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Interleukin 2 induces tyrosine phosphorylation and activation of p72–74 Raf-1 kinase in a T-cell line

(lymphokine/serine/threonine kinase/protooncogene)

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ABSTRACT **Interleukin 2 (IL-2) is a lymphokine, produced by T cells upon antigenic or mitogenic stimulation, that is a critical regulator of T-cell proliferation. Although the binding of IL-2 to its receptor has been well characterized, the molecular mechanisms by which IL-2 transmits its signal from the membrane to the interior of the cell are poorly understood. Like most other growth factors, IL-2 causes rapid phosphorylation of proteins within its target cells. Unlike many other growth factors, however, the known subunits of the IL-2 receptor lack tyrosine-specific kinase activity, and little is known about the kinases whose activities are regulated by IL-2. Here we show that IL-2 (but not IL-4) induces rapid phosphorylation of the p72–74 serine/threonine-specific kinase encoded by the c-*Raf-1* protooncogene in an IL-2-dependent murine T-cell line, CTLL-2, and that this phosphorylation is associated with increased kinase activity in p72–74 Raf-1-containing immune complexes. The concentration dependence of IL-2-mediated elevations in Raf-1 kinase activity correlated well with IL-2-stimulated proliferation of CTLL-2 cells. Furthermore, much of the IL-2-stimulated phorphorylation of p72–74 Raf-1 occurred on tyrosines. To our knowledge, the Raf-1 kinase represents the first endogenous substrate of an IL-2-regulated tyrosine kinase to be identified.**

Interleukin 2 (IL-2) is thought to be a principal regulator of *in vivo* immune responses. Despite extensive biochemical characterization of the p50–55 (α) and p70–75 (β) chains of the IL-2 receptor, molecular cloning and sequencing of their genes, and the reconstitution of high-affinity receptors in heterologous systems through gene transfer (1–5), few details are available regarding the mechanism by which the IL-2 signal is transmitted from the surface of the cell to its interior. Unlike many other growth factor receptors that possess ligand-dependent, tyrosine-specific kinase activity, neither the α chain nor the β chain of the IL-2 receptor appears to have intrinsic kinase activity (4, 6, 7). Also, none of the known second messenger pathways that regulate kinase activity through the production of cyclic nucleotides or elevations in cytosolic Ca^{2+} appear to be directly involved in IL-2 signal transduction (for reviews, see refs. 8 and 9). And yet, both tyrosine and serine/threonine phosphorylation of intracellular proteins are rapid events in IL-2-stimulated T cells (10–14). Clearly, therefore, IL-2 and its receptor must regulate the activity of kinases in lymphocytes, but it has remained obscure as to which ones are involved.

Here, we demonstrate that IL-2 specifically induces the phosphorylation and activation, in an IL-2-dependent T-cell clone, CTLL-2, of the p72–74 kinase encoded by the c-*Raf-1* protooncogene. This serine/threonine-specific kinase has recently been implicated in signal-transduction events mediated by several tyrosine kinase growth factor receptors and oncogene products (15, 16). Gene transfer experiments using v-*raf* have previously suggested a role for the Raf-1 kinase in the regulation of mitogenesis and malignant transformation in several types of cells, including IL-2-dependent T lymphocytes (17, 18). The findings presented here strongly suggest that Raf-1 is an important participant in IL-2-mediated signal transduction events leading to cellular proliferation. Furthermore, because the Raf-1 kinase isolated from IL-2-stimulated T cells is heavily phosphorylated on tyrosine, studies of Raf-1 may serve as a useful starting point for eventually linking this kinase to upstream protein tyrosine kinases whose activities are controlled directly by the IL-2 receptor.

