Stimulation of the T3-T Cell Receptor-associated Ca\textsuperscript{2+} Influx Enhances the Activity of the Na\textsuperscript{+}/H\textsuperscript{+} Exchanger in a Leukemic Human T Cell Line*

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Three monoclonal antibodies reactive with different structural domains of the T3-T cell receptor complex of the human T cell leukemia line, HPB-ALL, were previously shown to activate a membrane potential-sensitive, La\textsuperscript{3+}-inhibitable Ca\textsuperscript{2+} influx (Oettgen, H. C., Terhorst, C., Cantley, L. C., and Rosoff, P. M. (1985) Cell 40, 583-590). OKT3 (anti-T3), WT-31 (anti-receptor constant region), and T40/25 (anti-receptor variable region) also enhance the activity of the Na\textsuperscript{+}/H\textsuperscript{+} exchanger in these cells. The associated rise in pH, was dependent on the presence of external Ca\textsuperscript{2+} and Na\textsuperscript{+}, was inhibited by dimethylamiloride and La\textsuperscript{3+}, and was maintained for at least 20 min. Phorbol esters, which are co-mitogenic in T cells and activate protein kinase C, also stimulated the exchanger, but by a mechanism not requiring an elevation in cytoplasmic Ca\textsuperscript{2+}; the rise in pH, rapidly peaked and returned to baseline levels within 20 min. Pretreatment with phorbols prevented an increase in pH, by OKT3 although a transient additive effect was observed when the two were added simultaneously. Receptor function was maintained in the presence of phorbol esters as OKT3 still stimulated a Ca\textsuperscript{2+} influx. These data demonstrate the existence of two interdependent pathways to activate Na\textsuperscript{+}/H\textsuperscript{+} exchange in T lymphocytes and suggest a pathway of internal regulation of antigen-activated signal transduction.

Stimulation of undifferentiated or quiescent cells with growth factors and mitogens is accompanied by rapid alterations in the flux rates of several critical cations, often leading to dramatic changes in the intracellular ionic milieu. Many cell systems have been described in which binding of a specific mitogen to its receptor initiates a series of membrane transport events which eventually lead to increases in cytosolic pH, [Na\textsuperscript{+}], and free [Ca\textsuperscript{2+}] (Rosoff and Cantley, 1983; Rosoff et al., 1984; Cantley et al., 1984; Rosoff and Cantley, 1985a; Rozengurt and Heppel, 1975; Rozengurt, 1981; Rothenburg et al., 1983; Mix et al., 1984; Moolenaar et al., 1981; Moolenaar et al., 1984a). In LPS\textsuperscript{-}treated 70Z/3 pre-B lymphocytes and lectin and antibody-stimulated T cells, these changes appear to be rate-limiting for either differentiation or proliferation to proceed (Rosoff and Cantley, 1983; Rosoff et al., 1984; Deutsch and Price, 1982; Mastro and Smith, 1983; DeCoursey et al., 1984; Truneh et al., 1985; Weiss et al., 1984, a and b).

The increase in [Ca\textsuperscript{2+}], may be accomplished by at least two independent pathways: the opening of a membrane calcium channel which allows extracellular Ca\textsuperscript{2+} to flow down its concentration gradient as occurs in IgE-stimulated, antigen-primed mast cells (Beaven et al., 1984) or release of Ca\textsuperscript{2+} from a microsomal storage pool via an inositol 1,4,5-trisphosphate-mediated mechanism (Berridge et al., 1984; Joseph et al., 1984, a and b; Irvine et al., 1984). The latter pathway is coupled to the phosophatidylinositol turnover cycle which, in addition to inositol trisphosphate, generates diacylglycerol. This latter compound is essential for the activation of protein kinase C, the calcium-activated, phospholipid-dependent protein kinase (Berridge, 1984; Berridge and Irvine, 1984; Nishizuka, 1984). It is also possible that a mutual interaction between the two exists.

