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itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2

(protein-tyrosine kinases/T lymphocytes/lymphokines/lymphocyte activation)

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ABSTRACT **T lymphocytes are activated by interactions with antigens, lymphokines, and cell adhesion molecules. Tyrosine phosphorylation has been implicated as important in signaling through each of these pathways, but except for p56^{lck}, a member of the Src family that associates with CD4 and CD8, the protein-tyrosine kinases involved have not been defined. We describe here a tyrosine kinase gene that we have designated *itk* (for *I*L-2-inducible *T*-cell *k*inase). The *itk* gene specifies a 72-kDa protein-tyrosine kinase that is related to members of the Src family but lacks two features characteristic of Src kinases: an N-terminal myristoylation consensus sequence and a regulatory tyrosine residue near the C terminus. Analysis of mouse tissues and cell lines indicates that *itk* is specifically expressed in the T-cell lineage, suggesting that the tyrosine kinase encoded by *itk* functions in a signal transduction pathway unique to T lymphocytes. On addition of IL-2 to responsive T cells, *itk* RNA increases in parallel with that of IL-2Rα, implicating *itk* in T-cell activation.**

T-cell proliferation and differentiation are governed by several receptor–ligand interactions, of which the best understood are recognition of antigen–MHC complexes by the T-cell antigen receptor (TCR), engagement of the accessory molecules CD4 and CD8 by major histocompatibility complex (MHC) molecules, and binding of the mitogenic peptide interleukin 2 (IL-2) to intermediate- or high-affinity IL-2 receptors (IL-2Rs). Protein-tyrosine phosphorylation has been implicated in the signaling of each of these interactions, but except for the Src family member Lck, which is coupled to CD4 and CD8, the protein-tyrosine kinases involved have not been defined.

Signaling through the TCR is accompanied by a rapid rise in tyrosine phosphorylation of several proteins, including phospholipase C-γ, the TCR ζ chain, and a protein of about 70 kDa (ZAP-70) that is associated with the ζ chain (ref. 1 and references therein). Increases in phospholipid hydrolysis and intracellular Ca^{2+} that follow TCR engagement are blocked by agents that inhibit protein-tyrosine kinases, suggesting that protein-tyrosine phosphorylation is a prerequisite for these later events (2). The early consequences of TCR stimulation can be reconstituted in T cells by a chimeric protein containing the cytoplasmic portion of ζ (3), which associates physically with a tyrosine kinase(s) of unknown identity.

The high-affinity IL-2R is composed of three polypeptides, IL-2Rα, IL-2Rβ, and IL-2Rγ (ref. 4 and references therein). In lymphoid cells, a proliferative signal can be transduced in the absence of IL-2Rα (5), but all three polypeptides are apparently necessary for high-affinity binding to IL-2 (4). Resting T cells express IL-2Rβ but little or no IL-2Rα. In response to antigen or IL-2, expression of IL-2Rα is induced, with a concomitant increase in the density of high-affinity receptors at the cell surface (6). Two observations implicate tyrosine phosphorylation in signal transduction through the IL-2R: (*i*) within several minutes after binding of IL-2 to the high-affinity receptor or to IL-2Rβ, the tyrosine phosphorylation of several proteins, including IL-2Rβ itself, is increased (7); (*ii*) the IL-2R is associated with tyrosine kinase activity (8). This interaction is apparently noncovalent, as the IL-2R subunits do not carry tyrosine kinase consensus sequences. The tyrosine kinase Lck, a member of the Src family that is preferentially expressed in T lymphoid cells, can form a stable complex with IL-2Rβ (9). However, association with Lck is apparently not obligatory for receptor function, because a mutation in IL-2Rβ that disrupts its association with the 56-kDa *lck* gene product (p56^{lck}) has little or no effect on signaling in a pro-B-cell line (9).

We describe here the molecular cloning and characterization of a tyrosine kinase gene, *itk* (*I*L-2-inducible *T*-cell *k*inase).† The *itk* gene encodes a 72-kDa protein-tyrosine kinase (p72^{itk}) that is related to members of the Src family but lacks two features characteristic of Src kinases: an N-terminal myristoylation consensus sequence and a regulatory tyrosine residue near the C terminus. Analysis of mouse tissues and cell lines indicates that *itk* is specifically expressed in the T-cell lineage, suggesting that the tyrosine kinase encoded by *itk* functions in a signal transduction pathway unique to T lymphocytes. On addition of IL-2 to responsive T cells, *itk* RNA increases in parallel with that of IL-2Rα, implicating *itk* in T-cell activation.

