Phosphoinositide 3-Kinase Regulation of T Cell Receptor-mediated Interleukin-2 Gene Expression in Normal T Cells*

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Phosphoinositide (PI) 3-kinase has been implicated in T cell receptor (TCR) signaling, either as a positive or a negative regulatory molecule. Here, we show that for normal mouse lymph node T cells, PI 3-kinase activity is required for interleukin-2 (IL-2) production following TCR-mediated activation. Furthermore, in normal T cells, inhibition of PI 3-kinase prevented activation of enzymes in the extracellular signal-regulated protein kinase (ERK) signaling pathway (MEK-1 and ERK-2). Overexpression of a dominant-negative mutant of PI 3-kinase and pharmacological inhibitors of PI 3-kinase prevented transcriptional activation of AP-1 and NF-AT, transcription factors regulated by ERK-2 and pivotal for IL-2 gene expression. Although a constitutively active form of Akt kinase, a downstream mediator of PI 3-kinase function, enhanced TCR-induced IL-2 gene transcription, it could not bypass the requirement for PI 3-kinase activity. Therefore, PI 3-kinase is likely to be involved in signaling for IL-2 production in at least two steps in the TCR-initiated signaling pathway.

The T cell receptor for antigen (TCR) is a multisubunit complex whose ligation initiates a series of signaling events that lead to gene activation, lymphokine production, and cell division. The earliest biochemical event following TCR activation is activation of intracellular protein-tyrosine kinases (1, 2). The tyrosine phosphorylation events are regulated by two protein-tyrosine kinases. The Src family (Lck and Fyn) and the Syk/ZAP-70 family. Lck and Fyn phosphorylate tyrosine residues present within a signaling motif in the cytoplasmic regions of the CD3 γ, δ, and ε and TCR β chains, called the immunoreceptor tyrosine-based activation motif. Phosphorylation of immunoreceptor tyrosine-based activation motifs is followed by the recruitment, tyrosine phosphorylation, and activation of ZAP-70 and Syk. This TCR-associated protein-tyrosine kinase activation is coupled to the phosphorylation and activation of downstream signaling molecules such as phospholipase Cγ1. Hydrolysis of inositol phospholipids by phospholipase Cγ1 results in the generation of inositol polyphosphates and diacylglycerols, second messengers that lead to activation of protein kinase C family members and to an increased level of intracellular Ca2+. Increased intracellular Ca2+ in turn activates the phosphatase calcineurin, resulting in the nuclear translocation of the transcription factor NF-AT. Another signaling pathway emanating from the TCR involves the small GTP-binding protein Ras and a kinase cascade that it activates (the Ras/mitogen-activated protein kinase pathway). This pathway results in the activation of the transcription factors Elk1 and subsequently AP-1. Interleukin-2 (IL-2) gene expression, a common measure of productive T cell activation, cannot be induced by a single signaling pathway: it requires the coordinate action of these and other pathways that integrate at the level of multiple transcription factors, including NF-AT, AP-1, NF-κB, and Oct-1 (3).

Another enzyme that has recently been implicated in TCR signaling is phosphoinositide (PI) 3-kinase (4, 5). Class IA PI 3-kinase is a heterodimer composed of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110). The regulatory subunit p85 contains an N-terminal SH3 domain, two proline-rich sequences, and two SH2 domains. The SH2 domains bind to phosphorylated tyrosines on receptor and non-receptor tyrosine kinases and kinase substrates and thus recruit the p85/p110 dimer to activated receptors. The region that separates the two SH2 domains (inter-SH2 domain) mediates the interaction of p85 with the catalytic subunit p110 and is required for PI 3-kinase enzymatic activity. Thus, in activated cells, p110 is targeted to the membrane where its substrates, phosphatidylinositol-3-phosphate (PtdIns(3)P), PtdIns(4,5)P2 at the D3 position of the inositol ring, yielding PtdIns(3,4,5)P3, respectively. In eukaryotic cells, PtdIns(3)P is constitutively present, and its levels are largely altered upon cellular stimulation. In contrast, PtdIns(3,4)P2 and PtdIns(3,4,5)P3 are absent from resting cells. A wide variety of stimuli induce a rapid appearance of PtdIns(3,4,5)P3 in intact cells, followed shortly thereafter by PtdIns(3,4)P2 (6). Thus, the preferred substrate in vivo is likely to be PtdIns(4,5)P2. The rapid appearance of the PI 3-kinase lipid products and their resistance to cleavage by any known phospholipase suggest that they themselves may act as second messengers (7, 8).

