Phosphatidylinositol 3-Kinase Links the Interleukin-2 Receptor to Protein Kinase B and p70 S6 Kinase*

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Phosphatidylinositol 3-kinase (PI 3-kinase) is activated by the cytokine interleukin-2 (IL-2). We have used a constitutively active PI 3-kinase to identify IL-2-mediated signal transduction pathways directly regulated by PI 3-kinase in lymphoid cells. The serine/threonine protein kinase B (PKB/Akt) can act as a powerful oncogene in T cells, but its positioning in normal T cell responses has not been explored. Herein, we demonstrate that PKB is activated by IL-2 in a PI 3-kinase-dependent fashion. Importantly, PI 3-kinase signals are sufficient for PKB activation in IL-2-dependent T cells, and PKB is a target for PI 3-kinase signals in IL-2 activation pathways. The present study establishes also that PI 3-kinase signals or PKB signals are sufficient for activation of p70 S6 kinase in T cells. PI 3-kinase can contribute to, but is not sufficient for, activation of extracellular signal-regulated kinases (Erks) and Erk effector pathways. Therefore, PI 3-kinase is a selective regulator of serine/threonine kinase signal transduction pathways in T lymphocytes, and this enzyme provides a crucial link between the interleukin-2 receptor, the protooncogene PKB, and p70 S6 kinase.

The high affinity interleukin-2 receptor (IL-2R), which comprises α-, β-, and γ-subunits controls G1 to S progression, T cell clonal expansion, and functional differentiation (1–3). The IL-2R orchestrates downstream effector pathways by protein tyrosine kinase-dependent activation mechanisms engaging the Src family tyrosine kinases Lck and Fyn (4) and the Janus kinases 1 and 3 (5–7). Signaling cascades integrated by the action of these tyrosine kinases include activation of the Ras/Raf/extracellular-signal regulated kinase (Erk) pathway (8–10), activation of the transcription factors STAT3 and STAT5 (11), and the regulation of phosphatidylinositol 3-kinase (PI 3-kinase) (12).

PI 3-kinase is a ubiquitously expressed enzyme that catalyzes the phosphorylation of phosphoinositides at the D-3 hydroxyl of the myo-inositol ring generating PI 3-phosphate, PI 3,4-bisphosphate, and PI 3,4,5-trisphosphate (13, 14). The form of PI 3-kinase involved in protein-tyrosine kinase-dependent receptor signal transduction comprises a regulatory 85-kDa subunit that contains two Src homology 2 domains and at its N terminus one Src homology 3 domain and a catalytic 110-kDa subunit. Following IL-2R stimulation, several mechanisms have been proposed to recruit PI 3-kinase to the plasma membrane, where its cellular substrate PI 4,5-bisphosphate is located: engagement of the IL-2R leads to binding of the p85 regulatory subunit of PI 3-kinase to tyrosine 392 in the IL-2R β-chain (15); in addition, interleukin-2 (IL-2) stimulation results in the interaction of PI 3-kinase with the Src family kinases Fyn (16) and Lck (17).