The rise in cell Na\textsuperscript{+} and pH is due to the increased activity of the Na\textsuperscript{+}/H\textsuperscript{+} antiporter (Rosoff et al., 1984; Boron, 1984; Paris and Pouysségur, 1983, 1984). We and others have shown that tumor-promoting phorbol esters, which directly activate protein kinase C by substituting for endogenous diacylglycerol, can stimulate Na\textsuperscript{+}/H\textsuperscript{+} exchange by a calcium-independent pathway and can, in some cells, induce proliferation or differentiation (Rosoff et al., 1984; Moolenaar et al., 1984b; Besterman and Cuatrecasas, 1984; Burns and Rozengurt, 1983). The antiport may also be activated by a Ca\textsuperscript{2+}-dependent pathway as demonstrated by Villereal and his colleagues (Owen and Villereal, 1982; Villereal, 1981).

Quiescent T lymphocytes may be induced to proliferate by treatment with lectins, calcium ionophores, or anti-T3 antibodies, in the presence of monocytes or phorbol esters. These agents cause an increase in cell Ca\textsuperscript{2+} (Tsien et al., 1982; Weiss et al., 1984a; Metcalfe et al., 1980; Akerman and Andersson, 1984). Treatment with mitogenic lectins such as Con A has been reported to cause an increase in pH, as well (Gerson and Kiefer, 1982; Gerson et al., 1982, a and b; Hesketh et al., 1985). Neither monocytes nor phorbol esters cause an increase in [Ca\textsuperscript{2+}]. (Tsien et al., 1982). These agents apparently provide a second parallel signal for the induction of cell growth. It has recently been reported from this laboratory and others that monoclonal antibodies directed against the T3-T cell receptor complex of human T lymphocytes induce a membrane potential-sensitive calcium influx that is La\textsuperscript{3+}-inhibitable (Oettgen et al., 1985; Weiss et al., 1984, a and c; Imboden et al., 1985). In at least one case, treatment with these agents is also accompanied by an increase in phospha-
tidilyositol turnover and release of Ca\textsuperscript{2+} from intracellular stores (Imboden et al., 1985). These authors have suggested that resulting increased liberation of diacylglycerol activates protein kinase C which in turn may affect the Ca\textsuperscript{2+} influx. We have utilized monoclonal antibodies that bind to distinct epitopes residing on the T3-T cell receptor complex: OKT3 reacts with the T3 complex present on all mature human T cells and WT-31 and T40/25 which react with the constant and variable domains, respectively, of the 90-kDa heterodimeric T cell receptor for antigen on HPB-ALL cells (Meuer et al., 1984; Oettgen et al., 1984; Tax et al., 1983; Kappler et al., 1983). In this report, we show that these monoclonal antibodies activate Na\textsuperscript{+}/H\textsuperscript{+} exchange that is dependent on the presence of extracellular Ca\textsuperscript{2+} and is inhibited by dimethylamiloride and La\textsuperscript{3+}. Phorbol esters stimulate the antiporter by a calcium-independent pathway in these cells and can block the effects of anti-T3 antibodies on Na\textsuperscript{+}/H\textsuperscript{+} exchange. These data provide further insight into the triggering process by a calcium-independent pathway in these cells and can suggest a mechanism for internal regulation of these pathways.

MATERIALS AND METHODS

Cells

HPB-ALL cells were used for all experiments. They are a human T cell leukemia line, the characteristics of which have been described elsewhere (Minowada, 1985). They were maintained in continuous suspension culture in RPMI-1640 medium supplemented to 5% with fetal calf serum (v/v), 10 mM Na\textsuperscript{+}-pyruvate, 10 mM Hepes, 100 units/ml of penicillin, 100 units/ml of streptomycin, and 0.5 mM amphotericin B. Quin2/AM (the acetomethoxy ester of Quin2) was from Sigma and stored as a 30 mM stock solution in dimethyl sulfoxide at -20 °C. Quin2/AM (the acetomethoxy ester of Quin2) was stored at -20 °C in humidified air, 5% CO\textsubscript{2}.

