TRANSMEMBRANE SIGNALLING BY THE T CELL ANTIGEN RECEPTOR

Perturbation of the T3–Antigen Receptor Complex Generates Inositol Phosphates and Releases Calcium Ions From Intracellular Stores

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Under appropriate conditions, monoclonal antibodies (mAb),[1] either to the T cell antigen receptor or to its associated structures, T3, can mimic the effects of antigen and activate human T cells (1, 2). Previous studies (3, 4) from this laboratory indicate that this activation depends on the ability of the T3–antigen receptor complex to increase free cytoplasmic calcium ions ($[Ca^{2+}]_i$). This conclusion is based on the following observations: In the presence of phorbol myristate acetate (PMA), mAb either to the antigen receptor heterodimer or to T3 activate the human T cell line, Jurkat, to produce the lymphokine interleukin 2 (IL-2) (2–5). These same antibodies elicit prompt, sustained increases in $[Ca^{2+}]_i$ when added to Jurkat cells that are loaded with the Ca^{2+}-sensitive fluor, quin 2 (3, 4). Comparable increases in $[Ca^{2+}]_i$, induced by the Ca^{2+} ionophore, ionomycin, activate Jurkat in the presence of PMA, and also activate mutants of Jurkat which fail to express the T3–antigen receptor complex (4). Stimulation of Jurkat with either ionomycin or antibodies to the T3–antigen receptor complex lead to the appearance of the same new phosphoproteins, suggesting that the ionophore and the antibodies activate the same protein kinases (4). These findings are not restricted to Jurkat. Using antibodies against T3, similar results indicate that increases in $[Ca^{2+}]_i$ play a crucial role in receptor-mediated activation of a second malignant T cell line, Hut 78, as well as of bulk cultures of human peripheral blood T cells (our unpublished observations).

In order to investigate further the process of transmembrane signalling by the T3–antigen receptor complex, we studied the mechanism by which this complex increases $[Ca^{2+}]_i$. Herein, we demonstrate that the initial receptor-mediated increase in $[Ca^{2+}]_i$ is due to the release of Ca^{2+} from intracellular stores. This release of Ca^{2+}, in turn, appears to be mediated by receptor-induced increases in inositol trisphosphate (IP_3), the putative mobilizer of intracellular Ca^{2+} for a variety of hormone receptors (6–10). The process of transmembrane signalling by the T3–antigen receptor complex, therefore, is intimately linked to the

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[1] *Abbreviations used in this paper:* $[Ca^{2+}]_i$, free cytoplasmic calcium ions; DG, diacylglycerol; IL-2, interleukin 2; IP_3, inositol triphosphate; mAb, monoclonal antibodies; PIP_2, phosphatidylinositol biphosphate; PMA, phorbol myristate acetate.

activation of a phosphodiesterase that is capable of hydrolyzing polyphosphoinositides to inositol phosphates (6). These findings tie the T cell antigen receptor to a potent membrane transducing mechanism, because the hydrolysis of polyphosphoinositides generates a number of potential intracellular messengers, including diacylglycerol and arachidonate, as well as IP_3 (reviewed in 6 and 11).

Materials and Methods

Cells and mAb. Jurkat E6-1 was passaged as described (3). C305, an IgM-kappa mAb, recognizes the antigen-receptor heterodimer on Jurkat (5). C373, another IgM-kappa mAb, reacts with the E rosette receptor on human T cells (5). OKT3 was obtained from Ortho Pharmaceutical (Raritan, NJ).