MATERIALS AND METHODS

Cell Cultures and Stimulations. The murine cell line CTLL-2 is an IL-2-dependent cytolytic T-cell line (19). Experiments with CTLL-2 cells were initiated 3 days after plating, when the cells had reached plateau phase of growth and had consumed most of the IL-2 in cultures. Cells were washed with RPMI 1640 medium three times to remove growth factor and incubated at 37°C for 5–7 hr before stimulating with IL-2, IL-4, or 12-*O*-tetradecanoylphorbol 13-acetate (TPA; phorbol 12-myristate 13-acetate). Purified recombinant IL-2 (rIL-2) was provided by Cetus (20). Culture supernants from a HeLa cell line transfected with a murine IL-4 cDNA (H28 cell line) were employed as a source of IL-4 (21). Proliferation of these T cells in response to stimulation was assessed by measuring relative levels of DNA synthesis ([^{3}H]thymidine incorporation) as described (22).

Immunoprecipitation and Immunoblotting. CTLL-2 cells ($5–10 \times 10^6$) were washed three times with PO_4-free RPMI, incubated in PO_4-free RPMI containing 1% dialyzed serum for 1 hr, and then labeled with $^{32}PO_4$ (0.5–1 mCi/5 ml; 1 Ci = 37 GBq) for 5–6 hr prior to stimulation with IL-2. Labeled cells were then washed twice with ice-cold phosphate-buffered saline and lysed in 1.0–1.5 ml of modified RIPA lysis buffer [0.1% Triton X-100, 0.5–1% sodium deoxycholate, 0.1% SDS, 50 mM Tris (pH 7.5), 150 mM NaCl, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, aprotinin (0.23 units/ml), 10 μM leupeptin, and 1 mM sodium orthovanadate] at 4°C for 20 min. Immunoprecipitations were then performed as described (15, 16, 23) with several different preparations of anti-SP63 RAF peptide rabbit antiserum (23), the anti-v-Raf murine monoclonal antibody URP30S3 (24), and the anti-phosphotyrosine monoclonal antibody MA2G8 (25). For immunoblots, immunoprecipitated proteins (prepared from unlabeled CTLL-2 cells) were transferred from gels to nitrocellulose filters. Blots were then incubated with anti-Raf antibodies (diluted 1:500, vol/vol) or an anti-phosphotyrosine monoclonal antibody SB72 (26) (diluted 1:50, vol/vol)

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Abbreviations: IL, interleukin; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; rIL-2, recombinant IL-2.

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followed by ^{125}I-labeled protein A (0.1–0.5 μCi/ml; no. IM.144; Amersham). SB72 antibodies (gift of J. Brugge, Univ. of Pennsylvania) were prepared using phosphotyrosine conjugated to keyhole limpet hemocyanin for the antigen and were affinity purified on a phosphotyrosine-Sepharose column (27, 28).

***In Vitro* Raf Kinase Assays.** The *in vitro* kinase activity of immunoprecipitated p72–74 Raf was measured essentially as described by Morrison *et al.* (16), except that a different peptide substrate (IVQQFGFQRASDDGKLTD) was used. Reactions were terminated by spotting onto Whatman P81 phosphocellulose paper. Dried filters were washed extensively in 1% phosphoric acid prior to Cerenkov counting.

Phospho Amino Acid Analysis. ^{32}P-labeled Raf-1 proteins were immunoprecipitated from four or five replicate samples, electrophoresed in 7.5% SDS/PAGE gels, recovered from gel slices, acid hydrolyzed, and subjected to phospho amino acid analysis by the method of Hunter and colleagues (29). Phospho amino acid standards were included in samples and visualized with ninhydrin.

RESULTS

IL-2 Induces Phosphorylation of the c-Raf-1 Kinase. We examined the effects of IL-2 on the Raf-1 kinase in CTLL-2 cells, contrasting this lymphokine with the phorbol ester TPA and another T-cell growth factor, IL-4. Previous studies in fibroblasts had demonstrated that TPA and some growth factors induce rapid phosphorylation of the p72 Raf-1 protein, resulting in an increase in its apparent molecular mass to 74 kDa in SDS/PAGE gels (15). We therefore labeled CTLL-2 cells with $^{32}PO_4$ and then stimulated these T cells with IL-2, IL-4, or TPA, prior to immunoprecipitation of Raf-1 protein and SDS/PAGE analysis. The anti-RAF antisera used for these experiments were prepared against a synthetic Raf peptide (peptide SP63) corresponding to the last 12 amino acids (315–326) of the p74 c-Raf protein (23). Thus, addition of competing peptide SP63 to some of the cellular lysates prior to immunoprecipitation provided a specificity control.