MATERIALS AND METHODS

Cells and Cell Lines. The IL-2-dependent murine T-cell line CTLL-2 (10) was maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 μM 2-mercaptoethanol (RPMI-10), and 100 units of IL-2 per ml. Splenocytes were isolated from C57BL/6 female mice by centrifugation through Ficoll/Hypaque. T lymphocytes were lysed by treatment with a cocktail of antibodies to T-lymphocyte-specific antigens (anti-CD4, anti-CD8, anti-CD3, anti-Thy-1; gift of Drew Pardoll, Johns Hopkins University School of Medicine) and rabbit complement; lysed cells were removed by centrifugation through Ficoll/Hypaque. The efficacy of T-cell removal was assessed by flow cytometry.

Amplification of Protein-Tyrosine Kinase Sequences by PCR. Protein-tyrosine kinase sequences were amplified with four upstream oligonucleotide primers and one downstream primer. The sequences of the upstream primers are 5′-CC-AGC-GGC-CGC-GTN-CAY-CGN-GAY-CTN-GC-3′; 5′-CC-AGC-GGC-CGC-GTN-CAY-AGR-GAY-TTR-GC-3′,

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Abbreviations: TCR, T-cell antigen receptor; IL-2, interleukin 2; IL-2R, IL-2 receptor; MHC, major histocompatibility complex.

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†The nucleotide sequence of *itk* cDNA has been deposited in the GenBank data base (accession no. L00619).

5′-CC-AGC-GGC-CGC-GTN-CAY-CGN-GAY-TTR-GC-3′, and 5′-CC-AGC-GGC-CGC-GTN-CAY-AGR-GAY-CTN-GC-3′. Each upstream primer carries a *Not* I restriction site at its 5′ end, followed by codons specifying the amino acid sequence VHRDLA. The nucleotide sequence of the downstream primer is 5′-C-CAG-CGG-CCG-CCC-RAA-NSW-CCA-NAC-RTC-3′. This primer carries a *Not* I site at its 5′ end, followed by the reverse complement of codons specifying the amino acid sequence DVWSFG.

Poly(A)$^+$ RNA isolated from CTLL-2 cells maintained in medium with IL-2 was annealed to random hexanucleotides, and negative-strand cDNA was synthesized (cDNA Synthesis System Plus, Amersham). CTLL-2 cDNA was amplified in four reaction mixtures, each containing the downstream primer and one of the four upstream primers described above. Amplification mixtures (50 μl) contained 50 mM KCl, 10 mM Tris Cl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM each dNTP, 5 μM upstream primer, 5 μM downstream primer, 4 μl of cDNA synthesis product, and 2.5 units of *Taq* polymerase. Mixtures were incubated as follows: 1 cycle at 92°C for 2 min, 40°C for 2 min, 72°C for 2 min; 28 cycles at 92°C for 30 sec, 40°C for 2 min, 72°C for 2 min; and 1 cycle at 92°C for 30 sec, 40°C for 2 min, 72°C for 4 min. Products were purified by gel electrophoresis and cloned into the *Not* I site of pBluescript (Stratagene).

Antibodies. Antibody 679 was directed against a synthetic peptide (SDJS4) spanning amino acid residues 55–74 of p72^{itk}; antibody 680 was raised against a peptide (SDJS5) corresponding to residues 605–625. Coupling of peptides to carrier protein, immunizations, and purification of anti-p72^{itk} antibodies by adsorption to the SDJS4 or SDJS5 peptides were performed as described (11).