Reagents. Ionomycin was a gift from Squibb Corp. (Princeton, NJ). Purified IP_3 and inositol bisphosphate (IP_2), which were prepared by the method of Grado and Ballou (12) from phosphatidylinositol-4,5-bisphosphate and phosphatidylinositol-4-monophosphate, respectively, were a kind gift of Dr. Steven Watson (12). All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Determination of Free Cytoplasmic Calcium. Jurkat cells were loaded with 5 μM of the acetoxymethyl ester of quin 2 (Amersham Corp., Arlington Heights, IL) and the fluorescence of the cellular suspension (10^7 cells/ml) was monitored with a Perkin-Elmer 650-40 spectrofluorimeter as described (3). $[Ca^{2+}]_i$ was calculated by the method of Tsien et al. (13). Measurements were made in a solution (pH 7.40) containing 125 mM NaCl, 5 mM KCl, 1 mM Na_2HPO_4, 1 g/liter glucose, 25 mM Hepes, 0.1% bovine serum albumin, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$ ("saline"). The cellular quin 2 content in these experiments was 60–80 pmol of quin 2 per 10^6 cells. With extracellular Ca^{2+} depleted, substantial blunting of the C305-induced increase in $[Ca^{2+}]_i$ was observed with higher quin 2 contents (e.g. 0.3–0.4 nmol quin 2 per 10^6 cells), presumably due to the predicted ability of quin 2 to buffer transient increases in $[Ca^{2+}]_i$ (data not shown) (13). In the Ca^{2+}-depletion experiments, EGTA (final concentration, 10 mM) was added to the cellular suspension from a 200 mM stock (pH 7.40) 1–10 min before the addition of C305. The extracellular Ca^{2+} concentration in these experiments was estimated by adding EGTA (to 10 mM) in the same "saline" solution containing 2 μM quin 2 (free acid form; Calbiochem-Behring, San Diego, CA) in the absence of cells.

Measurement of [^{3}H]Inositol Phosphates. [^{3}H]Inositol phosphates were separated and quantified by the methods described previously (9, 14, 15). Incorporation of [^{3}H]inositol into phospholipid was achieved by incubating Jurkat cells (10^7 cells/ml) with 40 μCi/ml of [^{3}H]inositol (37 MBq/ml; Amersham Corp.) for 3 h at 37°C, followed by extensive washing, resuspension at 10^7 cells/ml in "saline", and incubation for an additional 60 min at 37°C. Aliquots (1 ml) of this cellular suspension were then added to 5-ml culture tubes, and the appropriate additions were made. Antibodies were added to a final dilution of 1:400, and ionomycin was added to 1 μM (from stock 200 μM in dimethyl sulfoxide). For 30-s time points, cells were stimulated in microfuge tubes and directly processed. For other time points, incubations were continued at 37°C, then the cells were transferred to microfuge tubes and sedimented in an Eppendorf 5414 centrifuge. After aspiration of the media, 0.750 ml of chloroform-methanol (1:2) was added to the cellular pellet. The phases were separated by the addition of 0.250 ml H_2O and 0.250 ml chloroform, and the upper phase transferred to a borosilicate tube containing 2.3 ml H_2O. This diluted aqueous phase was then applied to columns made from 1.2 ml of a 0.5 g/ml aqueous slurry of Dowex 1-X8 100–200 mesh (Sigma Chemical Co.) in formate form. [^{3}H]Inositol and [^{3}H]glycerophosphorylinositol were eluted with 12 ml of 60 mM sodium formate plus 5 mM disodium tetraborate, and were not routinely counted. The column was then washed with an additional 8 ml of the same buffer. During this wash, the eluted radioactivity fell to background levels. Inositol phosphate (IP_1), IP_2, and IP_3 were then sequentially eluted with 4 ml of 0.2 M ammonium formate plus 0.1 M formic acid, 18 ml of 0.4 M ammonium formate plus 0.1 M formic acid, and 16 ml 1 M ammonium formate plus 0.1 M formic acid, respectively. After the collection of each peak, the column was

washed with five to eight column volumes of the same buffer prior to the elution of the next peak; during these washes, eluted radioactivity returned to background levels. Radioactivity was determined by scintillation counting in Aquasol (New England Nuclear, Boston, MA). This method unequivocally separates IP_1, IP_2, and IP_3 from one another, but does not allow one to distinguish among the various possible isomers of each of these inositol phosphates (14).