Reagents

Antibodies—OKT3 was the gift of Dr. Cox Terhorst (Dana-Farber Cancer Institute, Boston, MA). T40/25 was generously supplied by Dr. W. J. Tax (St. Radboudziekenhuis, Nijmagen, The Netherlands).

Reagents—Dimethylcarboxyfluorescein diacetate and its free acid were from Molecular Probes Inc., Junction City, Oregon. Phorbol esters were from L. C. Services Corp., Woburn, MA. DMA was kindly provided by Dr. E. J. Cragoe, Jr. of Merck Research Labs, West Point, PA. It was kept in a stock solution at 23 mM in dimethyl sulfoxide at -20 °C. Quin2/AM (the acetomethoxy ester of Quin2) was from Sigma and stored as a 30 mM stock solution in dimethyl sulfoxide, frozen at -20 °C. All other chemicals used were of reagent grade.

Determination of pH,

Intracellular pH measurements were made using the pH-sensitive fluorescent dye, DMCF, using a modification of our previously reported method (Rosoff et al., 1984). This compound has a pK\textsubscript{a} of approximately 7.05 which makes it ideal for the study of pH. HPB-ALL cells were collected, washed, and resuspended to 5 x 10\textsuperscript{6} cells/ml in Buffer A (145 mM NaCl, 4 mM KCl, 1 mM NaH\textsubscript{2}PO\textsubscript{4}, 0.8 mM MgSO\textsubscript{4}, 1.8 mM CaCl\textsubscript{2}, 25 mM Hepes, 10 mM glucose, pH 7.4). DMCF-diacetate was added to 50 μM from a 0.5 mM stock made up in Buffer A (DMCF-diacetate powder was first dissolved in 50 μl of absolute ethanol, then brought up to the appropriate stock concentration in Buffer A) and incubated for 45 min at 37 °C. At the end of the loading period, the cells were washed 3 times in fresh Buffer A (37 °C), resuspended in 1 ml of the same buffer, and kept on ice as a stock suspension. Cells kept in this manner for up to 4 h did not exhibit any loss of viability as determined by the exclusion of trypan blue.

pH\textsubscript{e} was determined in a Kontron Uvikon 810 dual beam spectrophotometer utilizing the specific pH-dependent absorbance properties of this fluor. The parent compound of DMCF, carboxyfluorescein, has a peak absorbance at λ\textsubscript{em} 505 nm and an isosbestic point at λ\textsubscript{em} 470 nm (Thomas et al., 1982). To determine the analogous spectral properties of DMCF, the hydrolyzed acid was suspended to 0.5 μM in 1 ml of a solution containing 130 mM KCl, 1 mM MgCl\textsubscript{2}, and one of the following buffers: 25 mM Mes (to pH 6.5), 25 mM Hepes (to pH 6.3, 7.1, 7.3, and 7.5), and 25 mM Tris base (to pH 7.9 and 8.3) and absorbance scans were run. The results are shown in Fig. 1. There is an absorbance peak at λ\textsubscript{em} 505 nm and an isosbestic point at λ\textsubscript{em} 470 nm. These values were used for all further measurements and calculations. The ratio of absorbance at λ\textsubscript{em} 505 nm/470 nm (after subtracting away the absorbance at these wavelengths of an equal concentration of cells that had not been loaded with the dye) was used to determine pH, by comparison to a standard calibration curve generated by permeabilizing DMCF-loaded HPB-ALL cells with 5 μg/ml nigericin in variable pH/KCl buffers as described (Rosoff et al., 1984; Thomas et al., 1982). The calibration curve used for this report is shown in the inset of Fig. 1. For each experiment 1 x 10\textsuperscript{6} DMCF-loaded HPB-ALL cells were suspended in 1 ml of the appropriate buffer in 1 ml disposable polypropylene microcuvettes (American Scientific) in a 37 °C constant temperature cuvette chamber and allowed to equilibrate for 5 min. Data were collected using the multiple wavelength program feature of the Uvikon 810 with a 2-9 integration time for λ\textsubscript{em} 505 nm and λ\textsubscript{em} 470 nm (2 nm bandwidth). Absorbance spectra from 550 nm to 400 nm for the appropriate buffer and any added reagents were baseline-subtracted for each experiment. For experiments done in the absence of extracellular Ca\textsuperscript{2+} or Na\textsuperscript{+}, Buffers B and C were used, respectively (Buffer B: 145 mM NaCl, 4 mM KCl, 1.0 mM NaH\textsubscript{2}PO\textsubscript{4}, 0.8 mM MgSO\textsubscript{4}, 10 mM glucose, 25 mM Hepes, 0.5 mM EGTA, pH 7.4; Buffer C: 145 mM choline Cl, 3 mM KCl, 1.0 mM KH\textsubscript{2}PO\textsubscript{4}, 0.8 mM MgSO\textsubscript{4}, 1.8 mM CaCl\textsubscript{2}, 10 mM glucose, 25 mM Hepes acid, pH 7.4).