As shown in Fig. 1, stimulation of CTLL-2 cells for 15 min with IL-2 induced increased phosphorylation and retarded mobility of the Raf-1 protein. In contrast, IL-4 produced no detectable alteration in p72 Raf-1 (Fig. 1), whereas TPA induced only a minor shift in Raf-1 migration. Cerenkov counting of gel slices corresponding to the p72–74 Raf-1 band demonstrated a 5- to 10-fold increase in the $^{32}PO_4$ content of the Raf-1 protein isolated from IL-2-stimulated cells. The IL-2-induced increase in the intensity of the p74 Raf band seen with $^{32}PO_4$-labeled CTLL-2 cells was not attributable to increased synthesis of Raf-1 protein, since immunoblot analysis of Raf immunoprecipitates derived from unlabeled cells demonstrated no IL-2-mediated alterations in the levels of Raf-1 proteins. Furthermore, treatment of Raf immunoprecipitates with calf alkaline phosphatase demonstrated that the retarded gel mobility of c-Raf-1 proteins isolated from IL-2-stimulated CTLL-2 cells was due primarily to increased phosphorylation (data not shown).

IL-2 Stimulation Results in Elevated Raf-1 Kinase Activity. To determine whether phosphorylation of the Raf-1 protein affects its serine/threonine-specific kinase activity, we employed an *in vitro* kinase assay that uses a synthetic peptide (IVQQFGFQRASDDGKLTD) as a substrate. This peptide corresponds to a potential autophosphorylation site in the Raf-1 kinase (U.R., unpublished results), with the exception that amino acid number 7 has been changed from tyrosine to phenylalanine, thus obviating potential problems with promiscuous tyrosine kinase contaminants. Data were normalized with respect to kinase assays performed with a control peptide containing an alanine substitution, which removed the serine phosphate acceptor (IVQQFGFQRAADDGKLTD). As shown in Table 1, IL-2 produced a 5- to 10-fold increase in the activity of Raf-1 kinase as measured by this assay. In contrast, TPA treatment resulted in only a 3- to 5-fold increase. Thus, IL-2-induced phosphorylation of p72 Raf-1 was associated with increased kinase activity in Raf-containing immunoprecipitates. As shown in Fig. 1, the relative increases in Raf-1 kinase activity detected by this kinase assay generally correlated well with the increases in Raf-1 phosphorylation seen in SDS/PAGE analyses (Fig. 1). Note that IL-4 induced no elevations in Raf-1 kinase activity, in parallel with the phosphorylation results (Fig. 1). Similar results were obtained when histone (H1) was substituted for synthetic peptide in kinase assays (data not shown).

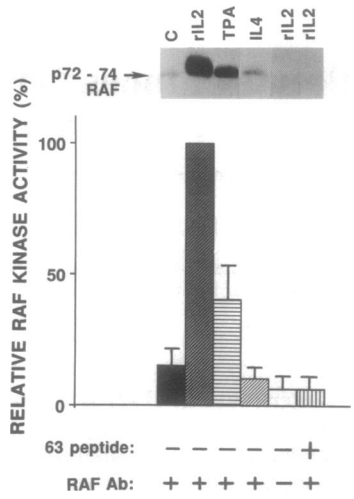

FIG. 1. IL-2-induced phosphorylation and activation of Raf-1 kinase. CTLL-2 cells were stimulated for ≈10 min with IL-2 (100 units/ml), TPA (20 ng/ml), or IL-4 (20% vol/vol) prior to performing immunoprecipitation analysis (*Upper*) or *in vitro* kinase assays (*Lower*) with anti-Raf-1 antisera. In some cases competing Raf peptide SP63 was added to cellular lysates before addition of anti-Raf antibodies. Unstimulated, quiescent CTLL-2 cells (C) are shown as a control. (*Upper*) For SDS/PAGE analysis, CTLL-2 cells were labeled with $^{32}PO_4$ prior to stimulation, and the immunoprecipitated $^{32}PO_4$-labeled proteins were size-fractionated by electrophoresis in 7.5% gels before exposure to x-ray film at −70°C. (*Lower*) For kinase assays, Raf-1 was immunoprecipitated from unlabeled CTLL-2 cells. Levels of Raf-1 kinase activity were calculated as a percentage relative to the IL-2-induced response (arbitrarily set at 100%). Data shown represent the mean ± SE for three experiments.