The activation of PI 3-kinase is a response that IL-2 shares with other cytokines that control lymphoid cell growth and development such as IL-4 and IL-7 (18, 19). It is also clear that PI 3-kinase activation is necessary for the growth- and differentiation-inducing properties of these cytokines (20–23). However, despite the pivotal role of PI 3-kinase in lymphoid cells, there is only a preliminary and incomplete understanding of the targets for this enzyme in the mitogenic signaling pathways regulated by the hematopoietin family of cytokines. To date, the identification of biochemical targets for PI 3-kinase in T cells stems mainly from studies employing the PI 3-kinase inhibitor wortmannin or the LY294002 compound (10, 20). Hence, IL-2 activation of the mitogen-activated protein (MAP) kinase Erk is sensitive to wortmannin (10). Similarly, IL-2 activation of the serine/threonine kinase p70 S6 kinase (p70S6k) is prevented by these PI 3-kinase inhibitors (20). In addition, IL-2 activation of p70S6k is impeded by the immunosuppressant rapamycin, which targets another member of the PI 3-kinase family of enzymes, Frap (for FKBP12-rapamycin-associated protein) also termed “mammalian target of rapamycin” (mTor) (24, 25). Observations that wortmannin and rapamycin have identical inhibitory effects on IL-2 activation of p70S6k generated a model for the p70S6k signaling pathway in which PI 3-kinase acts as an upstream regulator of Frap (24, 25). However, this model has been challenged by a recent study showing that the action of Frap is directly inhibited by wortmannin and LY294002 (26). These results raise the issue of whether PI 3-kinase itself has any upstream regulatory role in p70S6k activation in T lymphocytes. Similar caution must be applied to interpretations of data involving PI 3-kinase in Erk activation in T cells. In this context, expression of an active PI 3-kinase is sufficient for Erk activation in Xenopus oocytes (27), but it would be fallacious to extrapolate data obtained in Xenopus cells to T cells, since the role of PI 3-kinase as an upstream regulator of kinase pathways can vary depending on the cell system; to this end, PI 3-kinase signals did not stimulate Erk activity in a variety of fibroblasts and in a monoblast.
Plasmid DNA. The amounts of plasmid DNA were kept constant per construct, rCD2p110, that induces accumulation of cellular levels of PI 3,4-bisphosphate and PI 3,4,5-trisphosphate (29) as a tool to explore the regulation of serine/threonine kinase pathways by PI 3-kinase in T lymphocytes. We show that activation of PI 3-kinase is sufficient to stimulate p70S6k activity, although PI 3-kinase signals were not sufficient to induce activation of the MAP kinase Erk2 in T cells. The present study also characterizes a previously unrecognized IL-2-mediated signal transduction pathway in T cells that involves the serine/threonine protein kinase B (PKB) also known as c-Akt or Rac protein kinase (32–34). PKB was originally identified as the cellular homologue of the directly transforming oncogene of the murine retrovirus AKTS, which causes thymic lymphomas (35). Herein, we demonstrate that PKB is rapidly activated by IL-2 via a wortmannin- and LY294002-sensitive but rapamycin-insensitive pathway. PI 3-kinase signals alone were sufficient to activate PKB in T cells, and expression of a constitutively active pathway. PI 3-kinase signals were not sufficient to induce activation of the MAP kinase Erk2 in T cells, and this enzyme is a selective regulator of serine/threonine kinase signal transduction pathways in T lymphocytes, and this enzyme is an upstream regulator of the IL-2-activated kinases PKB and p70S6k.

EXPERIMENTAL PROCEDURES

Reagents—Phorbol 12,13-dibutyrate (PdBu) and wortmannin were from Calbiochem. LY294002 was a gift from Zeneca. PDD08509 was from New England Biolabs. Rapamycin was a gift from G. Thomas (FMI, Basel). [14C]Acetyl coenzyme A (at 50 mCi/mmol), [γ-32P]ATP (3000 Ci/mmol), and 125I-conjugated protein A were from Amersham Corp.

Antibodies—Ox34 monoclonal antibody (mAb) is raised against rat CD2 (rCD2) (29); 12CA5 mAb is reactive with hemaggutinin (HA), and 9E10 mAb is reactive with the Myc epitope (36); anti-human S6 kinase M5 antisemur (37) was from Santa Cruz Biotechnology; M1 antiserum reactive with p70S6k (37) was a gift from G. Thomas; Rac-PK-CT Ab (Upstate Biotechnology, Inc.) is reactive with PKB.

Plasmids and Reporter Constructs—HA-p70S6k (pBJ5) (38); HA-PKB (pSG5) and gagPKB (pSG5) (32); HA-Erk2 (pCEP4) (39); Myc-V12Rac (pEF), Myc-V12Cd42 (pEF), and Myc-V14Rho (pEF) (40); and Ha-v-vas (pEF) (29) vector constructs have been described. The described rCD2p110, rCD2p110-R/P, and rCD2p85 chimeras (29) were subcloned into the pEF-BOS expression vector. The reporter plasmids Nlxl.ELk-1 (pEF) and 2lexoptk. CAT (41) as well as Nlx.C2 (pMLV) (42) have been described.

Cell Culture and Transient Transfections—The Kit225 T leukemic cell line (43) was maintained in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum supplemented with 20 ng/ml of recombinant IL-2 (rIL-2) (Eurocetus) under normal growth conditions. For IL-2 activation assays of endogenous proteins, Kit225 cells were washed three times with phosphate-buffered saline A to remove the IL-2 and cultured further in RPMI supplemented with 5% fetal calf serum in the absence of rIL-2 for 48–72 h prior to IL-2 activation assays. When Kit225 cells were transfected, cells were treated as above but only deprived of rIL-2 for 24 h prior to transfection.