HPB-ALL cells were collected, washed, and loaded with the permeant acetomethoxy ester of Quin2 and fluorescence intensity was measured as described (Oettgen et al., 1986).

RESULTS

OKT3, WT-31, and T40/25 Antibodies Stimulate Na\textsuperscript{+}/H\textsuperscript{+} Exchange in HPB-ALL Cells—We have previously shown that

![Fig. 1. Absorbance spectra for dimethylcarboxyfluorescein dependence on pH. DMCF (free acid) was diluted to 0.5 μM in buffers of varying pH as described under "Materials and Methods" and absorbance scans were performed. Inset: calibration curve of λ\textsubscript{em} 505 nm/470 nm versus pH in permeabilized HPB-ALL cells.](Image)
monoclonal antibodies directed against different regions of the T3-T cell receptor complex in HPB-ALL cells stimulate a membrane potential-sensitive Ca\(^{2+}\) influx (Oettgen et al., 1985) that is inhibited by both EGTA and La\(^{3+}\). Both OKT3, which binds to an invariant heterotrimer present on all mature human T cells (Meuer et al., 1984), and WT-31, which recognizes a constant region domain of the 90-kDa heterodimer antigen receptor, are mitogenic for resting T cells (Tax et al., 1983). Increases in Na\(^+/H^+\) exchange have been shown to be intimately associated with cell activation; in at least one case, stimulation of this antiport appears to be essential for the induction of differentiation (Rosoff and Cantley, 1983; Rosoff et al., 1984). In addition, the presence of extracellular Na\(^+\) has been shown to be necessary for mitogen activation of T cells (Deutsch et al., 1981, 1984). We therefore wished to see if specific stimulation of the T3-T cell receptor complex with these monoclonal reagents could stimulate the Na\(^+/H^+\) antiport in these cells.

OKT3, T40/25, and WT-31 all caused a rapid and sustained increase in pH within 30 s after addition to a suspension of DMCF-loaded HPB-ALL cells (Fig. 2). The pH change was detected as an increase in the absorbance ratio at \(\lambda_590\) nm/\(\lambda_470\) nm of DMCF (see “Materials and Methods”). The resting pH in these cells is approximately 7.15 ± 0.05, in good agreement with data reported elsewhere using a different technique to measure basal pH in nontransformed T cells (Gerson and Kiefer, 1982). The pH increased by about 0.15 pH unit after treatment with the antibodies. WT-82, a monclonal reagent reactive with the T8-T lymphocyte surface antigen that is expressed on these cells, had no effect on pH in HPB-ALL cells (Fig. 2A). The increase in pH, was sustained for at least 15 min (see below) and was initiated on the same time scale as the antibody-stimulated Ca\(^{2+}\) influx (Oettgen et al., 1985). In addition, the absolute increase in pH, measured here is similar to that noted in LPS-treated 707/3 pre-B lymphocytes (Rosoff et al., 1984) and epidermal growth factor-stimulated A431 cells (Rothenburg et al., 1983), and serum-stimulated fibroblasts (Burns and Rozenburg, 1983).

