of $[Ca^{2+}]_i$ after stimulation of T cells by LPS. Also, the prevention of cytoplasmic alkalinization is not due to an inhibition of the increase of $[Ca^{2+}]_i$ in T cells [Mills et al., 1990].

In summary, the results demonstrate that LPS directly increases PKC activation and subsequent intracellular alkalinization in T cells. However, by lacking the machinery to stimulate the mitogenic signal on $[Ca^{2+}]_i$ elevation, LPS failed to stimulate the proliferation of T cells. Whether the PKC activation by LPS is clinically significant in T-cell activation (e.g., during Gram-negative sepsis or endotoxemia) remains to be investigated.

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REFERENCES

Alexander DR, Graves JD, Lucas SC, Cantrell DA, Crumpton MJ. 1990. A method for measuring protein kinase C activity in permeabilized T lymphocytes by using peptide substrates. Biochem J 268:303–308.

Astashkin EI, Khodorova AB, Surin AM. 1993. Arachidonic acid abolishes the mitogen-induced increase in cytosolic free Ca^{2+} and intracellular pH_i in rat thymocytes. FEBS Lett 329:72–74.

Berry N, Nishizuka Y. 1990. Protein kinase C and T cell activation. Eur J Biochem 189:205–214.

Berry N, Ase K, Kishimoto A, Nishizuka Y. 1990. Activation of resting human T cells requires prolonged stimulation of protein kinase C. Proc Natl Acad Sci USA 87:2294–2298.

Chopra RK, Nagel JE, Chrest EJ, Adler WH. 1987. Impaired phorbol ester and calcium ionophore induced proliferation of T cells from old humans. Clin Exp Immunol 70:456–462.

Clayton LK, Lerner A, Diener AC, Hussey RE, Koyasu S, Reinherz EL. 1992. T-cell-receptor isoforms. Int J Cancer 7:1–5.

Clevers HC, Hoeksema M, Gmelig-Meyling FH, Ballieux RE. 1985. Calcium ionophore A23187 induces interleukin 2 reactivity in human T cells. Scand J Immunol 22:633–638.

Defranco AL, Gold MR, Jakway JP. 1987. B-lymphocyte signal transduction in response to anti-immunoglobulin and bacterial lipopolysaccharide. Immunol Rev 5:161–176.

Ebanks R, Roifman C, Mellors A, Mills GB. 1989. The diacylglycerol analogue, 1,2-sn-dioctanoylglycerol, induces an increase in cytosolic free Ca^{2+} and cytosolic acidification of T lymphocytes through a protein kinase C-independent process. Biochem J 258:689–698.

Gelfand EW, Cheung RK, Grinstein S. 1984. Role of membrane potential in the regulation of lectin-induced calcium uptake. J Cell Physiol 121:533–539.

Gelfand EW, Cheung RK, Grinstein S, Mills GB. 1986. Characterization of the role for calcium influx in mitogen-induced triggering of human T cells. Identification of calcium-dependent and calcium-independent signals. Eur J Immunol 16:907–912.

Gelfand EW, Mills GB, Cheung RK, Lee JW, Grinstein S. 1987. Transmembrane ion fluxes during activation of human T lymphocytes: role of Ca^{2+}, Na^+/H^+ exchange and phospholipid turnover. Immunol Rev 95:59–87.

Gelfand EW, Cheung RK, Mills GB, Grinstein S. 1988. Uptake of extracellular Ca^{2+} and not recruitment from internal stores is essential for T lymphocyte proliferation. Eur J Immunol 18:917–922.

Gerson DF, Kiefer H, Eufe W. 1982. Intracellular pH of mitogen-stimulated lymphocytes. Science 216:1009–1010.

Grinstein S, Cohen S, Goetz JD, Rothstein A, Gelfand EW. 1985. Characterization of the activation of Na^+/H^+ exchange in lymphocytes by phorbol esters: change in cytoplasmic pH dependence of the antiport. Proc Natl Acad Sci USA 82:1429–1433.

Grinstein S, Smith JD, Onizuka R, Cheung RK, Gelfand EW, Benedict S. 1988. Activation of Na^+/H^+ exchange and the expression of cellular proto-oncogenes in mitogen- and phorbol ester-treated lymphocytes. J Biol Chem 263:8658–8665.