Immunoprecipitation and Kinase Assays. Fresh mouse thymocytes were lysed in RIPA buffer [150 mM NaCl/50 mM Tris Cl, pH 7.4/1% sodium deoxycholate/1% Nonidet P-40/0.1% SDS/10 mM NaF/1 mM Na_3VO_4/58 μM phenylmethanesulfonyl fluoride with aprotinin (10 μg/ml), leupeptin (10 μg/ml), pepstatin (1 μg/ml), antipain (1 μg/ml), and chymostatin (1 μg/ml)] (1 ml per 10^7 cells). Immunoprecipitation reaction mixtures contained 200 μl of lysate, 15 μg of affinity-purified antibody 679, and a 10- or 100-fold molar excess of homologous (SDJS4) or heterologous (SDJS5) peptide competitor. Antigen–antibody complexes were precipitated with protein A-Sepharose. To immunoprecipitates were added 40 μl of kinase buffer (20 mM Tris Cl, pH 7.4/10 mM $MgCl_2$/1 mM Na_3VO_4), 4 μl of 0.03% enolase, and 5 μCi of [γ-^{32}P]ATP (3000 Ci/mmol; 1 Ci = 37 GBq). The mixtures were incubated for 25 min at room temperature and 5 min at 37°C. Products were fractionated by SDS/10% PAGE, transferred to nitrocellulose, and detected by autoradiography for 16 hr at −70°C with an intensifying screen. Phospho amino acid analysis of kinase reaction products was performed as described (12). The thin-layer plate was exposed to a BaFBr/Eu^{2+} storage phosphor screen for 21 hr and the screen was scanned with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS AND DISCUSSION

Identification of *itk* and Other Protein-Tyrosine Kinase Genes by Selective Amplification of CTLL-2 cDNA. Degenerate oligonucleotide primers were used in the PCR to amplify tyrosine kinase coding sequences expressed in the IL-2-responsive T-cell line CTLL-2 (10). The primers were designed to include all known tyrosine kinase genes except for members of the *src* family, which were excluded. Amplified products were molecularly cloned and the nucleotide sequences of 67 clones were determined. These were found to represent 10 different cDNAs, and 6 appeared, on the basis of sequence, to represent protein-tyrosine kinases not found in the GenBank data base (January 1992) (J.D.S. and S.V.D., unpublished data).

One of these six cDNA clones represented a gene that we have called *itk*. To obtain a complete cDNA for *itk*, a mouse thymocyte cDNA library was screened with the partial clone that was obtained by PCR. Three overlapping cDNA clones (52-2.1, 52-15.2, and 52-4.1) defined a DNA sequence of 4295 base pairs (bp), including the flanking *Eco*RI restriction sites and a poly(A) tract of 22 residues (data not shown; GenBank accession no. L00619). An open reading frame of 625 codons extends from a Met codon at nucleotides 93–95 to a stop codon at 1968–1970. The Met codon at 93–95 occurs in a context favorable for translation (13). *In vitro* transcription and translation of *itk* yielded a 72-kDa polypeptide, as predicted by the nucleotide sequence; no polypeptides of comparable size were detected in reactions directed by antisense transcripts (data not shown). The 3′ untranslated region is 2297 bp long, exclusive of the poly(A) sequence, and contains four copies of the sequence ATTTA, which has been shown to destabilize the mRNAs of several lymphokines, cytokines, and protooncogenes (14).

The protein encoded by *itk* (p72^{itk}) exhibits the hallmarks of protein kinases and contains two sequence motifs, LAAR (residues 488–491) and PVKW (residues 526–529) that distinguish protein-tyrosine kinases from protein-serine/threonine kinases (15) (Fig. 1). The closest known relative of p72^{itk} is encoded by Dsrc29A, a gene in *Drosophila melanogaster* that was first identified on the basis of its homology to c-*src* (18, 19). All three proteins contain the Src homology regions SH3 and SH2 (residues 182–231 and 233–352 of p72^{itk}, respectively) and share the conserved tyrosine residue (Tyr^{517} of p72^{itk}) that is a site of autophosphorylation (Tyr^{416}) in p60$^{c\text{-}src}$ (15). The greatest similarity among the three proteins is found in the catalytic region, where p72^{itk} is 53% identical to p65^{Dsrc29A} and 39% identical to p60$^{c\text{-}src}$ (Fig. 1). Despite these similarities,