Several protein kinases have been placed downstream of PI 3-kinase in receptor-stimulated signaling. Protein kinases Ca2+/CaM, Cε, Cε, Ce, and C7 are calcium-independent members of the protein kinase family and are activated in vitro by PtdIns(3,4)P2 and PtdIns(3,4,5)P3 (9, 10). In intact cells, platelet-derived growth factor activates protein kinases Ce and Cα by a mechanism that requires PI 3-kinase (11, 12). Activation of the serine/threonine kinase p70s6k, which plays an important role in the progression of cells from G1 to S phase of the cell cycle, also involves PI 3-kinase (13). At this time, the most convincing evidence for a downstream mediator of PI 3-kinase signaling is the serine/threonine protein kinase Akt (14). PtdIns(3,4)P2
binds and activates Akt in vitro and in vivo. This requires that Akt have a functional pleckstrin homology domain, leading to the hypothesis that binding of the lipid to the pleckstrin homology domain targets Akt to the membrane where activating phosphorylation events, which are also PI 3-kinase-dependent, take place. Recently, phosphoinositide-dependent kinase-1, a protein kinase that phosphorylates Akt on one of the two sites required for Akt activity, has been purified and cloned (15–17). Phosphoinositide-dependent kinase-1 has a pleckstrin homology domain and is activated by PtdIns(3,4,5)P3. One substrate of Akt in vivo is glycogen-synthase kinase-3 (18). Glycogen-synthase kinase-3 phosphorylates NF-AT, resulting in nuclear exit of NF-AT and thus opposing the effect of calcineurin (19). Phosphorylation of glycogen-synthase kinase-3 by Akt inactivates glycogen-synthase kinase-3 (18). Glycogen-synthase kinase-3 phosphorylates NF-AT, resulting in nuclear exit of NF-AT and thus opposing the effect of calcineurin (19). Phosphorylation of glycogen-synthase kinase-3 by Akt inactivates glycogen-synthase kinase-3, allowing nuclear localization of NF-AT and transcription of NF-AT-dependent genes such as IL-2. The Bcl-2 family member Bad is another in vivo substrate of Akt; phosphorylation of Bad blocks its death-promoting activity and results in cell survival (20, 21).

Recent studies have implicated PI 3-kinase in regulating IL-2 production. In T cells, PI 3-kinase associates with the TCR upon activation due to either a direct interaction between p85 and the phosphorylated immunoreceptor tyrosine-based activation motif of the (22, 23) or ε (24) chain or an association between p85 and Src family protein-tyrosine kinases (25). Once bound, PI 3-kinase becomes activated, resulting in the accumulation of PI 3-kinase products (26). One study with the Jurkat T cell line has suggested that inhibition of PI 3-kinase actually induces IL-2 production in the absence of any other stimulus (27), whereas another study found that blocking PI 3-kinase inhibits IL-2 production (28). To address the physiologic role of PI 3-kinase in coupling TCR signaling to transcription of the IL-2 gene, we have used a combination of pharmacological and molecular approaches to characterize its role in primary lymph node T cells. We find that activation of PI 3-kinase is in fact essential for ERK activation and the initiation of IL-2 gene transcription and that this requirement cannot be bypassed by independent activation of Akt.

MATERIALS AND METHODS

Mice and Reagents—C57BL/6 × B10.D2F1, mice were intercrossed for 17–19 generations. Animals of either sex were killed at 6–10 weeks after treatment with soluble 2C11 (10 μg/ml) for the indicated time periods, cells were lysed in 30 μl of 20 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 200 μM ATP, 5 μCi of [γ-32P]ATP in 30 μl of 20 mM Tris-HCl, pH 6.8, and 10 mM MnCl2 (ZAP-70 kinase buffer) using 1 μg of Cfb3 as a substrate. After incubating the beads at 37 °C for 10 min, the reaction was stopped by the addition of SDS-PAGE sample buffer.