Kit225 cells were transfected by electroporation with 20–40 μg of plasmid DNA. The amounts of plasmid DNA were kept constant per cuvette by adding vector plasmid. Kit225 cells (1.5 × 10^6 cells/0.625 ml) were pulsed at 320 V and 960 microfarads using a Gene Pulser (Bio-Rad). The amounts of plasmid used were as follows (unless indicated otherwise): 7.5 μg of HA-p70S6k; 12.5 μg of HA-PKB; 10 μg of HA-Erk2; 20 μg of the plasmid pEF empty, rCD2p110, rCD2p110-R/P, Ha-v-vas, V12Rac, V12Cdc42, gagPKB, or rCD2p85; 7.5 μg of 2lexoptk.CAT; and 15 μg of pEF Nlx.ELk-1 or pMLV/Nlx.C2. For gene reporter assays, cells were stimulated as indicated 2–4 h after transfection. Cells were collected 14–18 h after transfection.

Immunoprecipitation, p70S6k Assays, and Western Blot Analysis—After stimulations as indicated, Kit225 cells were lysed in lysis buffer 1 (120 mM NaCl, 50 mM Tris pH 8.0, 20 mM NaF, 1 mM benzamidine, 1 mM EDTA, 6 mM EDTA, 7.5 mM PMSF, 15 mM p-nitrophenol phosphate, 1% Nonidet P-40, 0.1 mM phenylmethylsulfonyl fluoride, and 0.1 mM
with the vehicle dimethyl sulfoxide (DMSO). Kit225 cells starved of rIL-2 for 72 h were pretreated for 30 min with LY294002, but not rapamycin, inhibit IL-2-dependent activation of PKB. PI 3-kinase activity was measured in immune complex kinase assays using H2B as a substrate. [32P]Phosphate incorporation into H2B was quantified (44) using 9E10, Ox34, or specific Abs.

**PKB Assays**—Cells were treated as for p70S6k assays except that the reaction buffer contained 120 mM NaCl, 50 mM Hepes, pH 7.4, 10 mM NaF, 1 mM EDTA, 40 mM β-glycerophosphate, pH 7.5, 1% Nonidet P-40, 0.1 mM phenylmethylsulfonl fluoride, 0.1 mM Na3VO4, and 0.1 mM Na3VO4 was used to lyse cells. The immunoprecipitate was washed twice in lysis buffer 2, twice in high salt wash buffer (500 mM LiCl, 100 mM Tris, pH 7.5, 1 mM EDTA, pH 7.5), and once in PKB assay buffer (50 mM Tris, pH 7.5, 10 mM MgCl2, 1 mM dithiothreitol). The reaction was initiated by the addition of 15 μl of PKB reaction buffer containing 3 μCi of [γ-32P]ATP, 50 μM ATP, 7.3 mM MgCl2, 730 μM dithiothreitol, 500 nM protein kinase inhibitor (Sigma), 40 mM Tris, pH 7.5, and 2.5 μg of histone 2B (H2B) (Boehringer Mannheim). After 30 min at 25 °C, the reaction was terminated by adding reducing SDS-PAGE sample buffer and boiling. Proteins were resolved by SDS-PAGE, and the gel was treated as for p70S6k assays. To detect PKB proteins, Western blot analysis was performed with Rac-PK-CT Abs.

**Erk Assays**—Cells and cell extracts were processed as for p70S6k assays. HA-tagged Erk2 was immunoprecipitated with 12CA5 mAbs. Precipitated immune complexes were washed three times with lysis buffer 2 and then with 20 mM NaCl. The Erk assay was initiated by the addition of 10 μl of Erk reaction buffer containing 4 μCi of [γ-32P]ATP, 20 mM MgCl2, 2 mM MnCl2, 5 mM p-nitrophenyl phosphate, 500 nM protein kinase inhibitor (Sigma), 30 mM Tris, pH 8.0, and 15 μg of myelin basic protein (Sigma). After 30 min at 37 °C, the reaction was terminated by adding reducing SDS-PAGE sample buffer and boiling. Proteins were resolved by SDS-PAGE, and the gel was treated as for p70S6k assays. To detect Erk proteins, Western blot analysis was performed with 12CA5 Abs as primary Ab, rabbit anti-mouse IgG as secondary Ab, and [125I]-conjugated protein A.