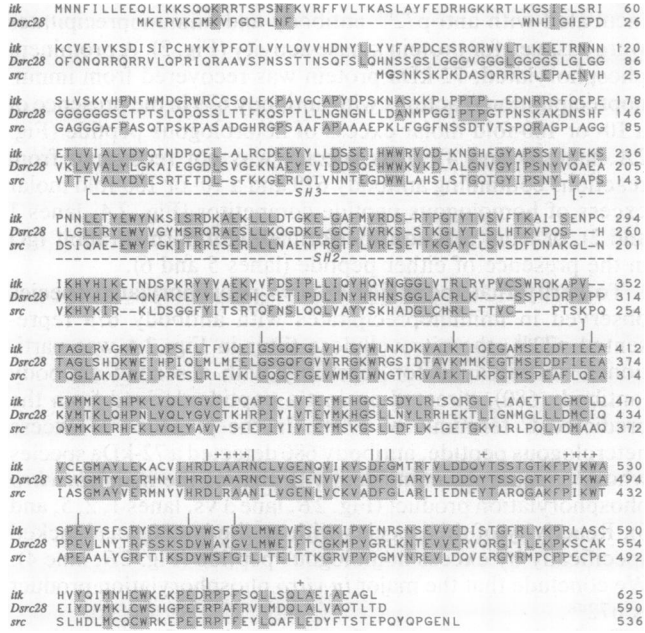

FIG. 1. The *itk* gene encodes a 72-kDa protein with homology to protein-tyrosine kinases. Conceptual translation of the single long open reading frame of *itk* is shown in single-letter amino acid code. Sequence identities between Dsrc29A (19) and *src* (17) are shaded. Amino acids that are conserved among protein kinases are indicated by vertical lines. Residues that distinguish tyrosine kinases from serine/threonine kinases are marked (+). SH2 and SH3 Src homology domains are indicated by dashed underlines. Amino acids are numbered at right. Dashes have been placed within sequences to maximize identity.

p72^{itk}, like p65^{Dsrc29A}, differs from p60$^{c\text{-}src}$ and other members of the Src family in several ways. First, the proteins of the Src family contain a unique N-terminal region of 50–90 amino acid residues. The N-terminal sequence unique to p72^{itk} is much longer (181 residues) and lacks the N-terminal consensus myristoylation sequence found in all Src family kinases. In particular, p72^{itk} lacks Gly at position 2, which is absolutely required for myristoylation (20). Second, p72^{itk} lacks a tyrosine residue near the C terminus (Tyr^{527} of p60$^{c\text{-}src}$) that is conserved among kinases of the Src family. In Src kinases, phosphorylation of this residue is correlated with decreased enzymatic activity; mutations that convert this Tyr to Phe confer increased kinase activity and the ability to transform fibroblasts (21). Third, all Src kinases carry the sequence HRDLRAAN (residues 384–391 of p60$^{c\text{-}src}$); all other protein-tyrosine kinases have HRDLAARN at the analogous position (15). p72^{itk} conforms to the latter pattern. These characteristics suggest that p72^{itk} may differ from kinases more closely related to p60$^{c\text{-}src}$ with respect to its intracellular localization and regulation. The *csk* gene (22) encodes a putative Src-kinase kinase that, like the Itk and Dsrc29A kinases, lacks a myristoylation signal and a putative regulatory tyrosine residue near its C terminus. The Csk kinase differs, however, from the Itk and Dsrc29A kinases in that it also lacks the putative autophosphorylation site, which is retained in Itk and Dsrc29A. The *syk* gene (23), like *itk*, encodes a 72-kDa non-receptor protein-tyrosine kinase that is preferentially expressed in lymphoid tissues. p72^{itk} differs markedly, however, from the kinase encoded by *syk*, which lacks an SH3 region and contains a duplication of the SH2 region.

Immunoprecipitation of p72^{itk} from Thymus and Tyrosine Phosphorylation *in Vitro*. To detect p72^{itk} in normal T-lineage cells and to demonstrate an association between this protein and kinase activity, polyclonal antibody (antibody 679) was raised against a peptide derived from the predicted unique region of p72^{itk}. Normal murine thymocyte extracts were incubated with anti-p72^{itk} antibody and immunoprecipitates were assayed for protein kinase activity (Fig. 2). A prominent phosphorylated, 72-kDa protein was recovered from immunoprecipitation reactions with antibody 679 in the presence of a 10- or 100-fold molar excess of heterologous peptide (Fig. 2*A*, lanes 3 and 4). This species was not recovered from reactions containing antibody 679 and a 10- or 100-fold molar excess of homologous peptide competitor (Fig. 2*A*, lanes 1 and 2), or from reactions performed with normal rabbit IgG in the presence of either peptide (lanes 5 and 6).