The fact that the anti-T3-T cell receptor complex antibodies induce an increase in intracellular pH does not, by itself, mean that this is due to enhanced Na\(^+/H^+\) exchange. We therefore examined the ability of the antibodies to increase pH, in the presence of DMA, a potent and specific inhibitor of the Na\(^+/H^+\) antiport (Zhuang et al., 1984). DMA alone caused a rapid decrease in pH, to a new steady state value of 6.60 after about a 4-min incubation at 37 °C (Fig. 2A), suggesting that the antiport is in a partially active state in the unstimulated cells. Although there was some variability in the rate of decrease in pH, with the addition of DMA between experiments, the eventual steady state pH of 6.60 was reproducibly achieved and addition of antibodies against the T3-T cell receptor complex caused no significant deviation from this low value. These results are in agreement with our observations on the effect of antibody on pH, in 70Z/3 cells (Rosoff et al., 1984). The concentration of DMA used (50 μm) was well below the \(K_f\) for inhibition of protein kinase C as recently reported by Besterman et al. (1985). The basal pH was not affected by exposure to 1% dimethyl sulfoxide, the solvent in which DMA was dissolved (data not shown). These data all suggest that the antibodies are stimulating the Na\(^+/H^+\) antiport to produce a rise in pH.

Further evidence that these reagents are causing an increase in Na\(^+/H^+\) exchange is provided by the dependence of the increase in pH, on external Na\(^+\). DMCF-loaded HPB-ALL cells were placed in a Na\(^+-\)free, choline C\(^+\) buffer (Buffer C), allowed to equilibrate to the 37 °C temperature of the cuvette chamber for 5 min, treated with OKT3, WT-31, or T40/25, and the pH was followed. The results are shown in Fig. 3. Elimination of extracellular Na\(^+\) by itself without antibody treatment caused a decrease in pH, to approximately 6.6, very similar to the decrease observed in the presence of DMA (Figs. 2 and 3). As in the case of DMA addition, the scatter in the data was greater in the Na\(^+-\)free medium; however, the antibodies against the T3-T cell receptor complex did not appear to cause a significant increase in cytoplasmic pH under these conditions. The regulation of cytoplasmic pH in unstimulated cells is dependent on extracellular Na\(^+\). The antibody-induced alkalization is also dependent on extracellular Na\(^+\), indicating the role of the plasma membrane Na\(^+/H^+\) exchange system in this regulation.

The increase in pH, stimulated by OKT3, WT-31, and T40/25 appears to result from the stimulated Ca\(^{2+}\) influx. The effects of antibody treatment on pH, in the absence of extracellular Ca\(^{2+}\) are shown in Fig. 3. Preventing the influx of Ca\(^{2+}\) that is associated with antibody treatment by removal of its external source completely eliminated the stimulation of Na\(^+/H^+\) exchange. Removal of extracellular Ca\(^{2+}\) had no effect on pH, by itself over the 15-min time course of the experiment. We have previously shown that La\(^{3+}\) inhibits the antibody-stimulated Ca\(^{2+}\) influx (Oettgen et al., 1985). This cation also blocked the ability of the antibodies to induce an increase in pH, (data not shown). These results suggest that the rise in pH, is a process initiated or dependent upon the Ca\(^{2+}\) influx stimulated by receptor activation.

**Phospholipid Esters Stimulate Na\(^+/H^+\) Exchange by a Mechanism Which Does Not Require Elevation of Cytoplasmic Ca\(^{2+}\)**