Permeabilized Cell Experiments. The ability of purified IP_3 to release Ca^{2+} from intracellular stores in Jurkat was tested by a method adapted from that used by Joseph et al. (8) with hepatocytes. Jurkat cells (2×10^7 cells/ml) were permeabilized with saponin (60 μg/ml) in thermostatically maintained (37°C) quartz cuvettes in a medium (pH 7.20) containing 110 mM KCl, 10 mM NaCl, 1 mM KH_2PO_4, 5 mM $KHCO_3$, 20 mM Hepes, 20 mM creatine phosphate, 10 U/ml creatine kinase, and 0.3 mM $MgCl_2$. The Ca^{2+} concentration in the cuvette was monitored with 20 μM quin 2 (free acid form) in a Perkin-Elmer spectrofluorimeter (excitation at 339 nm, emission at 492 nm). After permeabilization was complete, Mg^{2+}-ATP was added to a final concentration of 3 mM. IP_3 was added from a 100 μM stock. At the end of each experiment, maximal fluorescence was determined by the addition of 1 mM Ca^{2+}, and the minimal fluorescence by the subsequent addition of 10 mM EGTA and sufficient 1 M Tris Base to raise the pH to >8.3. The Ca^{2+} concentration was then calculated by the method of Tsien et al. (13). It should be noted that, as the cells are permeabilized, this technique measures the Ca^{2+} concentration in the entire suspension. Changes in this Ca^{2+} concentration, therefore, cannot be compared directly to the measurements of $[Ca^{2+}]_i$ using intracellularly trapped quin 2.

Results

Antibodies to the T3–antigen receptor complex cause substantial increases in $[Ca^{2+}]_i$ when added to Jurkat cells that are loaded with the Ca^{2+} indicator quin 2 (Fig. 1*B*) (3, 4). Such receptor-mediated increases in $[Ca^{2+}]_i$ can be due to uptake of extracellular Ca^{2+}, release of Ca^{2+} from intracellular stores, or a combination of these processes. To distinguish among these possibilities, we tested the ability of C305, an IgM mAb against the antigen receptor heterodimer on Jurkat, to increase $[Ca^{2+}]_i$ when extracellular Ca^{2+} is depleted. Basal $[Ca^{2+}]_i$ in Jurkat is ~80 nM (3, 4). When extracellular Ca^{2+} is reduced to <60 nM by 10 mM EGTA, C305 still elicits a substantial increase in $[Ca^{2+}]_i$ (Fig. 1*A*). Furthermore, the initial rate of the increase in the fluorescence of quin 2 is identical to that seen in the presence of 1 mM extracellular Ca^{2+}. This initial receptor-mediated increase in $[Ca^{2+}]_i$, occurring in the absence of a Ca^{2+} gradient across the plasma membrane, must be due to the release of Ca^{2+} from intracellular stores.

The T cell antigen receptor, therefore, generates some signal that, in turn, releases Ca^{2+} from intracellular sites. As IP_3 has been implicated as such a messenger for a number of hormone receptors, we measured inositol phosphates in Jurkat by loading these cells with [^{3}H]inositol and separating the different [^{3}H]inositol phosphates by anion-exchange chromatography (6–10, 14, 15). As shown in Fig. 2, the addition of C305 to Jurkat results in a nearly twofold increase in IP_3, as well as a 40% increase in IP_2, within 30 s (it is not technically possible to measure earlier time periods). The levels of IP_3 and IP_2 continue to rise during the first 10 min after the addition of C305 (Fig. 3) and are still elevated at 30 min (data not shown). Increased levels of IP_1 are not detected until at least 60 s after the addition of antibody (Fig. 2), but rise thereafter (Figs. 2 and 3).