To verify that the major *in vitro* phosphorylated species observed in immunoprecipitates with antibody 679 represented p72^{itk}, the nitrocellulose filter in Fig. 2*A* was partitioned and assayed for reactivity with a second antibody (antibody 680), directed against a peptide derived from the predicted C terminus of p72^{itk}. In the presence of excess heterologous peptide, antibody 680 detected a 72-kDa species whose appearance coincided with that of the major *in vitro* phosphorylation product (Fig. 2*B*, lane 3 vs. lanes 1, 2, 5, and 6). Reactivity of this species with antibody 680 was blocked specifically by excess homologous peptide (Fig. 2*B*, lane 4). We conclude that the major *in vitro* phosphorylation product is p72^{itk}.

To determine whether phosphorylation of p72^{itk} had occurred on tyrosine, immunoprecipitations were carried out with anti-p72^{itk} antibody and *in vitro* kinase reactions were performed as above. Products were fractionated by electrophoresis and transferred to a poly(vinylidene difluoride) filter. The region of the filter containing phosphorylated p72^{itk} and the corresponding region in the control lane were excised for phospho amino acid analysis. Radiolabeled phosphotyrosine was recovered from p72^{itk} (Fig. 2*C*, lane 1), but not from the control sample (lane 2).

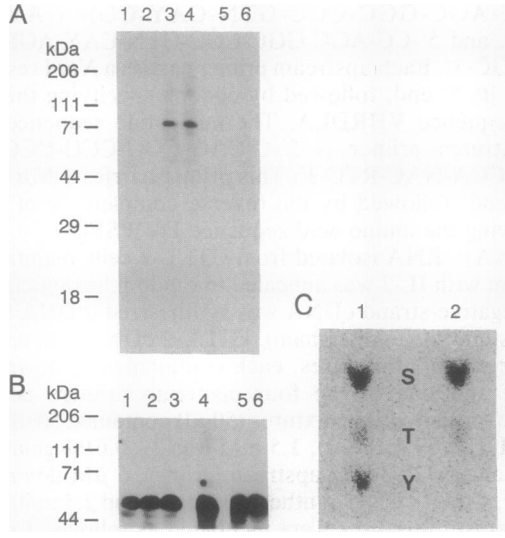

FIG. 2. Immunoprecipitation of p72^{itk} from thymus and phosphorylation *in vitro*. (*A*) Immunoprecipitation and *in vitro* kinase assay. Lanes 1–4, immunoprecipitation with 15 μg of antibody 679; lanes 5 and 6, immunoprecipitation with 15 μg of normal rabbit IgG (Southern Biotechnology Associates, Birmingham, AL). Immunoprecipitation reaction mixtures contained homologous (SDJS4) or heterologous (SDJS5) peptides as follows: a 10-fold (lane 1) or 100-fold (lanes 2 and 5) excess of homologous peptide (SDJS4) or a 10-fold (lane 3) or 100-fold (lanes 4 and 6), excess of heterologous peptide (SDJS5). (*B*) Identification of p72^{itk} in immunoprecipitates by reactivity with a second anti-p72^{itk} antibody. The nitrocellulose filter in *A* was partitioned and individual sets of lanes were assayed for reactivity to antibody 680, raised against a C-terminal p72^{itk} peptide (SDJS5). The enhanced chemiluminescence assay (Amersham) for antibody binding was performed with antibody 680 at 3 μg/ml and horseradish peroxidase-conjugated protein A at 167 ng/ml. Lanes 1–6 correspond to lanes 1–6 of *A*. Lane 4 was incubated in the presence of homologous peptide; all other lanes were incubated in the presence of heterologous peptide. The intense band at about 50 kDa in all lanes is the heavy chain of the immunoprecipitating antibody. (*C*) Phosphorylation of p72^{itk} on tyrosine. Immunoprecipitation was performed with anti-p72^{itk} antibody in the presence of heterologous (SDJS5) or homologous (SDJS4) competitor peptide, kinase reactions were performed as above, and products were fractionated by electrophoresis and transferred to a poly(vinylidene difluoride) filter. The region of the filter containing phosphorylated p72^{itk} and the corresponding region in the control lane were excised and subjected to phospho amino acid analysis. Lane 1, p72^{itk}; lane 2, control. Positions of phosphoserine (S), phosphothreonine (T), and phosphotyrosine (Y) markers are identified.

Taken together with the sequence data presented above, the simplest interpretation of this experiment is that p72^{itk} has intrinsic protein-tyrosine kinase activity and is capable of autophosphorylation. Because radiolabeled phosphoserine and phosphothreonine were recovered from both samples, the experiment does not resolve whether p72^{itk} is also associated with serine/threonine kinase activity. That p72^{itk} is itself a serine/threonine kinase seems unlikely, however, because of its lack of similarity to serine/threonine kinases and its homology to other protein-tyrosine kinases.