**Gene Expression Analysis**—Fourteen to 16 h after inductions, as indicated, Kit225 T cells were harvested and cells were lysed in 200 μl of lysis buffer (0.65% Nonidet P-40, 10 mM Tris, pH 8.0, 1 mM EDTA, 150 mM NaCl). Gene expression assays were carried out as described (45). The data are presented as percentage of conversion.
expression of the rCD2p110 chimera was confirmed by flow cytometric immunofluorescence analysis with rCD2 mAbs (data not shown). The HA-tagged p70S6k was immunoprecipitated from transiently transfected cells and assayed for its ability to phosphorylate S6 ribosomal subunits (Fig. 1B). Expression of the active PI 3-kinase, rCD2p110, resulted in constitutive IL-2-independent p70S6k activation (Fig. 1B). p70S6k was not constitutively activated in cells expressing “kinase-dead” rCD2p110-R/P, confirming that the p70S6k activation requires the kinase activity of the p110 subunit (Fig. 1C). The expression of rCD2p110-R/P was noted in some experiments to suppress rIL-2 inducibility of p70S6k, indicating that this chimera may be an inhibitory mutant of PI 3-kinase pathways.

**IL-2 and Membrane-localized PI 3-Kinase Activate PKB—**PKB can be activated by receptor tyrosine kinases such as the platelet-derived growth factor receptor and has been identified as a target of PI 3-kinase in fibroblasts (28, 32, 33). However, whether this pathway is conserved in the hematopoietic system has not been explored. In particular, although PKB can become oncogenic and initiate thymic tumors, its regulation and significance for normal T cell growth processes is not known. Since cytokine receptors have essential functions in the development and maintenance of the hematopoietic system, we were interested to assess whether members of the hematopoietin receptor family, such as the prototypical IL-2R, regulate PKB. To examine whether IL-2 activates PKB, immunoprecipitates of this kinase were prepared from rIL-2-deprived and rIL-2-activated Kit225 cells and subjected to in vitro kinase assays using H2B as a substrate. The data in Fig. 2A show that IL-2 induced a rapid activation of PKB. A 2–3-fold increase in enzyme activity over basal levels was sustained for more than 60 min in response to rIL-2. PKB activity is regulated by phosphorylation as indicated by the reduced electrophoretic mobility of PKB isolated from rIL-2-activated cells (Fig. 2A). PKB activity was not induced by exposure of Kit225 cells to phorbol esters that activate PKC (Fig. 2A). The data in Fig. 2B show the failure of rIL-2 to stimulate PKB in cells pretreated with LY294002 and wortmannin, two well characterized PI 3-kinase inhibitors that bind to the ATP or lipid binding sites on the p110 catalytic subunit, respectively. These inhibitors also prevent the autokinase activity of Frap/mTOR (26), a member of the PI 3-kinase family (47), which is the cellular target for the drug rapamycin and which prevents IL-2-coordinated cell cycle progression and proliferation of T lymphocytes (24, 25). Frap activity is absolutely required for p70S6k action in T cells (24, 25). We therefore assessed whether Frap function was necessary for IL-2-induced stimulation of PKB. Rapamycin had no effect on IL-2-triggered activation of PKB (Fig. 2B), although rapamycin completely abolished IL-2- or PI 3-kinase-controlled induction of p70S6k (data not shown). Thus, the inhibition of PKB by wortmannin and LY294002 cannot be caused by prevention of Frap activity and indicate that IL-2 regulation of PKB employs PI 3-kinase pathways.