ZAP-70 Kinase Assay—After treatment with soluble 2C11 (10 μg/ml) for the indicated time, cells were lysed in buffer containing 150 mM NaCl, 25 mM Tris, pH 7.5, 1% Triton X-100, 5 mM EDTA, 1 mM Na3VO4, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10 μM phenylmethylsulfonyl fluoride. Cell lysates were incubated on ice for 30 min and then cleared by centrifugation at 14,000 × g for 10 min. Lysates were then incubated with protein A-Sepharose beads that had been preincubated with polyclonal anti-ZAP-70 antibody. After 2 h at 4 °C, the beads were washed twice with lysis buffer and once with kinase buffer. The beads were then subjected to a ZAP-70 kinase assay with 10 μCi of [γ-32P]ATP in 30 μl of 20 mM Tris-HCl, pH 6.8, and 10 mM MnCl2 (ZAP-70 kinase buffer) using 1 μg of Cfb3 as a substrate. After incubating the beads at 37 °C for 10 min, the reaction was stopped by the addition of SDS-PAGE sample buffer.

Mitogen-activated Protein Kinase Assay—After treatment with soluble 2C11 (10 μg/ml) for the indicated time periods, cells were lysed in buffer containing 150 mM NaCl, 25 mM HEPES, pH 7.4, 1 mM Na3VO4, 1% Triton X-100, 0.5 mM dithiothreitol, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 10 μM phenylmethylsulfonyl fluoride. Cell lysates were incubated on ice for 30 min and then cleared by centrifugation at 14,000 × g for 10 min. Lysates were then incubated with protein A-Sepharose beads that had been preincubated with polyclonal anti-ERK-2 antibody. After 2 h at 4 °C, the beads were washed twice with lysis buffer and once with kinase buffer. The kinase reaction was carried out in 30 μl of 20 mM HEPES, pH 7.4, 10 mM MgCl2, 20 μM ATP, and 2 mM EDTA (MAPK kinase buffer) containing 2 μCi of [γ-32P]ATP

In Vitro Transcription and Translation—In vitro translated proteins were prepared using the TNT coupled reticulocyte lysate system (Promega) using 30 μCi of [35S]methionine (1000 Ci/mmol/sample. Typically, 0.5 μg of circular plasmid DNA was used. After 1.5 h at 30 °C, the in vitro translated proteins were either immediately analyzed by SDS-PAGE and autoradiography or first immunoprecipitated and then analyzed by SDS-PAGE and autoradiography to detect protein-protein interactions.
PI 3-Kinase Is Required for IL-2 Production

PI 3-Kinase Antagonists Inhibit IL-2 Production Induced by TCR Ligation—The effects of wortmannin, a fungal metabolite that irreversibly blocks PI 3-kinase catalytic activity (31, 32), on activation-induced IL-2 production were determined. Freshly isolated murine lymph node cells were stimulated with anti-CD3 antibody in the presence of increasing concentrations of wortmannin, and IL-2 was measured (Fig. 1A). Wortmannin inhibited TCR-induced IL-2 production in a dose-dependent fashion, with half-maximal inhibition at a concentration of ~20 nM and complete inhibition at 100 nM, concentrations of wortmannin that inhibit PI 3-kinase activity (33, 34). Since wortmannin can inhibit at least two other enzymes, PI 4-kinase (35, 36) and phospholipase A2 (37), at nanomolar concentrations, it is possible that the effect of this reagent on T cell activation was independent of its ability to inhibit PI 3-kinase. Therefore, similar experiments were performed with the structurally unrelated PI 3-kinase inhibitor LY294002 (38) (Fig. 1B). As with wortmannin, LY294002 inhibited IL-2 production at concentrations that completely and specifically abolish PI 3-kinase activity (38). Neither wortmannin nor LY294002 was toxic at the concentrations used, as determined by staining with trypan blue (data not shown). These results indicate that PI 3-kinase activity is necessary for IL-2 production in lymph node T cells following activation via the TCR.