Selective Expression of *itk* in Normal T Cells and T-Cell Lines. A radiolabeled probe specific for *itk* was hybridized to RNA from the CTLL-2 cell line and from various murine tissues. The *itk* probe detected a 4-kilobase (kb) RNA expressed in CTLL-2 (Fig. 3, lane 9), thymus (Fig. 3, lane 1), and to a lesser extent in spleen (Fig. 3, lane 2; Fig. 4), but not in liver, kidney, gut, heart, or brain (Fig. 3, lanes 4–8). Upon prolonged exposure, *itk* RNA was also detectable in the sample from lung (Fig. 3, lane 3), probably because of contamination with hilar lymph nodes. The preferential expression of *itk* RNA in thymus and spleen suggested that this

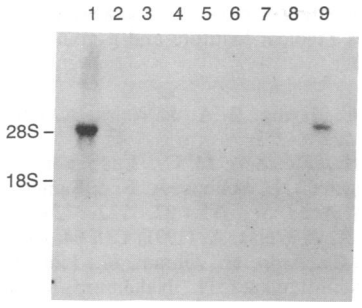

FIG. 3. Expression of *itk* in normal murine tissues. Total cellular RNA (20 μg per lane) was fractionated by electrophoresis, transferred to nitrocellulose, and assayed for hybridization to the ^{32}P-labeled *itk* probe obtained by PCR. Lane 1, thymus; lane 2, spleen; lane 3, lung; lane 4, liver; lane 5, kidney; lane 6, gut; lane 7, heart; lane 8, brain; lane 9, CTLL-2 cell line. Similar amounts of RNA were loaded in each lane as assessed by staining with ethidium bromide and hybridization to a β-tubulin probe. Positions of 28S and 18S RNA are indicated.

gene was expressed preferentially or exclusively in lymphoid cells. To determine whether *itk* was expressed in both T and B cells or in T cells alone, we first examined expression of *itk* in congenitally athymic (*nu/nu*) mice; such mice have 90–95% fewer T cells than normal controls (24). Steady-state levels of *itk* RNA in lymph nodes and spleen from *nu/nu* mice (Fig. 4*Aa*, lanes 3 and 4) were greatly diminished relative to levels in the same organs from C57BL/6 mice (Fig. 4*Aa*, lanes 1 and 2); RNA for TCR β chain showed a similar reduction in *nu/nu* mice (Fig. 4*Ab*). The samples yielded comparable levels of β-tubulin RNA (Fig. 4*Ac*). While this result suggested that *itk* was preferentially expressed in T cells, it remained possible that the low level of *itk* RNA seen in *nu/nu* mice was derived in part from non-T splenic