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**Fig. 2. Effects of OKT3, WT-31, and T40/25 on HPB-ALL cell pH\(^{-}\) inhibition by dimethylamiloride.** HPB-ALL cells were loaded with the diacete ester of DMCF as described under “Materials and Methods” and equilibrated for 5 min at 37 °C in 1 ml of Buffer A at 1 x 10⁵ cells/mL. Cytoplasmic pH was periodically monitored by scanning the absorbance spectrum of DMCF at discrete wavelengths as described under “Materials and Methods.” At the time indicated by the arrow, antibody was added to 2.5 μg/mL. When present, dimethylamiloride (50 μM final concentration) was added to a fraction of cells 2 min prior to the addition of the antibody (open circles). Data points represent means of at least 4 experiments.
Phorbol esters are potent co-mitogens for lectin and anti-T3 antibody stimulation and mediate their effects via protein kinase C activation (Berridge and Irvine, 1984). These effects require the presence of extracellular Ca\(^{2+}\) and are dependent on the requirement for monocytes/macrophages to be present in the culture system. We have previously shown that phorbol esters enhance amiloride-sensitive Na\(^+/H^+\) exchange in 702/3 cells (Tsien et al., 1982; Moolenaar, 1985). We therefore wished to determine the effects of TPA on pHi in HPB-ALL cells.

DMC\(^{-}\)-loaded HPB-ALL cells were exposed to either 50 nM TPA or PDD (a biologically inactive phorbol ester) and cytoplasmic pH was monitored. The results are shown in Fig. 4. TPA stimulated an increase in pHi that was independent of the presence of extracellular Ca\(^{2+}\), but was dependent on the presence of external Na\(^+\). In addition, as with the rise in pHi, the effects of OKT3 and TPA were completely blocked by the addition of 50 \(\mu\)M DMA. Curiously, unlike the response to the antibodies, where pHi remained elevated for at least 20 min (see below), after 8–10 min of exposure to TPA, the pHi returned to the prestimulated level. The biologically inactive phorbol, PDD, and 0.1% dimethyl sulfoxide (v/v), the solvent in which both TPA and PDD were dissolved, had no effect on pHi. These data suggest that there are at least two separate mechanisms for stimulation of the Na\(^+/H^+\) antiport in these cells: one via a Ca\(^{2+}\)-dependent pathway through stimulation of the T3-T cell receptor complex-associated Ca\(^{2+}\) channel and the second via a Ca\(^{2+}\)-independent pathway mediated by protein kinase C.

**Pretreatment with Phorbol Esters Prevents OKT3 Stimulation of Na\(^+/H^+\) Exchange**—It has recently been reported that even though phorbol esters cause a small increase in cytoplasmic pH in A431 human epidermoid carcinoma cells, pretreatment with phorbol esters blocks the epidermal growth factor stimulation of Na\(^+/H^+\) exchange (Whitley et al., 1984). We therefore wished to see if pretreatment of HPB-ALL cells with TPA would abolish the effect of anti-T3-T cell receptor antibodies on Na\(^+/H^+\) exchange.

When added simultaneously, TPA and OKT3 had apparent additive effects on the pHi of DMC\(^{-}\)-loaded HPB-ALL cells (Fig. 5). However, as with TPA treatment alone, the pH rapidly increased and then returned to baseline levels within 10 min. When these cells were exposed to 50 nM TPA for 15 min at 37 °C (by which time the pHi had returned to baseline) and then treated with OKT3, there was complete inhibition.
of the ability of the antibody to stimulate Na+/H+ exchange (Fig. 5). The converse condition produced opposite results: preincubation with OKT3 did not block the effects of subsequently added TPA on pH. It can be seen from Fig. 5 that a small increase in pH occurs when TPA is added subsequent to OKT3 pretreatment but, as observed with simultaneous addition of the two agents, the pH returns to baseline after 8–10 min. Thus, the effect of phorbol esters on pH is transient whereas that of the antibodies is more prolonged. With OKT3 addition alone, the pH remains elevated above that of control cells for at least 20 min. The results in Fig. 5 indicate that while TPA enhances the effect of OKT3 on pH, at short times after addition, it eliminates the response at longer times, thus converting a step increase in pH into a transient pulse.