Time Course and Concentration Dependence of IL-2-Induced Phosphorylation and Activation of Raf-1 Kinase. The kinetics of IL-2-induced phosphorylation and elevated activity of the Raf-1 kinase were next investigated. On the basis of results of several experiments (Fig. 2 and data not shown), maximal phosphorylation of p72 Raf-1 appeared to occur within 5–15 min and declined to baseline within 2 hr after exposure of T cells to IL-2 (Fig. 2 *A* and *B*). The time course of IL-2-induced increases in Raf-1 kinase activity paralleled the phosphorylation results, with maximal activity occurring within 15 min and declining to baseline within 1–2 hr after exposure of T cells to IL-2 (Fig. 2*C*). These kinetics are similar to that reported previously for platelet-derived growth factor-stimulated fibroblasts (15, 16).

We also examined the concentration dependence of IL-2-induced elevations in Raf-1 kinase activity and correlated this with IL-2-stimulated proliferation of CTLL-2 cells. As shown in Fig. 3*A*, IL-2 stimulated elevations in the activity of Raf-1 kinase in a concentration-dependent manner. Half-maximal levels of Raf-1 kinase activity were induced by purified rIL-2 at ≈1 unit/ml, and maximal levels were inducted by rIL-2 at ≈15–30 units/ml. As shown in Fig. 3*B*, these IL-2-induced increases in Raf-1 kinase activity correlated well with in-

Table 1. *In vitro* kinase assays of Raf-containing immunoprecipitates

Stimulation		Peptides			Raf kinase activity		
					Total	Corr.	Rel.
IL-2	TPA	SP63	Ser^+	Ser^-	cpm	cpm	cpm
			Experiment 1				
−	−	−	+	−	11,328	4,335	1.0
−	+	−	+	−	19,472	12,479	2.9
+	−	−	+	−	28,498	21,505	5.0
+	−	−	−	+	6,993	0	—
			Experiment 2				
−	−	−	+	−	8,018	1,448	1.0
+	−	−	+	−	22,409	15,839	10.9
+	−	−	−	+	6,570	0	—
			Experiment 3				
−	−	−	+	−	26,950	2,970	1.0
−	+	−	+	−	39,502	15,522	5.2
+	−	−	+	−	46,202	22,222	7.5
+	−	+	+	−	23,980	0	—

CTLL-2 cells were lysed ≈10 min after stimulation with rIL-2 (100 units/ml) or TPA (20 ng/ml). Immunoprecipitations were performed with *raf*-1 antiserum in the presence or absence of competing peptide SP63. Immunoprecipitates were then incubated with either a *raf* substrate peptide (Ser^+) (IVQQFGFQRASDDGKLTD) or a peptide lacking serine (Ser^-) (IVQQFGFQRAADDGKLTD) in an *in vitro* kinase assay, and the phosphorylated substrate peptides were collected on phosphocellulose filters and quantified by Cherenkov counting. Corrected cpm were obtained by subtraction of values resulting from kinase assays performed with the serine-deficient peptide substrate (Ser^-) or with immunoprecipitates performed with SP63 competing peptide. Preliminary experiments verified that the substrate peptide was present in excess when used at 100 μM and demonstrated that phosphorylation of this peptide was linear through 20 min in kinase reactions performed at 30°C (data not shown). Corr., corrected; Rel., relative.

creased T-cell proliferation (measured by [^{3}H]thymidine incorporation). Fig. 3*B* also shows the effects of IL-4 and TPA on CTLL-2 cell proliferation, demonstrating that both of these agonists stimulated significant proliferation of these T cells. Comparison of the relative effects of IL-2 and TPA on Raf-1 kinase activity and CTLL-2 cellular proliferation (see Figs. 1–3 and Table 1) revealed a close correlation between regulation of the kinase and stimulation of DNA synthesis in these T cells. These data thus suggest a role for Raf-1 kinase in the control of T-cell proliferation. Our findings with IL-4, however, indicate that there may also be Raf-1 kinase-independent pathways that are sufficient for at least modest growth, since this lymphokine failed to induce phosphorylation or increased activity of Raf-1 yet did stimulate some CTLL-2 proliferation.