To investigate directly whether PI 3-kinase signals are sufficient to activate PKB, rIL-2-deprived Kit225 cells were co-transfected with either rCD2p110 or rCD2p110-R/P expression vectors together with an expression vector encoding HA epoicen-tagged PKB. In addition, the ability of activated forms of the small GTPases Ha-v-ras and V12Rac to activate PKB was assessed. Immunoprecipitates of HA-tagged PKB were assayed for kinase activity using H2B as a substrate. The constitutively active PI 3-kinase rCD2p110 induced a robust activation of PKB (Fig. 2C). This stimulatory effect of rCD2p110 was dependent on the kinase activity of the chimera, since co-expression of kinase-inactive rCD2p110-R/P did not stimulate PKB activity. As observed previously in other cell
systems (28, 33, 48), co-expression of activated Ha-v-ras but not of active V12Rac led to a moderate rise in PKB activity in Kit225 T cells.

Co-expression of an Activated Form of PKB Stimulates p70S6k in Kit225 T Cells—p70S6k is activated by multiple serine/threonine phosphorylation in response to mitogenic stimuli. The retroviral oncogene v-Akt is a chimeric molecule, consisting of the retroviral Gag protein fused to the N terminus of c-Akt, which is myristoylated, and hence v-Akt is predominantly found at the plasma membrane, which may give raise to its oncogenicity (49). The expression of constitutively active PKB, gagPKB, has been described as activating p70S6k in Rat-1 cells (32) and COS1 cells (33). Nevertheless, the ability of phorbol esters to stimulate p70S6k without any discernible activation of PKB indicated that PKB-independent pathways for activation of p70S6k must exist in T cells. To determine the role of PKB in p70S6k activation in T cells, rIL-2-deprived Kit225 cells were co-transfected with a gagPKB expression vector together with an expression vector encoding HA epitope-tagged p70S6k. p70S6k activity was analyzed in anti-HA tag immune complexes with S6 ribosomal subunits as a substrate. Co-expression of constitutively active PKB induced a strong activation of p70S6k that was comparable with increases in p70S6k activity seen by co-expression of rCD2p110 (Fig. 3A). A rCD2p85 construct that does not regulate cellular levels of D-3 phosphoinositides (29) did not stimulate p70S6k. In contrast to data described in fibroblasts (48), co-expression of V12Rac and V12Cdc42 had no effect on p70S6k activity in Kit225 cells (Fig. 3A). To confirm that V12Rac and V12Cdc42 are active in Kit225 cells, we tested their ability to activate the stress-activated protein kinases, also known as c-Jun N-terminal kinases (50, 51). Stress-activated protein kinase and hence Rac/Cdc42 activity can be measured by the ability of stress-activated protein kinases to phosphorylate the transcription factor ATF-2 (42). To monitor ATF-2 transcriptional activity, a fusion protein comprising the N terminus of ATF-2 (termed C2) linked to the LexA repressor (42) was co-transfected into Kit225 cells together with a LexA operator-controlled chloramphenicol acetyltransferase (CAT) reporter gene. V12Rac and V12Cdc42 potently stimulated ATF-2/LexA-C2 transcriptional activity in Kit225 T cells (Fig. 3B).

IL-2 Regulates the Transcription Factor Elk-1 in Kit225 Cells in a PI 3-Kinase-dependent Fashion—In T cells, a PI 3-kinase-sensitive pathway for regulating the activity of Erk kinase (Mek) and the Erks has been reported to co-exist alongside the PI 3-kinase/p70S6k pathway (10). While p70S6k is thought to exert its mitogenic function by controlling translation initiation and protein synthesis, the MAP kinase Erk is implicated in regulating the phosphorylation and activity of certain transcription factors. One well characterized cellular substrate for Erks in fibroblasts and T cells is the transcription factor Elk-1 (40, 52, 53). We therefore tested the ability of IL-2 to regulate Elk-1 transcriptional activity and hence Erk in Kit225 cells. To monitor Elk-1 transcriptional activity, a fusion protein comprising the C terminus of Elk-1 linked to the LexA repressor (41) was co-transfected into Kit225 cells with a LexA operator-controlled CAT reporter gene. The data in Fig. 4A demonstrate that IL-2 can regulate Elk-1 transcriptional activity in Kit225 cells. To confirm that Elk-1 transcription activation is induced by a Mek/Erk-sensitive pathway, we investigated the ability of the well characterized inhibitor of Mek activation, PD098059 (54), to prevent IL-2-mediated activation of Elk-1. Treatment of Kit225 cells with the PD098059 component inhibited stimulation of Elk-1 transcriptional activity triggered by rIL-2 (Fig. 4B). Moreover, rIL-2 induction of Elk-1 activity was prevented by the PI 3-kinase inhibitor wortmannin in a dose-dependent manner (Fig. 4C), which corroborates earlier studies indicating that Erk activation by IL-2 requires PI 3-kinase function (10). Treatment of Kit225 cells with rapamycin did not affect Elk-1 transcriptional activity in Kit225 cells (Fig. 4D). To assess whether constitutively active PI 3-kinase and the in vivo production of D-3 phosphoinositides could induce MAP kinase signaling pathways in T cells, the activity of rCD2p110 to induce transcriptional activation of Elk-1 was analyzed. LexA-Elk-1 transcriptional activity was low in quiescent Kit225 cells but could
be instigated by co-expression of active Ha-v-ras and by stimulation with phorbol esters, whereas expression of rCD2p110 did not stimulate phorbol ester induction of the transcriptional activity of Elk-1. This potentiating effect was not observed in cells expressing the kinase-dead rCD2p110-R/P and was thus dependent on the integrity of the lipid kinase and the cellular production of D-3 phosphoinositides. Moreover, gagPKB cannot mimic the effects of PI 3-kinase on the Erk/Elk-1 pathway (Fig. 4E).