The fact that OKT3 can no longer increase pH after preincubation of HPB-ALL cells with TPA could be due to modification of the T3-T cell receptor complex with a corresponding functional inhibition. We therefore measured the ability of OKT3 to stimulate a Ca2+ influx in Quin2-loaded HPB-ALL cells as previously described (Oettgen et al., 1985). The results are shown in Fig. 6. It is clear that prolonged exposure to TPA had no effect on the ability of OKT3 to increase [Ca2+]i, a function associated with stimulation of the T3-T cell receptor complex and a prerequisite for antibody stimulation of Na+/H+ exchange. The [Ca2+]i rose from a baseline value of 150 nM to about 300 nM, as previously observed (Oettgen et al., 1985). Both WT-31 and T40/25 antibodies were also able to stimulate a Ca2+ influx after TPA preincubation (data not shown). These results suggest that the inhibitory action of phorbol esters is directed against a step distal to the T3-T cell receptor complex-associated Ca2+ influx and argues against a feedback regulatory role of protein kinase C on the T3-T cell receptor complex.

**DISCUSSION**

In this paper we show that monoclonal antibodies directed against the variable and constant domains of the T cell receptor (T40/25 and WT-31, respectively) and the associated T3 antigen (OKT3) of the human T cell leukemia line HPB-ALL rapidly stimulate Na+/H+ exchange, leading to an increase in pH. The antibody-induced rise in pH is dependent on the presence of extracellular Na+ and Ca2+ and is blocked by dimethylamiloride, an inhibitor of Na+/H+ exchange (Figs. 2 and 3), and La3+, an inhibitor of Ca2+ channels. We have previously demonstrated that these same antibodies activate a membrane potential-sensitive Ca2+ influx in HPB-ALL cells that produces a rise in cytosolic free [Ca2+] (Oettgen et al., 1985). These data therefore suggest that stimulation of the T3-T cell receptor complex-associated Ca2+ influx is a necessary prerequisite for activation of the antiporter in T lymphocytes.

We also show that TPA, a potent tumor-promoting phorbol ester, stimulates DMA-inhibitable Na+/H+ exchange in these cells (Fig. 4). This agent, which does not activate the T3-T cell receptor Ca2+ channel (Oettgen et al., 1985), enhances antiporter activity by a mechanism which does not require elevation of cytoplasmic Ca2+. TPA and OKT3 appear to have
additive, but transitory, effects on pH when added to HPB-ALL cells simultaneously (Fig. 5). However, when the cells are preincubated with TPA prior to the addition of the antibody, OKT3 is incapable of stimulating an increase in pH.

The converse situation gives different results: preincubation with OKT3 does not prevent further activation of Na+/H+ exchange by TPA. The fact that phorbol esters can enhance Na+/H+ exchange suggests a role for receptor-stimulated phosphatidylinositol turnover which has been shown to increase in response to anti-T3 and T cell receptor antibodies (Innboden and Stobo, 1985). These data also suggest the existence of a feedback regulatory pathway mediated by protein kinase C.

It is not surprising that the antibody-stimulated Ca2+ influx should lead to enhanced Na+/H+ exchange by a Ca2+-dependent mechanism. Several investigators have shown that treatment of splenic and thymic lymphocytes (Gerson et al., 1982; Gerson and Kiefer, 1982; Heskeh et al., 1985) with the T cell mitogen, con A, produces an increase in pH. As con A treatment causes a rise in [Ca2+], that is dependent on the presence of extracellular Ca2+ (Tsien et al., 1982; Heskeh et al., 1985), it is reasonable to suppose that a pathway similar to the one described here is operative in these nontransformed cells. However, it has yet to be proven that con A is exerting its effects by stimulating the antigen receptor.

It has been suggested that the increase in Na+/H+ exchange resulting from serum or growth factor stimulation of quiescent fibroblasts is due to activation of a Ca2+-calmodulin-dependent pathway resulting from a primary increase in [Ca2+]. (Villereal, 1981; Owen and Villereal, 1982; Vincentini et al., 1984). These workers have shown that the calcium ionophore A23187 activates an amiloride-sensitive Na+ influx into treated cells that is blocked by the calmodulin inhibitor, trifluoperazine, but not by the phospholipase C inhibitor, mepacrine. The interpretation of these data is complicated by the relatively nonspecific effects of these drugs on a variety of Ca2+ and lipid-dependent enzymes (Nishizuka, 1984). Sifert et al. (1984) also show that A23187 stimulates amiloride-sensitive Na+/H+ exchange in platelets by measuring H+ efflux from treated cells. Using the spectrophotometric methodology to measure intracellular pH as described here, we have been unable to use Ca2+ ionophores to study their effects on pH, since they work by exchanging H+ for Ca2+. However, these results suggest that there may be at least two complementary pathways for increasing the activity of the Na+/H+ antiport in quiescent cells.