IL-2 Induces Tyrosine and Serine Phosphorylation of Raf-1 Kinase. We initially explored the question of tyrosine phosphorylation of Raf-1 in IL-2-stimulated T cells by immunoblot analysis using anti-phosphotyrosine antibodies. For these experiments, Raf-1 was immunoprecipitated from unlabeled CTLL-2 cells at various times after stimulation with IL-2. The resulting immunoprecipitated proteins were then split into two samples, size-fractionated in parallel in the same SDS/PAGE gels, and transferred to nitrocellulose filters. These Western blots were then incubated either with anti-Raf antibodies or with an anti-phosphotyrosine antibody (SB72). As shown in Fig. 4*A*, IL-2 induced increases in the tyrosine phosphorylation of p72–74 Raf-1 that were detectable within 2 min and maximal at 5–20 min after stimulation. Probing replicate protein blots with anti-Raf-1 antibody (Fig. 4*B*) verified that the increased signal detected with anti-

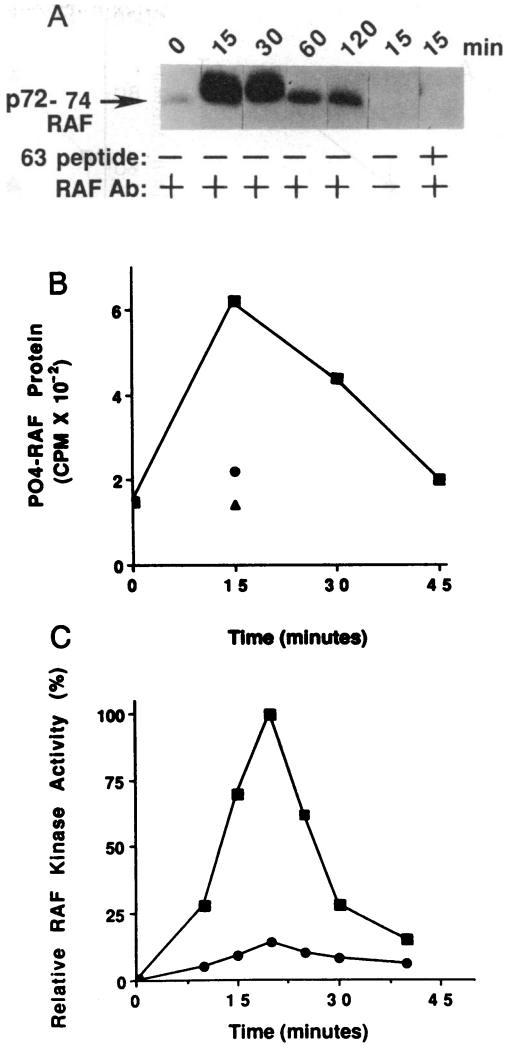

FIG. 2. Time course of IL-2-induced phosphorylation and activation of Raf-1 kinase. CTLL-2 cells were stimulated with rIL-2 (100 units/ml) for 0, 15, 30, 60, or 120 min prior to lysing cells and performing Raf immunoprecipitation analysis from $^{32}PO_4$-labeled cells for SDS/PAGE analysis (*A* and *B*) or from unlabeled cells for kinase assays (*C*). (*B*) Relative levels of Raf-1 protein phosphorylation were quantified by Cerenkov counting of the Raf-containing bands excised from the SDS/PAGE gels. Results for CTLL-2 cells stimulated with rIL-2 (■), IL-4 (▲), and TPA (●) are shown. (*C*) Kinase assays were performed with either a substrate peptide containing serine (■) or a serine-deficient peptide (●). The percentage of Raf-1 kinase activity was calculated relative to the maximum IL-2-induced response (mean of three experiments).

phosphotyrosine antibody was not due to loading of more Raf-1 protein for the later time points.