Perturbation of the antigen receptor leads to an early and sustained increase

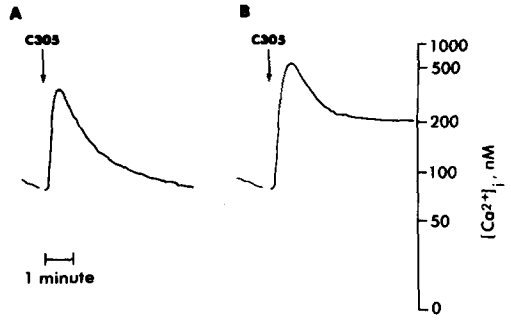

FIGURE 1. C305, an antibody against the antigen receptor heterodimer, increases $[Ca^{2+}]_i$ when extracellular Ca^{2+} has been depleted. Jurkat cells were loaded with the acetoxymethyl ester of quin 2, and the fluorescence of the cellular suspension monitored over time, as described in Materials and Methods. $[Ca^{2+}]_i$, calculated by the method of Tsien et al. (13), is displayed on the vertical axis. *A*, EGTA was added to a final concentration of 10 mM one minute prior to the addition of C305. This immediately reduced extracellular Ca^{2+} to <60 nM. Under these conditions $[Ca^{2+}]_i$ in unstimulated cells fell, over a period of 5 min, to ~40 nM (data not shown). Comparable increases in quin 2 fluorescence were seen after a 10 min incubation in 10 mM EGTA (data not shown). This tracing is representative of six separate experiments. *B*, C305 was added in the presence of 1 mM extracellular Ca^{2+}.

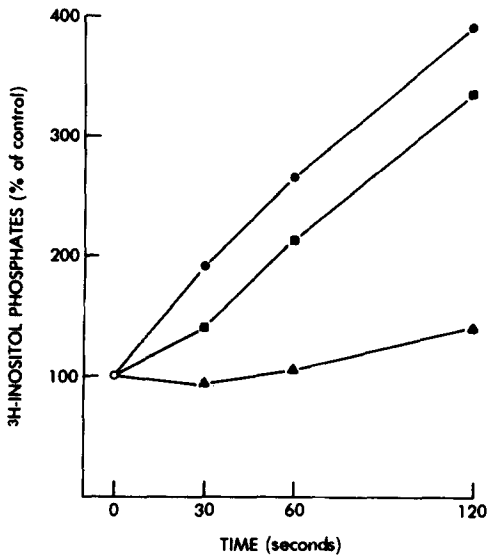

FIGURE 2. Perturbation of the antigen receptor heterodimer on Jurkat increases [^{3}H]inositol phosphates. The levels of [^{3}H]IP_3 (●), [^{3}H]IP_2 (■), and [^{3}H]IP_1 (▲) after the addition of C305 are expressed as a percentage of the values in unstimulated cells at time 0. After Jurkat cells (4×10^7 cells in 4 ml) were loaded with [^{3}H]inositol, the [^{3}H]inositol phosphates were extracted from 10^7 unstimulated cells, and C305 was added to the remainder. One milliliter aliquots were removed subsequently for each time point. The indicated time points are the interval from the addition of antibody to the lysis of the cells in chloroform-methanol. The inositol phosphates were separated by anion-exchange chromatography as outlined in Materials and Methods. Levels of [^{3}H]inositol phosphates in the unstimulated cells were: IP_3, 249 cpm; IP_2, 799 cpm; and IP_1, 466 cpm. These data are representative of three separate experiments.