A Deletion Mutant of p85 Does Not Associate with p110 and Blocks the Transcriptional Activity of the IL-2 Promoter—To further explore the relationship between PI 3-kinase and activation-induced IL-2 production, a dominant-negative form of p85 was used to inhibit the function of endogenous PI 3-kinase (39, 40). We constructed a deletion mutant of p85 (Δp85) lacking 102 amino acids of the inter-SH2 domain that confers binding to p110 (41, 42). To verify the absence of binding of Δp85 to p110, Δp85 and p110 were transfected and cotranslated in a reticulocyte translation system in the presence of [35S]methionine. Δp85 was immunoprecipitated from the lysate, and the immunoprecipitate was analyzed for the presence of p110 (Fig. 2A). When wild-type p85 and p110 were cotranslated, anti-p85 antibodies brought down both p85 and p110. In contrast, immunoprecipitation of Δp85 from lysates in which Δp85 and p110 were cotranslated resulted in the appearance of only Δp85. Therefore, the deletion of the inter-SH2 region of p85 did in fact disrupt the interaction between these two molecules.

To determine what effect repression of enzymatic function by dominant-negative Δp85 would have on activation-dependent signaling, T cell blasts were cotransfected with a reporter plasmid containing the minimal IL-2 promoter/enhancer upstream of the luciferase gene and either an empty vector or a vector encoding Δp85. The transfected cells were stimulated with anti-CD3 antibody, and induction of luciferase activity was assessed (Fig. 2B). As expected, the IL-2-luciferase reporter was induced by 2C11. When Δp85 was coexpressed, however, the anti-CD3 antibody-induced IL-2 activity was almost completely inhibited (87% inhibition). Importantly, when the Δp85-transfected T cell blasts were stimulated with phorbol 12-myristate 13-acetate and ionomycin, reagents that bypass the TCR to induce new gene transcription, IL-2-luciferase induction was not inhibited by Δp85. In addition, treatment of T cell blasts that had been transfected with the IL-2-luciferase reporter construct with wortmannin before stimulation with anti-CD3 antibody also inhibited IL-2 promoter activity (81% inhibition) (Fig. 2C). As a control, cells were cotransfected with a β-galactosidase expression plasmid, and β-galactosidase activity was measured in the same cell extracts used to analyze luciferase activity. In all samples analyzed, β-galactosidase activity never varied >2-fold. These results indicate that PI 3-kinase is a necessary mediator in TCR signaling for IL-2 gene transcription.

The IL-2 promoter contains binding sites for members of the NF-AT and AP-1 families of transcription factors (3). NF-AT proteins reside in the cytosol in resting T cells and translocate to the nucleus in TCR-activated T cells. Once in the nucleus, NF-AT and AP-1 form a functional transcription factor complex (43). Besides binding to the NF-AT/AP-1 site, AP-1 also binds to...
the presence of \(^{35}S\)methionine. The reaction mixtures were subjected in vitro to associate with p110. p110 was cotranslated with the transcriptional activity of the IL-2 promoter. p85 was cotransfected with 10 \(\mu\)g of pCI-\(\Delta p85\) or empty pCI vector and cultured for 3 h before transfer onto wells coated with 2C11. After 14 h, luciferase activity was assessed. The mean relative light units of the extracts obtained from cells cultured in medium were 32,583 (empty vector), 36,129 (\(\Delta p85\)), 47,076 (empty vector), and 80,606 (pCI-\(\Delta p85\)). B and D, lymph node T cell blasts were transfected with 10 \(\mu\)g of NF-AT-luciferase reporter construct (B) or 10 \(\mu\)g of AP-1-luciferase reporter construct (D), treated with wortmannin (Wort; 100 nM) for 30 min, and then cultured in wells coated with 2C11 for 15 h before being harvested. The mean relative light units of the extracts obtained from unstimulated cells were 49,592 (vehicle control), 55,847 (empty vector), 36,129 (vehicle control), and 43,539 (wortmannin). The results shown are representative of three independent experiments. The error bars represent the S.E. from quadruplicate samples.

CD3 antibody-induced NF-AT and AP-1 activation by 83 and 77%, respectively (Fig. 3, B and D).