Anti-phosphotyrosine antibodies were also employed for immunoprecipitation of Raf-1 protein. For these experiments, phosphotyrosine-containing proteins were immunoprecipitated from unlabeled CTLL-2 cells before and after stimulation with IL-2, using the anti-phosphotyrosine monoclonal antibody MA2G8. The anti-phosphotyrosine precleared lysates were then immunoprecipitated with anti-Raf antibody to recover non-tyrosine-phosphorylated Raf-1 proteins. Anti-Raf- and anti-phosphotyrosine-immunoprecipitated proteins were then size-fractionated side-by-side in SDS/PAGE gels and analyzed by immunoblotting using anti-Raf antibodies. As shown in Fig. 5, before stimulation with IL-2, no Raf-1 protein was immunoprecipitated by the anti-phosphotyrosine antibody (lane b), and all of the Raf-1 molecules appeared to be in the nonphosphotyrosine fraction (lane d). After stimulation

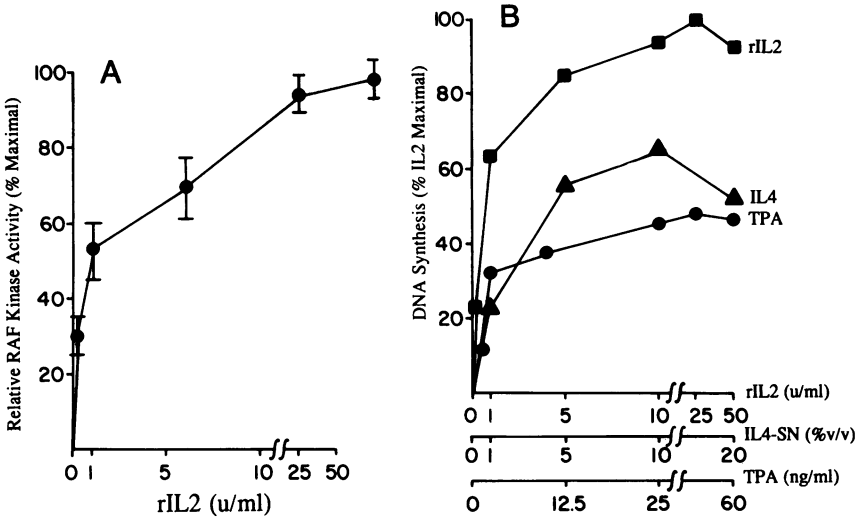

FIG. 3. Comparison of IL-2 concentration dependence of Raf-1 kinase activation and cellular proliferation. (*A*) CTLL-2 cells were stimulated with various concentrations of rIL-2 for 10 min, and Raf-1 kinase activity was determined as described for Table 1 (mean ± SE for at least two experiments). (*B*) Cellular proliferation in response to rIL-2 (■), IL-4-containing supernatants (IL4-SN) (▲), and TPA (●) was determined by measuring relative levels of DNA synthesis ([^{3}H]thymidine incorporation). Approximately 5×10^3 cells in 100 μl of medium were cultured in triplicate in 96-well flat-bottom plates. After 20 hr of culture, 1 μCi of [^{3}H]thymidine was added to each well, and the cells were harvested 8 hr later onto glass filters. Data represent the mean of triplicate determinations and are expressed as a percentage of the maximum IL-2-induced response (SE were <12% for all data).

with IL-2, in contrast, about 20–50% (depending on the experiment) of the immunologically detectable Raf-1 protein was now immunoprecipitated by the anti-phosphotyrosine antibody (Fig. 5, lane a). Recognizing the limitations of this approach employing anti-phosphotyrosine antibodies for immunoprecipitations (such as incomplete recovery of tyrosine-phosphorylated proteins and the conformation-dependence of binding of anti-phosphotyrosine antibodies to some target proteins), we conclude that IL-2 induces increased levels of phosphotyrosine on a significant fraction of the Raf-1 molecules in CTLL-2 cells.