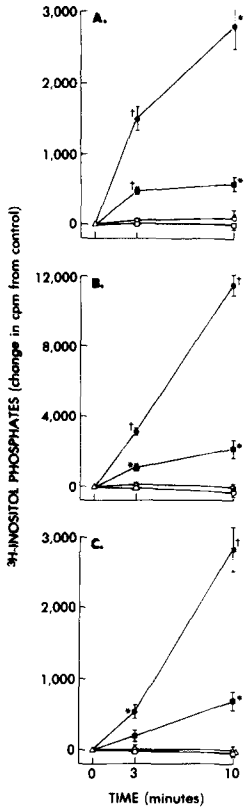

FIGURE 3. Antibodies against the T3–antigen receptor complex on Jurkat cells generate increased levels of: (*A*) [^{3}H]inositol trisphosphate, (*B*) [^{3}H]inositol bisphosphate, and (*C*) [^{3}H]inositol phosphate. Jurkat cells were loaded with [^{3}H]inositol as described in Materials and Methods, and then divided into aliquots, which received either C305 (●), OKT3 (■), C373 (○), or 1 μM ionomycin (□), or which were left unstimulated (△). Results are expressed as the change in cpm from unstimulated cells at time 0, and are expressed as the mean ± SEM for three separate experiments. Mean values for unstimulated values at time 0 were: IP_3, 193 ± 70 cpm; IP_2, 1,456 ± 598 cpm; and IP_1, 547 ± 82 cpm. Values that are significantly elevated over unstimulated cells, C373-treated cells, and ionomycin-treated cells are designated by * and † for p values <0.05 and <0.02, respectively (Student's paired t test). No values for C373-treated, or ionomycin-treated cells significantly differed from those of unstimulated cells. The mean value for unstimulated cells incubated throughout the course of the experiment is shown at the 10 min time point, and superimposes on the C373 mean values. [^{3}H]Inositol phosphates were separated and quantified as described in Materials and Methods.

in IP_3. To insure that this increase in IP_3 is not a consequence of the receptor-mediated increase in $[Ca^{2+}]_i$, we studied the levels of inositol phosphates in Jurkat cells that are stimulated with 1 μM ionomycin. This concentration of ionophore does not change the levels of IP_3, IP_2, or IP_1 (Fig. 3), even though it increases $[Ca^{2+}]_i$ to levels comparable to those seen after the addition of C305 (4). The specificity of the C305-induced increases in inositol phosphates was confirmed by studying two additional mAb. OKT3, an IgG mAb, recognizes a T3 determinant and increases $[Ca^{2+}]_i$ (3). As expected, this antibody generates significant increases in all three inositol phosphates (Fig. 3). In contrast, C373, an IgM mAb against the T cell E rosette receptor, binds to Jurkat, but does not change

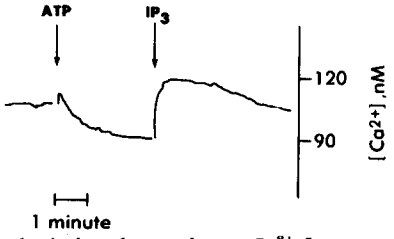

FIGURE 4. Purified inositol trisphosphate releases Ca^{2+} from saponin-permeabilized Jurkat cells. Jurkat cells (2×10^7 cells/ml) were permeabilized with saponin as described in Materials and Methods. The free Ca^{2+} concentration in the suspension, ($[Ca^{2+}]$), was monitored with quin 2 (free acid form). ATP was added to a final concentration of 3 mM. After the addition of ATP, $[Ca^{2+}]$ fell to 90 nM (which approximates the level of $[Ca^{2+}]_i$ in intact, unstimulated Jurkat cells). IP_3 was added from a 100 μM stock to a final concentration of 1 μM. This preparation of IP_3 did not contain detectable contaminating amounts of Ca^{2+}, as evidenced by the failure of IP_3 to change quin 2 fluorescence in the absence of cells (data not shown). These data are representative of 10 separate experiments.

$[Ca^{2+}]_i$ (4, 5). The addition of C373 to Jurkat does not affect the levels of any of the inositol phosphates (Fig. 3).

Increases in IP_3 and in $[Ca^{2+}]_i$ are both early events in the antigen receptor–induced activation of Jurkat. The ability of IP_3 to mobilize intracellular Ca^{2+} in other cells, however, suggests that these events are not independent and that the increase in IP_3 might be responsible for the increase in $[Ca^{2+}]_i$. Accordingly, we tested the ability of purified IP_3 to release Ca^{2+} from intracellular stores in Jurkat. In these experiments Jurkat cells are permeabilized with saponin, and the concentration of Ca^{2+} in the suspension is measured with quin 2. After permeabilization, the addition of ATP leads to uptake of Ca^{2+}, as evidenced by the fall in quin 2 fluorescence (Fig. 4). The subsequent addition of IP_3 results in a prompt increase in fluorescence, demonstrating that IP_3 releases Ca^{2+} from permeabilized Jurkat cells. Release of Ca^{2+} is detected with concentrations of IP_3 as low as 0.1 μM, and is maximal at 1 μM. This ability of IP_3 to release Ca^{2+} is enhanced considerably by the prior addition of ATP, suggesting that IP_3 mobilizes Ca^{2+} from an ATP-dependent pool of Ca^{2+}. In contrast, there was no detectable change in the Ca^{2+} concentration in any of the three experiments in which purified 1 μM IP_2 was added to permeabilized Jurkat cells (data not shown).