**Wortmannin Does Not Affect Proximal TCR Signaling**—The earliest biochemical response to TCR ligation is the sequential activation of protein-tyrosine kinases: first Src family kinases and then the Syk/ZAP-70 family. Therefore, to determine if inhibition of PI 3-kinase interfered at a very proximal level of signaling, lymph node cells were treated with 100 nM wortmannin prior to stimulation with anti-CD3 antibody. After stimulation, ZAP-70 was immunoprecipitated, and its kinase activity was assessed by measuring the phosphorylation of a 43-kDa substrate (cfb3). Wortmannin, at a concentration that inhibited CD3 antibody-induced NF-AT and AP-1 activation by 83 and 77%, respectively (Fig. 3, B and D). Wortmannin (Wort; 100 nM) for 30 min, and then cultured in wells coated with 2C11 for 15 h before being harvested. The mean relative light units of the extracts obtained from unstimulated cells were 49,592 (vehicle control), 55,847 (empty vector), 36,129 (vehicle control), and 43,539 (wortmannin). The results shown are representative of three independent experiments. The error bars represent the S.E. from quadruplicate samples.

**PI 3-Kinase Is Required for IL-2 Production**

**FIG. 2.** \(\Delta p85\), a deletion mutant of p85, and wortmannin block the transcriptional activity of the IL-2 promoter. A, \(\Delta p85\) does not associate with p110. p110 was cotranslated in vitro with p85 or \(\Delta p85\) in the presence of \(^{35}S\)methionine. The reaction mixtures were subjected to immunoprecipitation with anti-p85 antibodies. Bound p110 was detected by SDS-PAGE and autoradiography. B, lymph node T cell blasts were cotransfected with 10 \(\mu\)g of IL-2 promoter-luciferase reporter construct and 10 \(\mu\)g of pCI-\(\Delta p85\) or empty pCI vector. The cells were cultured for 3 h before being transferred into wells coated with 2C11. After 14 h, the cells were harvested, lysed, and assayed for luciferase activity. The mean relative light units of the extracts obtained from cells cultured in medium were 661 (empty vector) and 3785 (wortmannin). The data shown are the means ± S.E. of three independent experiments. C, lymph node T cell blasts were transfected with 10 \(\mu\)g of IL-2 promoter-luciferase reporter construct, treated with wortmannin (100 nM) for 30 min, and then cultured on wells coated with 2C11. After 15 h, cell extracts were prepared, and luciferase activity was quantitated. The mean relative light units of the extracts obtained from cells cultured in medium were 2803 (vehicle control) and 3785 (wortmannin). The data shown are from one of three independent experiments with similar results. The error bars represent the S.D. of duplicate samples.

**FIG. 3.** \(\Delta p85\) and wortmannin block NF-AT and AP-1 transcriptional activities. A and C, lymph node T cell blasts were cotransfected with 10 \(\mu\)g of pCI-\(\Delta p85\) or empty pCI vector and 10 \(\mu\)g of NF-AT-luciferase reporter construct (A) or with 10 \(\mu\)g of AP-1-luciferase reporter construct (C) and 10 \(\mu\)g of \(\Delta p85\) or empty vector and cultured for 3 h before transfer onto wells coated with 2C11. After 14 h, luciferase activity was assessed. The mean relative light units of the extracts obtained from cells cultured in medium were 2903 (vehicle control) and 3755 (wortmannin). The mean relative light units of the extracts obtained from cells cultured in medium were 2903 (vehicle control) and 3755 (wortmannin). The mean relative light units of the extracts obtained from cells cultured in medium were 2903 (vehicle control) and 3755 (wortmannin).
Therefore, the effects of wortmannin and LY294002 on ERK activation following anti-CD3 antibody activation were assessed. Freshly isolated lymph node cells were treated with wortmannin or LY294002 and then activated with anti-CD3 antibody. ERK-2 was immunoprecipitated, and its kinase activity was analyzed using myelin basic protein as a substrate. As expected, ERK-2 was activated by anti-CD3 antibody. In wortmannin- and LY294002-treated cells, however, the anti-CD3 antibody-induced ERK-2 activation was markedly inhibited (Fig. 5A). Treatment of the lymph node cells with wortmannin or LY294002 did not change the levels of ERK-2 protein, as visualized by immunoblotting of the reaction samples and probing with an anti-ERK-2 antibody (Fig. 5B). Furthermore, the addition of wortmannin or LY294002 directly to immunoprecipitated ERK-2 prior to and during the kinase assay had no effect on ERK-2 kinase activity (data not shown). Thus, wortmannin and LY294002 do not directly inhibit ERK-2, but rather inhibit a step necessary for ERK-2 activation.