**PI 3-Kinase Signals Synergize with Phorbol Esters to Induce Erk Activity in Kit225 Cells**—To assess the effect of membrane-localized PI 3-kinase on Erk activity directly, rIL-2-deprived Kit225 cells were co-transfected with expression vectors encoding rCD2p110 and HA epitope-tagged p42 Erk2, and cells were stimulated with phorbol esters or left untreated. Co-expression of rCD2p110 did not stimulate Erk2 activity, although Erk2 could be activated by co-expressing the activated Ras, Ha-v-ras (Fig. 5). These results thus confirm the data in Fig. 4E indicating that PI 3-kinase signals are not sufficient to activate the Erk/Elk-1 pathway. The data in Fig. 5 demonstrate that active PI 3-kinase markedly potentiated the level of Erk2 activation triggered by phorbol esters, an effect that was not observed in cells expressing the kinase-dead rCD2p110-R/P. PI 3-kinase signals did not enhance IL-2 activation responses on Erk (data not shown). Taken together, the results in Figs. 4E and 5 clearly demonstrate that although PI 3-kinase signals are not sufficient for Erk/Elk-1 activation, they can synergize with phorbol esters to induce a maximal response. These results are concordant with a model where PI 3-kinase signals bifurcate to activate the PKB rapamycin-sensitive/p70S6k pathway and independently contribute to the Mek/Erk/Elk-1 pathway via an as yet undefined mechanism (see Fig. 6).

**DISCUSSION**

The present study has used a membrane-targeted, constitutively active, catalytic subunit of PI 3-kinase as a tool to identify direct targets of PI 3-kinase action in IL-2 signal transduction pathways. We demonstrate that the serine/threonine kinase PKB/Akt can be activated by the cytokine IL-2 via a PI 3-kinase-dependent pathway. Importantly, PI 3-kinase signals alone are sufficient to activate PKB in T cells, demonstrating that PI 3-kinase acts as an upstream regulator of this serine/threonine kinase in lymphoid cells. PKB contains an N-terminal pleckstrin homology domain that can directly bind D-3 phosphoinositides (33, 55, 56), which may contribute to the regulation of the enzyme. Since PI 3-kinase signals are sufficient to substitute for IL-2 in PKB activation, PKB could be a direct target for PI 3-kinase signals during IL-2 signal transduction. PKB/c-Akt is highly expressed in the thymus (57), and the oncogenic form of this kinase causes thymic malignancies. Therefore, PKB has a pivotal role in controlling T cell proliferation/differentiation. The present data identify one function for PKB in T cells; PKB action is sufficient to stimulate p70S6k. Moreover, PI 3-kinase signals are sufficient for activation of p70S6k, which stresses the close link between PI 3-kinase and PKB in regulating p70S6k activity in T cells. Questions regarding the selectivity of the inhibitors that were first used to define a role for PI 3-kinase in T cell biology have challenged the involvement of this enzyme in the regulation of p70S6k in T cells (26). The present data resolve this controversy and provide unequivocal evidence that PI 3-kinase can function as an upstream regulator of p70S6k in T cells.