We have shown that phorbol esters can activate Na+/H+ exchange in 70Z/3 pre-B lymphocytes. This activation appears to be a requirement for the induction of differentiation (Rosoff and Cantley, 1983; Rosoff et al., 1984). Phorbol esters have been shown to have similar effects on several other cell types as well (Besterman et al., 1984; Moonaar et al., 1984b). In TPA-treated 70Z/3 cells, this activation does not require a prior elevation of cytoplasmic Ca2+ and actually decreases the [Ca2+]. (Rosoff and Cantley, 1985b). A decrease in [Ca2+] has also been observed in TPA-treated splenic T cells (Tsien et al., 1982) and neutrophils (Lagast et al., 1984; Korchak et al., 1984). These data suggest that the Na+/H+ antiport can be directly activated by protein kinase C via a pathway that does not require elevation of cytoplasmic Ca2+. By substituting for endogenously produced diacylglycerol, TPA lowers the Kc for Ca2+ of protein kinase C to the Ca2+, found in many unstimulated cells (Nishizuka, 1984). Our results with HPB-ALL cells also support this idea. TPA treatment leads to a rapid and transient increase in pH, that is dependent on Na+, inhibitable by DMA, and does not involve an increase in cytoplasmic [Ca2+].

Resting T cells may be induced to proliferate by anti-T3 antibodies, the calcium ionophore A23187, or mitogenic lectins such as con A, but only when a second stimulus such as TPA or monocytes are simultaneously present (Chang et al., 1982; Mastro and Smith, 1983). Since the first signals all produce a rise in [Ca2+], as well as pH, this argues that these changes alone are not sufficient to induce mitogenesis in these cells. The importance of the enhanced Na+/H+ exchange activity in mitogen activation of resting T cells is suggested by studies which have demonstrated the requirement for extracellular Na+ for con A and phytohemagglutinin-induced DNA synthesis (Deutsch et al., 1981; Deutsch et al., 1984) and the close association of mitogen treatment and increases in ouabain-insensitive Na+ uptake (Segal et al., 1979). Protein kinase C apparently performs an integral role in co-mitogenesis, distinct from its synergistic action on Na+/H+ exchange. It is of course possible that the ability of TPA to attenuate Na+/H+ exchange activation is an essential step in the proliferative response to TPA. The role of Na+/H+ exchange activation rather than continuous activation may be necessary for a proper cellular response to the stimulatory signal). However, it seems likely that protein kinase C causes additional cellular responses not involving Na+/H+ exchange.

The ability of TPA to attenuate the activation of Na+/H+ exchange in HPB-ALL cells suggests a possible feedback mechanism mediated by C kinase. TPA-activated protein kinase C has been shown to decrease the tyrosine kinase activity and cellular response of both the epidermal growth factor receptor (McCaffrey et al., 1984; Iwashita and Fox, 1984; Crochet et al., 1984; Whitely et al., 1984) and the insulin receptor (Jacobs et al., 1983; Takeyama et al., 1984). We have also found that TPA inhibits phosphatidylinositol turnover in 70Z/3 pre-B lymphocytes (Rosoff and Cantley, 1985b). The data presented in Fig. 5 show that the ability of the anti-T3-T cell receptor complex antibodies to stimulate Ca2+ influx is not affected by pretreatment with TPA. Thus, the step at which TPA attenuates antibody-activated Na+/H+ exchange is subsequent to the receptor-activated Ca2+ influx. Further work is required to determine the complex regulation of Na+/H+ exchange and its role in T cell activation.

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