MEK-1 is a dual-specificity kinase that is activated following TCR stimulation (44) and subsequently phosphorylates and thereby activates ERK-2. Freshly isolated lymph node cells were treated with wortmannin or LY294002 and then activated with anti-CD3 antibody. MEK-1 was immunoprecipitated, and its kinase activity was assessed using kinase-inactive ERK-2 as a substrate. MEK-1 was activated by anti-CD3 antibody, and this activation was markedly inhibited by the addition of wortmannin or LY294002 (Fig. 6A). This was a reflection of an inhibition of specific activity, as the levels of MEK-1 protein were not altered by treatment with wortmannin or LY294002 (Fig. 6B). The addition of wortmannin or LY294002 directly to immunoprecipitated MEK-1 prior to and during the kinase assay had no effect on MEK-1 kinase activity (data not shown). Taken together, these results indicate that PI 3-kinase activity is necessary for a step in the ERK signaling cascade that leads to MEK-1 and ERK-2 activation following TCR activation.

Constitutively Active Akt Enhances TCR-mediated Signaling for IL-2 Transcription, but Does Not Bypass the Requirement of PI 3-Kinase—PI 3-kinase may regulate MEK-1 activation directly or indirectly via one of its known targets. Since Akt has been described as a downstream target of PI 3-kinase (40, 45), we asked whether a constitutively active Akt could bypass the need for PI 3-kinase in T cell signaling. Lymph node T cell blasts were transfected with expression plasmids encoding the IL-2-luciferase reporter construct and v-Akt (30). Transient expression of v-Akt in T cell blasts in the absence of an anti-TCR stimulus did not induce IL-2 promoter activity (Fig. 7, see legend). Expression of v-Akt did, however, lead to augmented IL-2 promoter activation following TCR stimulation, suggesting that Akt participates in signaling for IL-2 gene transcription following TCR activation. Coexpression of v-Akt and Δp85 resulted in a marked decrease of IL-2 promoter activation when compared with expression of v-Akt alone. The level of TCR-mediated IL-2 promoter activation after coexpression of v-Akt and Δp85 was similar to the level observed after transfection of only Δp85. Thus, although v-Akt augments signaling via the TCR, it cannot bypass the need for PI 3-kinase in TCR-mediated signaling resulting in IL-2 gene transcription.

**DISCUSSION**

The study of PI 3-kinase function has been facilitated by the discovery of two structurally unrelated inhibitors, wortmannin and LY294002. Wortmannin covalently binds Lys-802 of p110(8) of p110 (46) and irreversibly inhibits PI 3-kinase at nanomolar concentrations (33, 34). LY294002, a competitive antagonist that prevents the binding of ATP to p110, inhibits PI 3-kinase with an IC₅₀ of 1.4 μM (38). Mutants of p85 in which the inter-SH2 domain has been deleted have proved to act as dominant-negative inhibitors, providing a non-pharmacological method of analyzing PI 3-kinase function in vivo (5). A number of investigators have used these reagents and the Jurkat T cell line to investigate the role of PI 3-kinase in T cell activation and have reported conflicting findings that support either a negative or a neutral role for PI 3-kinase in IL-2 production. In one study, for example, it was concluded that PI 3-kinase has a negative role in TCR-mediated activation because a constitutively active PI
but does not bypass the requirement for PI 3-kinase.