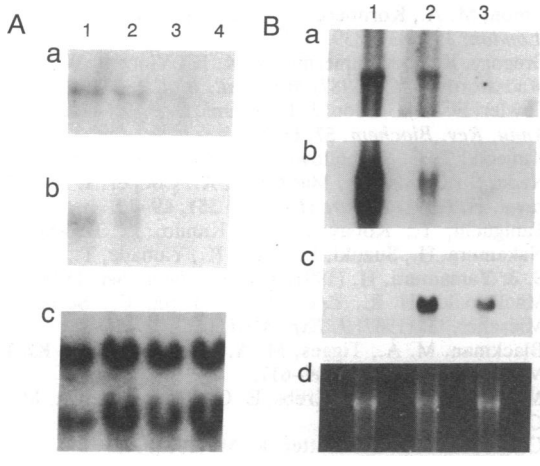

FIG. 4. Specific expression of *itk* RNA in T-lymphoid cells. (*A*) Reduced expression of *itk* in nude (*nu/nu*) mice. Total cellular RNA (20 μg) from lymph node (lane 1) and spleen (lane 2) of C57BL/6 mice and from lymph node (lane 3) and spleen (lane 4) of congenitally athymic (*nu/nu*) mice were fractionated by electrophoresis, transferred to nitrocellulose, and assayed for hybridization to a ^{32}P-labeled, 1.5-kb *Hin*dIII fragment from *itk* clone 52-2.1 (*a*). The same filter was hybridized to probes specific for TCR β-chain transcripts (*b*) and β-tubulin transcripts (*c*). (*B*) *itk* transcripts in unfractionated and T-cell-depleted splenocyte populations. Total RNA (17 μg) from thymus (lane 1), unfractionated splenocytes (lane 2), and T-cell-depleted splenocytes (lane 3) was electrophoresed, transferred to nitrocellulose, and assayed for hybridization to a ^{32}P-labeled 1.5-kb *Hin*dIII fragment from *itk* clone 52-2.1 (*a*). The same filter was then hybridized sequentially to probes specific for TCR β (*b*) and immunoglobulin κ (*c*) RNA. (*d*) Ethidium bromide stain of 28S RNA. In *a*, the exposure time was 24 hr for lane 1 and 72 hr for lanes 2 and 3.

mononuclear cells. To exclude this possibility, we assayed splenic mononuclear cells for *itk* expression before and after selective depletion of T cells. The depleted population contained fewer than 1% CD3$^+$ (T) cells and more than 98% B220$^+$ (B) cells. The 4-kb *itk* transcript was observed in RNA from thymus and from unfractionated splenocytes (Fig. 4*Ba*, lanes 1 and 2). Removal of T cells was accompanied by the loss of *itk* transcripts (Fig. 4*Ba*, lane 3) and a selective reduction in TCR β-chain RNA (Fig. 4*Bb*) relative to immunoglobulin κ transcripts (Fig. 4*Bc*). (The residual TCR β transcripts in the T-cell-depleted population are most likely contributed by natural killer cells.) We conclude that *itk* is expressed predominantly or exclusively in T lymphoid cells. This was supported by a survey of *itk* expression in cell lines: *itk* RNA was found in three T-cell lines examined (R1.1, EL-4, and CTLL-2) but not in any of three pro-B-cell lines, two pre-B-cell lines, six B-cell lines, four plasma cell lines, three myeloid cell lines, or six nonhematopoietic cell lines (data not shown).

Induction of *itk* RNA by IL-2. In responsive T cells, IL-2 increases expression of its own high-affinity receptor, through increased production of IL-2Rα RNA. IL-2 has a similar effect on the steady-state level of *itk* RNA. CTLL-2 cells were maintained in IL-2 and withdrawn from IL-2 for 48 hr. After 24 hours of deprival, a portion of the cell culture was reexposed to conditioned medium containing IL-2. Steady-state levels of RNA for *itk*, IL-2Rα, and β-tubulin were assessed. In CTLL-2 cells maintained in IL-2, *itk* RNA was low relative to IL-2Rα RNA (Fig. 5 *Aa* and *Ab*, lanes 1). By 48 hr after withdrawal of CTLL-2 cells from IL-2, IL-2Rα

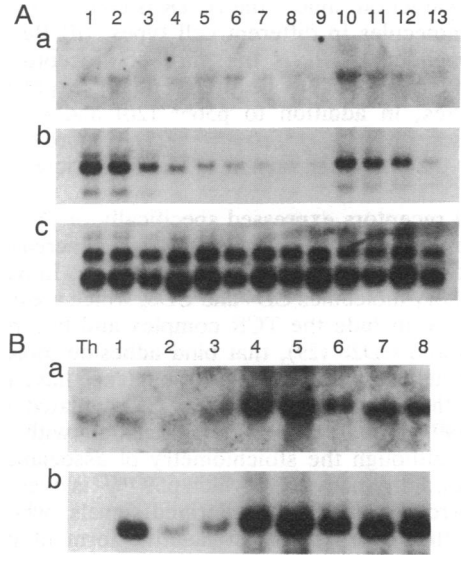

FIG. 5. Coordinate induction of *itk* and IL-2Rα transcripts by IL-2. (*A*) CTLL-2 cells were collected by centrifugation, washed in RPMI-1640, and cultured in RPMI-10 (see *Materials and Methods*) lacking IL-2. Samples were removed after 0, 1, 6, 12, 24, and 48 hr of IL-2 starvation (lanes 1–6). After 24 hr of starvation, IL-2 (100 units/ml) was added to a portion of the culture and cells were harvested after 0, 0.5, 1, 6, 12, 24, and 48 hr (lanes 7–13). Total cellular RNA (20 μg) was electrophoresed, transferred to nitrocellulose and hybridized with a ^{32}P-labeled *itk* probe (*a*). The same filter was hybridized to probes specific for IL-2Rα (*b*) and β-tubulin (*c*). (*B*) As in *A*, except that CTLL-2 cells were starved for IL-2 for 24 hr. Samples were taken after 12 hr (lane 1) and 24 hr (lane 2) of starvation. IL-2 (units/ml) was added and cells were harvested 1, 2, 4, 6, 8, and 12 hr later (lanes 3–8). Total RNA (20 μg) was assayed for hybridization to probes specific for *itk* (*a*) and IL-2Rα (*b*). Lane Th, 20 μg of total thymus RNA. For the experiment in *A*, IL-2 was provided by culture supernatant from a murine cell line that overexpresses IL-2 (16); for the experiment in *B*, recombinant IL-2 (Genzyme) was used.