Discussion

The studies presented here suggest that the process of transmembrane signalling, by which the T3–antigen receptor complex activates Jurkat, involves the generation of IP_3. Elevated concentrations of IP_3 release Ca^{2+} from intracellular stores and thereby increase $[Ca^{2+}]_i$. Previous studies have demonstrated that this increase in $[Ca^{2+}]_i$ provides an essential signal for the receptor-induced appearance of IL-2 transcripts and the subsequent production of IL-2 biological activity by Jurkat cells (2–4).

Several lines of evidence support these conclusions. Antibodies to the T3–antigen receptor complex on the T cell line, Jurkat, increase $[Ca^{2+}]_i$ (Fig. 1) (3, 4). These same antibodies generate significant elevations in the levels of IP_3, IP_2,

and IP_1 (Figs. 2 and 3). These increases in inositol phosphates appear to be a specific effect of perturbation of the receptor complex and are not merely the result of an interaction of mAb with the cellular surface (Fig. 3). A crucial question, then, is whether the link between the receptor-mediated generation of inositol phosphates and the receptor-mediated increase in $[Ca^{2+}]_i$ is merely a temporal association, or whether one effect is a consequence of the other. The elevated levels of inositol phosphates are not secondary to the receptor-induced changes in $[Ca^{2+}]_i$. The Ca^{2+} ionophore, ionomycin, in a concentration of 1 μM, increases $[Ca^{2+}]_i$ to levels that are comparable to those induced by antibodies to the T3–antigen receptor complex, and can substitute for these antibodies in activating Jurkat (4). This same concentration of ionophore does not affect the levels of any of the inositol phosphates (Fig. 3). The data in Figure 4 demonstrate that one of these inositol phosphates, IP_3, could function as a mobilizer of intracellular stores of Ca^{2+} in Jurkat. Purified IP_3, in concentrations between 0.1 and 1 μM, is able to release Ca^{2+} from permeabilized Jurkat cells, indicating that this compound might mobilize intracellular Ca^{2+} after perturbation of the T3–antigen receptor complex. That the T3–antigen receptor complex does indeed mobilize Ca^{2+} from intracellular stores is demonstrated by the ability of anti–antigen receptor antibody to increase $[Ca^{2+}]_i$ when extracellular Ca^{2+} is depleted (Fig. 1).

Further support for the role of IP_3 in mediating this increase in $[Ca^{2+}]_i$ derives from similar findings in other receptor systems. It is clear that a number of hormone receptors which mobilize intracellular Ca^{2+} also generate substantial elevations in inositol phosphates (reviewed in 6). For example, the hepatocyte receptors for vasopressin and α_1-adrenergic stimuli have been shown to stimulate hydrolysis of polyphosphoinositides, generate IP_3, and release Ca^{2+} from intracellular stores (8–10, 16, 17). Furthermore, purified IP_3 releases Ca^{2+} from permeabilized hepatocytes (8, 10). The concentrations of IP_3 that release Ca^{2+} from permeabilized hepatocytes are in the same range (0.1–1 μM) as the concentrations that are required to mobilize Ca^{2+} from permeabilized Jurkat cells (8, 10). These concentrations are substantially less than the intracellular levels of IP_3 (10–50 μM) that are estimated to occur in stimulated hepatocytes (10). Recent studies (7, 8, 10, 18) demonstrate that IP_3 mobilizes Ca^{2+} from an ATP-dependent, nonmitochondrial pool that is probably within the endoplasmic reticulum.