In contrast to these studies, the data in the present report demonstrate that PI 3-kinase activity is necessary for IL-2 production by normal T cells. The results show that PI 3-kinase regulates IL-2 production at the level of gene transcription since a dominant-negative PI-3 kinase and wortmannin inhibited TCR-mediated IL-2 promoter activation. The role of PI 3-kinase in IL-2 gene transcription was further defined with respect to the transcription factors that participate in IL-2 gene transcription. PI 3-kinase activity was found to be essential for the transcriptional activity of both NF-AT and AP-1. Furthermore, this effect appears to be exerted at the level of signaling molecules within the Ras/ERK pathway since PI 3-kinase activity was required for both MEK and ERK activation following TCR stimulation. Thus, in normal T cells, PI 3-kinase appears to be required for activation-induced up-regulation of MEK activity. We have used three different assays to determine if inhibition of PI 3-kinase alters activation of Ras. Unfortunately, although we can easily detect Ras activation in T cell lines, we have not been able to do so in primary T cells. Efforts are currently underway to develop a system in which the Ras signal can be enhanced to investigate what role, if any, PI 3-kinase plays in Ras activation in normal T cells.

The reason for the discrepancies between our results, indicating a positive role for PI 3-kinase in IL-2 production, and those obtained in the studies mentioned above is not clear, but is likely to reflect differences in TCR-mediated signaling and the regulation of IL-2 production in normal resting T cells compared with Jurkat cells. For example, phorbol ester is needed in addition to TCR ligation to stimulate Jurkat cells, but not normal T cells, to produce IL-2. Another difference is that treatment with wortmannin itself results in a low level of IL-2 production even without TCR activation in Jurkat cells (27), but does not induce IL-2 production or proliferation in normal T cells. In support of this distinction between normal T and Jurkat cells is that IL-2 production by antigen-stimulated TCR transgenic T cells has been shown to be inhibited by wortmannin (28). Surprisingly, inhibition was not observed when activation was achieved with immobilized anti-TCR antibodies. In contrast, we have found that anti-TCR antibody-induced activation is readily inhibited by both chemical and molecular inhibitors of PI 3-kinase activity. This difference may be explained by different modes of activation. Whereas we used immobilized anti-TCR antibodies only, Shi and co-workers (28) used either syngeneic B7- B cell blasts that had been lipopolysaccharide-stimulated and mitomycin C-treated, or phorbol 12-myristate 13-acetate together with anti-TCR antibodies. Of interest is the observation that splenic T cells that had been activated by anti-CD3 antibody and B7- B cells become wortmannin-sensitive when they are propagated in vitro (49). We have identified both MEK-1 and ERK-2 as downstream effectors for PI 3-kinase in normal T cells upon TCR activation. This is in contrast to one study in which it was found that TCR-induced MEK-1 activation in Jurkat cells was not inhibited by wortmannin and Δp85, although ERK-2 activation was inhibited by both reagents (50). Again, this discrepancy might be explained by differences between Jurkat and normal T cells.

There are at least two nonexclusive possibilities by which PI 3-kinase might regulate IL-2 gene transcription: directly, by regulating a component of one of the signaling cascades leading to IL-2 gene expression, or indirectly, through one of its known targets. We have tested one of these possibilities by asking whether the PI-3 kinase-dependent step involved the PI 3-kinase target Akt. Since a constitutively active Akt kinase did not
overcome the block in IL-2 promoter activity mediated by Δ85, we conclude that there is an additional target of PI 3-kinase that is necessary for IL-2 gene transcription. In addition, the data presented here indicate that the PI 3-kinase target Akt can participate in TCR signaling since constitutively active Akt synergizes with anti-TCR antibodies to induce IL-2 promoter activity in normal T cells. The involvement of Akt in TCR-mediated IL-2 gene expression presumably reflects its positive regulation of NF-AT since Akt inactivates glycogen-synthase kinase-3, resulting in decreased phosphorylation of NF-AT and allowing NF-AT to stay in the nucleus. The results of this study suggest that PI 3-kinase participates in TCR-mediated IL-2 gene transcription by at least two distinct pathways, one involving ERK activation and the other Akt/glycogen-synthase kinase-3. The ERK pathway results in the induction of the c-fos gene, which participates in the formation of AP-1, whereas the Akt/glycogen-synthase kinase-3 pathway prevents the nuclear exit of NF-AT. Both AP-1 and NF-AT are necessary for IL-2 gene transcription and thus can account for the essential role that PI 3-kinase has in IL-2 gene expression.

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