RNA had declined about 5-fold, while *itk* RNA was nearly unchanged (Fig. 5 *Aa* and *Ab*, lanes 6). *itk* and IL-2Rα RNA increased greatly after readministration of medium containing IL-2 (Fig. 5 *Aa* and *Ab*, lanes 7–13). β-Tubulin transcripts did not vary greatly (Fig. 5*Ac*). Hybridization of the same filter to a probe specific for *lck* revealed, in distinction to *itk*, a slight decline in RNA level upon administration of IL-2 (data not shown). Stimulation of CTLL-2 cells with recombinant IL-2 gave similar results (Fig. 5*B*). IL-2 induced *itk* and IL-2Rα RNAs with similar kinetics; by 2 hr these transcripts had increased 7- and 8-fold, respectively, relative to their levels before IL-2 administration (Fig. 5*B*, compare lanes 2 and 4). Thus, in CTLL-2 cells, *itk* and IL-2Rα transcripts are coordinately increased in response to IL-2. IL-2Rα and *itk* transcripts are not strictly coregulated, however, as evidenced by their disparate levels in cells maintained in IL-2 (above), and the ability of tumor necrosis factor α to induce *itk* RNA, but not IL-2Rα RNA, in the EL-4 cell line (J.D.S. and S.V.D., unpublished data).

Potential Functions of *itk*. We consider two settings in which *itk* might function. (*i*) The coinduction of IL-2Rα and *itk* transcripts by IL-2 suggests a direct role for the *itk* product in signaling through the IL-2R. While *itk* expression is clearly regulated by IL-2, *itk* expression is not absolutely required for signaling through the IL-2R. In the B-cell line BCL_1, IL-2 induces a program of differentiation culminating in expression of J-chain RNA and secretion of immunoglobulin (25). Induction of J-chain RNA by IL-2 in BCL_1 cells was not accompanied by induction of *itk* RNA, which remained undetectable (J.D.S. and S.V.D., unpublished). This observation apparently contradicts a direct role for *itk* in IL-2 signal transduction, unless the IL-2R is coupled to different effector molecules in different cell types. (*ii*) Because the protein encoded by *itk* is the third non-receptor protein-tyrosine kinase found to be preferentially expressed in T lymphocytes, in addition to $p56^{lck}$ (26) and a lymphoid-specific isoform of $p59^{fyn}$, $p59^{fyn(T)}$ (27), a more economical notion is that *itk* functions in a T-cell-restricted signaling pathway.

Several receptors expressed specifically or preferentially on T lymphoid cells are known to trigger increases in the phosphotyrosine content of cellular proteins. In addition to the accessory molecules CD4 and CD8, which are coupled to $p56^{lck}$, these include the TCR complex and two receptors, CD2 (28) and CD28 (29), that bind adhesion molecules on heterologous cells. Several lines of evidence have led to the proposal that $p59^{fyn}$ functions in TCR-mediated signaling. First, $p59^{fyn}$ can be found in association with the TCR complex, although the stoichiometry of association is low (30). Second, thymocytes in which $p59^{fyn(T)}$ is overexpressed are hyperresponsive to TCR-derived signals, whereas thymocytes that overexpress an inactive form of $p59^{fyn}$ are relatively resistant (31). Experiments with Fyn-deficient mice indicate, however, that expression of $p59^{fyn(T)}$ is not required for TCR-mediated signaling in peripheral T cells, despite its apparent requirement in thymocytes (32, 33). Thus the kinase(s) involved in signaling through the TCR, in addition to those that are activated upon engagement of CD2 and CD28, have not been defined. The T-cell-specific protein-tyrosine kinase that we describe here may function in one or more of these signaling pathways.

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