Results obtained recently with p110 constructs that were membrane-targeted by myristoylation or farnesyl signalings showed that PI 3-kinase signals are sufficient to activate PKB and p70S6k in COS cells (28). It has also been shown in fibroblasts that the GTPases Rac and Cdc42 induce p70S6k activation. We find no evidence for Rac/Cdc42 activation of p70S6k in T cells, indicating that cells of different lineages can differ.
markedly in their cellular mechanisms for kinase activation. Nevertheless, the present data show a striking conservation of the PI 3-kinase/PKB/p70S6k link in human T cells and simian fibroblasts. The conservation of the PI 3-kinase/PKB/p70S6k signaling cascade in T cells implies a physiological importance of this pathway, which has guaranteed its evolutionary conservation.

The role of PI 3-kinase as an upstream regulator of the Erk kinase pathways can also vary depending on the cell system; expression of an active PI 3-kinase is sufficient for Erk activation in Xenopus oocytes (27) but not in fibroblasts or mono- blasts (28–31). The present data show directly that PI 3-kinase can have a positive regulatory role in Erk activation in T cells (see Fig. 6). However, PI 3-kinase signals alone fail to stimulate Erk signaling pathways but markedly potentiate Erk responses in combination with phorbol esters. Erk regulation of downstream nuclear targets is hereby analyzed using the transactivation capacity of the ternary complex factor Elk-1, a well characterized substrate for Erks in fibroblasts and Jurkat T cells (40, 53). We establish that Elk-1 is regulated by IL-2 via Mek- and PI 3-kinase-sensitive pathways. Furthermore, as observed in direct Erk activation assays, PI 3-kinase signals potentely enhance phorbol ester induction of Elk-1 transcriptional activity. PI 3-kinase signals may thus be required for activation of MAP kinase pathways in IL-2-dependent T cells, but they are not sufficient and hence are one component of a more complex signaling network. We have not yet explored the PI 3-kinase effector pathways involved in Erk activation, although previous data have excluded the involvement of the Frap/p70S6k pathway, since activation of Erk is not sensitive to rapamycin inhibition. Moreover, PKB, which is a potent activator of p70S6k, cannot mimic the effects of activated PI 3-kinase on the Erk/Elk-1 pathway. These data best fit a model in which PI 3-kinase regulation of MAP kinases and PKB/p70S6k bifurcate prior to activation of PKB (Fig. 6). Members of the Rho family of GTPases can potentiate Erk activation pathways in fibroblasts (39). Activation of PI 3-kinase is sufficient to induce cytoskeletal rearrangements mediated by the GTPase Rac and Rho in Swiss 3T3 cells (29) and thus has the potential to regulate Rac/Rho signaling pathways in T cells. Accordingly, it is possible that Rac/Rho family GTPases could mediate PI 3-kinase regulation of Erk. However, several other candidate in vivo targets for D-3 phosphoinositides have been proposed including members of the novel PKC family (58) and the atypical PKC family, PKC-λ (59) and PKC-ζ (60), which has recently been implicated as a regulator of Mek and Erk activity in COS cells (61).

p70S6k plays a key role in cellular growth control mechanisms by coordinating protein biosynthesis via phosphorylation of the S6 subunit of 40 S ribosomes or via regulation of the activity of the eukaryotic initiation factor 4E binding protein, 4E-BP1 (24, 25, 62). Expression of an activated PKB can stimulate p70S6k activity in T cells, indicating that PKB substrates are part of the p70S6k activation pathways. Moreover, given the ability of PI 3-kinase signals to stimulate PKB and p70S6k, it seems probable that PKB mediates the PI 3-kinase activation of p70S6k in T cells. The immunosuppressive drug rapamycin inhibits the cell cycle progression and proliferation of T lymphocytes and has been shown previously to block IL-2 activation of p70S6k. Rapamycin forms a complex with the intracellular protein FKBP12, which subsequently provides a high affinity inhibitor of Frap. The Frap kinase is a member of the PI 3-kinase family of enzymes (47) and plays an established, but poorly defined, role as an upstream regulator of p70S6k (24, 25). Rapamycin prevents the activation of p70S6k induced in T cells by the constitutively active PI 3-kinase, rCD2p110, (data not shown) or by active PKB (32, 33), thus indicating that PI 3-kinase or PKB activation signals cannot bypass the role of Frap in p70S6k activation pathways. A simple interpretation of these data is that PI 3-kinase and PKB activation of p70S6k is mediated by Frap, although the possibility cannot be excluded that Frap regulates p70S6k by an essential signaling pathway operating in parallel with PI 3-kinase/PKB signals (Fig. 6). Frap controls p70S6k activation by regulating the phosphorylation of key residues in the enzyme (63, 64). Nevertheless, p70S6k is not a direct substrate for Frap, and some intermediate p70S6k kinase(s), as yet uncharacterized, must be invoked to explain the role of Frap in p70S6k activation. Although the evidence that PKB mediates PI 3-kinase effects on p70S6k is compelling, these data do not exclude the possibility that there are PKB-independent mechanisms for p70S6k activation of T cells. In this context, the present data show that activation of PKC by phorbol esters stimulates p70S6k without any discernible stimulatory effect on PKB.