The receptor-mediated generation of IP_3 is almost certainly due to the hydrolysis of the phospholipid, phosphatidylinositol bisphosphate (PIP_2), as this reaction is the only identified source of IP_3 in mammalian cells (Fig. 5) (6). The increased levels of IP_2 could be due either to dephosphorylation of IP_3 or to receptor-mediated hydrolysis of phosphatidylinositol phosphate (PIP). The lag in the appearance of IP_1 indicates that hydrolysis of phosphatidylinositol (PI) is not an early, receptor-mediated event, and suggests that accumulation of IP_1 is due to the breakdown of IP_3 and IP_2. The initial events in transmembrane signalling by the T3–antigen receptor complex, therefore, appear to follow the course that is shown schematically in Fig. 5. Appropriate stimulation of the receptor complex activates a phosphodiesterase that generates IP_3 from PIP_2.

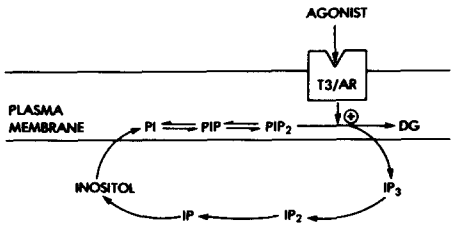

FIGURE 5. Model of the early events in antigen receptor–mediated activation. Appropriate stimulation of the T3–antigen receptor complex activates a phosphodiesterase that hydrolyzes the phospholipid, PIP_2. This reaction generates IP_3 and DG. IP_3, a water-soluble compound, is released into the cytoplasm, where it mobilizes Ca^{2+} from intracellular stores and thereby increases $[Ca^{2+}]_i$. IP_3 is subsequently broken down to IP_2 and then IP_1, leading to the accumulation of all three inositol phosphates after perturbation of the receptor complex. Receptor-mediated hydrolysis of PIP may be an additional source of IP_2. The lag in the appearance of IP_1 argues against direct breakdown of PI to IP_1. Inositol can be recycled into PI, and then converted to polyphosphoinositides by the action of specific kinases (6). Although shown as a single unit in this scheme, the nature of the physical association between T3 and the antigen receptor heterodimer during activation is not known. Also, at least one chain of the antigen receptor heterodimer, as well as all three T3 proteins, appear to span the plasma membrane (24, 25).

IP_3, a water-soluble compound, is released into the cytosol and mobilizes Ca^{2+} from intracellular stores, leading to an increase in $[Ca^{2+}]_i$.

While release of intracellular Ca^{2+} accounts for the early effects of the antigen receptor on $[Ca^{2+}]_i$, the sustained increase in $[Ca^{2+}]_i$ requires the presence of extracellular Ca^{2+} (Fig. 1). There are several possible explanations for this observation. The prolonged elevation in $[Ca^{2+}]_i$ could be maintained by slowing the mechanism of Ca^{2+} extrusion without a change in the membrane permeability to Ca^{2+} (under physiological conditions, the gradient of Ca^{2+} across plasma membranes is 10^4, and is maintained by active extrusion of Ca^{2+}) (19). Such a mechanism appears to maintain the elevations in $[Ca^{2+}]_i$ in hepatocytes after stimulation with either vasopressin or α_1-adrenergic stimuli (19). Alternatively, the sustained increase in $[Ca^{2+}]_i$ could be due to increased permeability of the plasma membrane to Ca^{2+}. In Jurkat, however, there does not appear to be any uptake of extracellular Ca^{2+} through a classical voltage-dependent Ca^{2+} channel after perturbation of the T3–antigen receptor complex. The antigen receptor–mediated changes in $[Ca^{2+}]_i$ are not affected by the Ca^{2+} channel blockers, nifedipine and verapamil, and can occur when extracellular Na^+ is replaced with nonexchangeable choline (i.e. under conditions in which the cell cannot depolarize with a Na^+ influx; our unpublished observations). Furthermore, depolarization of Jurkat by the addition of K^+ does not change $[Ca^{2+}]_i$, suggesting that Jurkat does not bear voltage-dependent Ca^{2+} channels (our unpublished observations). The possibility that IP_3 can translocate Ca^{2+} across the plasma membrane, to our knowledge, has not been tested directly. It is not clear whether the sustained increase in $[Ca^{2+}]_i$ is important for activation, or whether the initial peak increase in $[Ca^{2+}]_i$ suffices. This issue cannot be approached by depleting extracellular Ca^{2+}, as such treatment adversely affects cellular viability within several hours (our unpublished observation).