Finally, two-dimensional phospho amino acid analysis of gel-purified, radiolabeled Raf-1 protein biochemically confirmed these immunoblot results and demonstrated that IL-2 induces both tyrosine and serine phosphorylation of p72–74 c-Raf-1. As shown in Fig. 6, the induction of phosphorylation of these two amino acids occurred with similar time courses in IL-2-stimulated cells. In contrast to IL-2, stimulation of CTLL-2 cells with TPA induced serine phosphorylation but little tyrosine phosphorylation of p72–74 Raf-1 (data not shown). No threonine phosphorylation was detected in Raf-1 from IL-2-stimulated CTLL-2 cells, consistent with results obtained for other growth factor-induced phosphorylations of this kinase (15, 16).

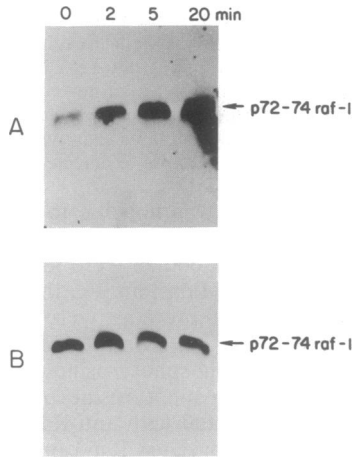

FIG. 4. Anti-phosphotyrosine immunoblot analysis of Raf-1 protein from IL-2-stimulated CTLL-2 cells. Raf-1 was immunoprecipitated from CTLL-2 cells at 0, 2, 5, and 20 min after stimulation with IL-2. Samples were then divided equally, subjected to SDS/PAGE, and transferred to nitrocellulose filters. Blots were incubated with either anti-phosphotyrosine (*A*) or anti-Raf-1 antibodies (*B*), followed by ^{125}I-labeled protein A.

DISCUSSION

Though IL-2 is known to induce the rapid tyrosine phosphorylation of several proteins in T cells (10–14, 26), heretofore the identity of any of these phosphoproteins has been unknown. Previous reports of tyrosine phosphorylation of the p70 β chain of the IL-2 receptor indicated that this was of low stoichiometry, and it was not demonstrated that the levels of p70 tyrosine phosphorylation varied with IL-2 stimulation (14, 30). In contrast, we found that tyrosine phosphorylation of p72–74 Raf was IL-2 inducible and appeared to occur with high stoichiometry (Figs. 4–6). To our knowledge, the Raf-1 kinase thus represents the first endogenous substrate of an IL-2-regulated tyrosine kinase to be identified. It remains to be determined whether tyrosine phosphorylation of p72–74 Raf-1 bears any relation to the tyrosine protein kinase activity recently detected in association with IL-2 receptor complexes (31).

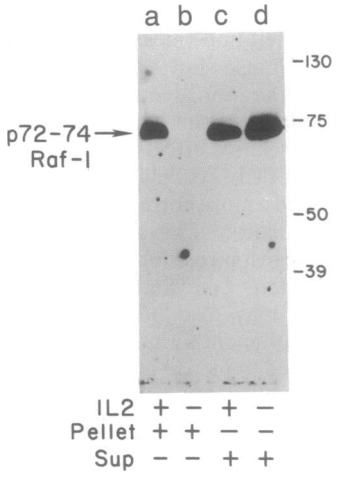

FIG. 5. Immunoprecipitation of Raf-1 from IL-2-stimulated cells using anti-phosphotyrosine antibody. CTLL-2 cells were lysed before (lanes b and d) or after (lanes a and c) stimulation for 10 min with IL-2. Anti-phosphotyrosine antibody was first added to lysates, and the resultant antigen–antibody complexes were collected with protein A-Sepharose (Pellet). Anti-Raf-1 antibody was subsequently added to the precleared cellular lysates, and these immune complexes were similarly recovered with protein A-Sepharose (Sup). Phosphotyrosine-containing proteins were released with 10 mM phenylphosphate [subsequent boiling in Laemmli buffer confirmed complete release of Raf-1 protein (data not shown)], whereas Raf-1 was eluted from immune complexes by boiling prior to 7.5% SDS/PAGE and subsequent transfer to nitrocellulose. Blots were incubated with anti-Raf-1 antibody followed by ^{125}I-labeled protein A. Molecular mass markers (in kilodaltons) are given at right.