Recent studies showing that cytokine activation of serine kinases is important for the regulation of apoptosis (65, 66) have focused attention on cytokine-induced serine kinase cascades. PI 3-kinase and PKB have been implicated in the prevention of apoptosis in other cell systems (67, 68). The present study demonstrates that PI 3-kinase can couple the IL-2R to a selective subset of serine/threonine kinase pathways in T cells, and in this respect, the PI 3-kinase/PKB link is intriguing, since PKB mediates activation of the Frap/p70S6k pathway but may also regulate other kinase cascades that bifurcate from the PKB/p70S6k pathway including glycogen synthase kinase-3 (GSK3) signaling pathways (69). Therefore, PI 3-kinase and/or PKB have the potential for pleiotropic functions in T cells, and their downstream effectors may include additional serine/threonine kinases evoked by IL-2R engagement.

Finally, PI 3-kinase is activated by members of the cytokine receptor family such as the IL-2R, the IL-4 receptor, the IL-7 receptor, and the IL-13 receptor. Signaling pathways regulated by PI 3-kinase can hence have an impact on lymphocyte biology at multiple points. Accordingly, it is important to establish the function of this enzyme in lymphoid cells. The IL-2R is a prototypical member of this hematopoietin receptor family. The present results directly define PI 3-kinase function in T cells and position PKB for the first time in a physiologically relevant cytokine-induced signal transduction pathway in lymphoid cells. The model described herein may also be applicable to serine/threonine kinase pathways regulated by other receptors that activate PI 3-kinase in T cells.

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Note Added in Proof—Recently, Alessi and colleagues described the purification of two upstream kinases that are likely to mediate PKB activation. The activity of at least one of these upstream kinases, PKD1, is regulated by binding D-3 polyphosphate phosphoinositides (Alessi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R. J., Reese, C. B., and Cohen, P. (1997) Curr. Biol. 7, 261–269).

REFERENCES
1. Nelson, B., Lord, J., and Greenberg, P. (1994) Nature 369, 333–338
2. Nakamura, Y., Russell, S., Mess, S., Friedmann, M., Erdoes, M., Francis, C., Jacques, Y., Adelstein, S., and Leonard, W. (1994) Nature 369, 330–333
3. Smith, K. A. (1988) Science 240, 1169–1176
4. Taniguchi, T. and Ihle, J. (1995) Science 268, 251–253
5. Miyazaki, T., Kawahara, A., Fujii, H., Nakagawa, Y., Minami, Y., Liu, Z.-J., Oishi, I., Silvennoinen, O., Wittthuhn, B., Ible, J., and Taniguchi, T. (1994) Science 266, 1045–1047
6. Russell, S., Johnston, J., Noguchi, M., Kawamura, M., Bacon, C., Friedmann, M., Berg, M., McVicar, D., Wittthuhn, B., Silvennoinen, O., Goldman, A., Schmalstieg, F., Ible, I., O'Shea, J., and Leonard, W. (1994) Science 266, 1042–1045
7. Beadling, C., Guschin, D., Wittthuhn, B. A., Ziemiecki, A., Ible, J. N., Kerr, I. M., and Cantrell, D. A. (1994) EMBO J. 13, 5605–5615
8. Izquierdo, M., Downward, J., Leonard, W. J., Otani, H., and Cantrell, D. A.