Perturbation of the T3–antigen receptor complex by C305 generates higher levels of inositol phosphates than does perturbation of the complex by OKT3

(Fig. 3). It is not known whether this difference is a function of the different specificities of these two antibodies, or whether this reflects perturbation of the complex by an IgM, as opposed to an IgG, antibody. The difference in the levels of inositol phosphates does not appear to be functionally important, as the two antibodies induce comparable changes in $[Ca^{2+}]_i$, and comparable amounts of IL-2 biological activity (3, 4). This result is to be expected if stimulated Jurkat cells attain concentrations of IP_3 that are similar to those in receptor-activated hepatocytes, for these levels are one to two orders of magnitude greater than the concentrations apparently required to release Ca^{2+} (10).

We previously demonstrated that two signals are required to activate Jurkat to produce IL-2 (2). Perturbation of the T3–antigen receptor complex by mAb transmits one signal by increasing $[Ca^{2+}]_i$ (3, 4). The second signal can be provided by PMA (2–4). PMA is a potent activator of protein kinase C, an enzyme that plays a prominent role in signal transduction by a number of receptors and that appears to act synergistically with increases in $[Ca^{2+}]_i$ (20–23). The putative physiological activator of kinase C is diacylglycerol (DG), a necessary product in the generation of inositol phosphates from phosphoinositides (Fig. 5) (6, 11, 23). In Jurkat, perturbation of the receptor complex by mAb increases $[Ca^{2+}]_i$, but apparently is not capable of sufficiently activating kinase C as well. Hence, PMA is required. It is possible, however, that, under other conditions, the T cell antigen receptor complex does generate all the signals required for activation. For example, the signalling capabilities of the receptor might be affected by better crosslinking, or by other maneuvers that more closely mimic interaction with antigen-presenting cells. Of considerable interest in this regard are the observations of Meuer et al (1). They are unable to activate alloreactive human T cell clones to produce IL-2 with either anti-T3 antibodies or clonotypic antibodies alone, but can activate them by using these same antibodies attached to sepharose beads. Under these conditions, then, the T cell antigen receptor complex does generate the signals necessary for activation. Previous studies (3, 4) indicate that one of these signals is an increase in $[Ca^{2+}]_i$. The data presented here help to clarify the mechanisms involved in mediating this increase in $[Ca^{2+}]_i$, and also provide an approach to a more complete understanding of the process of transmembrane signal transduction by the T3–antigen receptor complex.

Summary

Antibodies against the T3–antigen receptor complex can activate the human T cell line, Jurkat, to produce interleukin 2 (2–5). This activation is initiated by a receptor-mediated increase in the concentration of free cytoplasmic calcium ions $[Ca^{2+}]_i$ (3, 4). In this communication, we investigate the mechanism by which the receptor complex increases $[Ca^{2+}]_i$ in Jurkat cells. The initial receptor-mediated change in $[Ca^{2+}]_i$ can occur when extracellular Ca^{2+} is depleted by EGTA. Perturbation of the T cell antigen receptor, therefore, generates a signal which mobilizes Ca^{2+} from intracellular stores. As inositol trisphosphate appears to function as such a signal for certain hormone receptors, we measured the levels of inositol trisphosphate and of the other inositol phosphate compounds in Jurkat. Antibodies to either the antigen receptor heterodimer or T3 determinants result in marked elevations of all three inositol phosphates. These

changes in inositol phosphates are not secondary to the receptor-mediated increases in $[Ca^{2+}]_i$ as demonstrated by the inability of the Ca^{2+} ionophore, ionomycin, to affect the levels of any of these compounds. In concentrations between 0.1 and 1 μM, purified inositol trisphosphate releases Ca^{2+} from permeabilized Jurkat cells. Taken together, these data indicate that, during activation, perturbation of the T3–antigen receptor complex generates inositol trisphosphate. This compound functions as an intracellular signal to release Ca^{2+} from intracellular stores, leading to increases in $[Ca^{2+}]_i$.

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