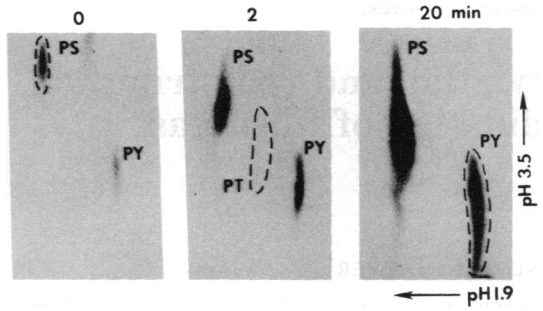

FIG. 6. Phospho amino acid analysis of p72–74 Raf-1 obtained from IL-2-stimulated CTLL-2 cells. $^{32}PO_4$-labeled Raf-1 was immunoprecipitated from CTLL-2 cells at 0, 2, or 20 min after IL-2 stimulation, gel-purified, and acid hydrolyzed for two-dimensional phospho amino acid analysis. The locations of ninhydrin-visualized phosphoserine (PS), phosphothreonine (PT), and phosphotyrosine (PY) standards are shown for the 0-, 2-, and 20-min determinations, respectively.

The specific induction of p72–74 Raf-1 phosphorylation by IL-2 but not by IL-4 in CTLL-2 cells (Fig. 1) is of interest, since the IL-2 receptor β chain and IL-4 receptor belong to the same family of hematopoietic growth factor receptors (32, 33). Sequence homologies for the IL-2 receptor β chain and IL-4 receptor, however, are confined largely to their extracellular ligand-binding domains and do not include the cytoplasmic intracellular portions of these transmembrane glycoproteins. In contrast, amino acid sequence homologies have been noted previously within the cytoplasmic domains of the receptors for IL-2 (p70 β chain), IL-3, IL-7, erythropoietin, and granulocyte/macrophage colony-stimulating factor (32, 33). Though the effects of IL-7 and erythropoietin on p72–74 Raf-1 have not been explored, IL-3 and granulocyte/macrophage colony-stimulating factor induced rapid tyrosine phosphorylation and activation of the Raf-1 kinase in myeloid cell lines (34). Thus, those hematopoietic growth factor receptors that have structural similarities in their cytoplasmic domains may make use of the same or similar kinases (such as Raf-1) to transduce and propagate their signals intracellularly. Given the recent demonstrations of ligand-inducible association of p72–74 Raf-1 with tyrosine kinase growth factor receptors, including those for platelet-derived growth factor and epidermal growth factor (16, 35), it is intriguing to speculate that Raf-1 kinase may interact directly either with hematopoietic growth factor receptors such as those for IL-2 and IL-3 or with tyrosine kinases that associate with these receptors. Thus, the Raf-1 kinase may serve as a useful starting point for working back toward the receptor to identify the relevant tyrosine kinases that initiated the cascades of protein phosphorylation responsible for mediating the intracellular actions of IL-2, IL-3, and other hematopoietic growth factors.

The finding of p72–74 Raf-1 in physical association with tyrosine kinase growth factor receptors for platelet-derived growth factor and epidermal growth factor also raises the possibility that the Raf-1 kinase functions proximally in the intracellular pathways of protein phosphorylation regulated by these growth factors. In this regard, gene transfer experiments using v-*raf*-containing retroviruses have demonstrated that an activated Raf kinase can act in concert with v-myc to abrogate the dependence of CTB6 T cells on IL-2 for long-term *in vitro* growth (36). This v-*raf*-assisted abrogation of growth factor requirements occurred through nonautocrine mechanisms. Though these studies utilizing viral *raf* genes may be only indirectly applicable to the normal Raf-1 kinase, when taken together with the findings presented here demonstrating IL-2-inducible activation of the Raf-1 kinase and its correlation with IL-2-stimulated proliferation of CTLL-2 cells, these previous gene transfer experiments imply that activation of the Raf-1 kinase contributes to the growth promoting effects of IL-2 on T cells.

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