Grynkiewicz G, Poenie M, Tsien RY. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450.

Gukovskaya AS, Zinchenko VP, Khodorov BI, Cragoe EJ. 1990. The relationship between mitogen-induced changes in the cytoplasmic pH and free Ca^{2+} concentration in rat thymocytes. Biochim Biophys Acta 1051:242–249.

Hengel H, Allig B, Wagner H, Heeg K. 1991. Dissection of signals controlling T cell function and activation: H7, an inhibitor of protein kinase C, blocks induction of primary T cell proliferation by suppressing interleukin (IL)2 receptor expression without affecting IL2 production. Eur J Immunol 21:1575–1582.

Hess SD, Oortgiesen M, Cahalan MD. 1993. Calcium oscillations in human T and natural killer cells depend upon membrane potential and calcium influx. J Immunol 150: 2620–2633.

Koyasu S, Suzuki G, Asano Y, Osawa H, Diamantstein T, Yahara I. 1987. Signals for activation and proliferation of murine T lymphocyte clones. J Biol Chem 262:4689–4695.

Lin CY, Lo SC. 1991. Treatment of hepatitis B virus-associated membranous nephropathy with adenine arabinoside and thymic extract. Kidney Int 39:301–306.

Lowry OH, Rosenborough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275.

Mattern T, Thanhäuser A, Reiling N, Toellner KM, Duchrow M, Kusumotok S, Rietschel ET, Ernst M, Brade H, Flad HD, Ulmer AJ. 1994. Endotoxin and lipid A stimulate proliferation of human T cells in the presence of autologous monocytes. J Immunol 153:2996–3004.

Mattern T, Flad H, Brade L, Rietschel ET, Ulmer AJ. 1998. Stimulation of human T lymphocytes by LPS is MHC unrestricted but strongly dependent on B7 interactions. J Immunol 160:3412–3418.

McKenna TM, Li S, Tao S. 1995. PKC mediates LPS- and phorbol-induced cardiac cell nitric oxide synthase activity and hypocontractility. Am J Physiol 269:H1891–H1898.

Mills GB, Cheung RK, Grinstein S, Gelfand EW. 1985a. Increase in cytosolic free calcium concentration is an intracellular messenger for the production of interleukin 2 but not for expression of the interleukin 2 receptor. J Immunol 134:1640–1643.

Mills GB, Cheung RK, Grinstein S, Gelfand EW. 1985b. Interleukin 2-induced lymphocyte proliferation is independent of increases in cytosolic-free calcium concentrations. J Immunol 134:2431–2435.

Mills GB, Cragoe EJ, Gelfand EW, Grinstein S. 1985c. Interleukin 2 induces a rapid increase in intracellular pH through activation of a Na^+/H^+ antiport, cytoplasmic alkalinization is not required for lymphocyte proliferation. J Biol Chem 260:12500–12507.

Mills GB, Hill M, McGill M, May C, Stanley J, Stewart DJ, Mellors A, Gelfand EW. 1990. Interrelationship between signals transduced by phytohemagglutinin and interleukin 1. J Cell Physiol 142:539–551.

Roskoski R. 1983. Assays of protein kinase. Method Enzymol 99:3–6.

Rosoff PM, Cantley LC. 1985. Lipopolysaccharide and phorbol esters induce differentiation but have opposite effects on phosphatidylinositol turnover and Ca^{2+} mobilization in 70Z/3 pre-B lymphocytes. J Biol Chem 260:9209–9215.

Truneh A, Albert F, Schmitt-Verhulst AM. 1985. Early steps of lymphocyte activation bypassed by synergy between calcium ionophores and phorbol ester. Nature 313:318–320.

Weiss A, Littman DR. 1994. Signal transduction by lymphocyte antigen receptor. Cell 76:263–274.

Whelan JP, Shearer WT, Gilliam EB, Hardy KJ. 1992. A protein kinase C-activating phorbol ester accelerates the T cell antigen receptor-stimulated phosphatidylinositol cycle in normal human $CD4^+$ T cells. J Immunol 148: 2872–2878.