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Immunology

Interleukin 2 regulates the activity of the lyn protein-tyrosine kinase in a B-cell line

(lymphokine/signal transduction)

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ABSTRACT **Recently, interleukin 2 (IL-2) has been shown to induce increased activity of the p56^{lck} protein-tyrosine kinase (PTK) in T-cell and natural killer cell lines, and evidence for a direct interaction between the p75 subunit of the IL-2 receptor (IL-2R) and this src-family kinase has been reported. Though these findings suggest a central role for lck in IL-2 signal transduction, one problem with this idea is that not all IL-2-responsive cells express the *lck* gene. For this reason, we examined the effects of IL-2 on the activity of src-like kinases in a pro-B cell line, F7, that lacks p56^{lck} but that displays high-affinity IL-2Rs and vigorously proliferates in response to this lymphokine. Of the eight known src-family PTKs, F7 cells were shown to contain only p53/56^{lyn}, p59^{fyn}, and a small amount of p62^{yes}. Stimulation of resting F7 cells with IL-2 induced a rapid (detectable within 1 min and maximal at 15 min) and concentration-dependent increase in the specific activity of p53/56^{lyn} kinase, as assessed by *in vitro* kinase assays. This effect of IL-2 on p53/56^{lyn} kinase was specific, since no IL-2-inducible changes were detected in the activities of the p59^{fyn} and p62^{yes} kinases. Furthermore, by using a monoclonal antibody specific for the ≈75-kDa β subunit of the IL-2R (referred to as p75/IL-2Rβ), evidence for physical association between the lyn kinase and the IL-2R complex was obtained, in that a small proportion of the p53/56^{lyn} kinase in F7 cells, but no detectable p59^{fyn} kinase, was coimmunoprecipitated with p75/IL-2Rβ. When combined with the recent evidence that IL-2 regulates p56^{lck} in T cells, these results indicate that some flexibility exists in the ability of various src-like PTKs to participate in IL-2 signal transduction mechanisms and raise the possibility that lineage-specific (T- versus B-cell) responses to IL-2 may be determined at least in part by the repertoire of src-like PTKs expressed in the cell.**

Interleukin 2 (IL-2) induces the rapid phosphorylation of tyrosine residues on several proteins in lymphocytes (1). Unlike many other growth factor receptors, such as those for platelet-derived growth factor, epidermal growth factor, and insulin, however, the known IL-2 receptor (IL-2R) subunits lack tyrosine kinase activity (2). Recently, the ≈75-kDa β subunit of the IL-2R complex (hereafter referred to as p75/IL-2Rβ) was shown to be capable of physically associating with p56^{lck}, a nonreceptor, membrane-associated protein-tyrosine kinase (PTK) of the src family (3). Furthermore, IL-2 has been shown to induce increases in the specific activity of p56^{lck} in T cells (4). By analogy to many other growth factor receptors (5) and to the CD4-lck and CD8-lck situation (6), these findings suggest that p56^{lck} may function essentially as the tyrosine kinase subunit of the IL-2R complex and thereby mediate the pleiotropic effects of IL-2 in lymphocytes.

One problem with the hypothesis that lck is a critical participant in IL-2 signal transduction is that not all IL-2-responsive cells express the *lck* gene. For example, *lck* expression is highest in T and natural killer (NK) cells, but B cells typically contain little or no *lck* mRNA (7). This problem prompted us to examine the effects of IL-2 on the activities of other src-like kinases in an IL-2-responsive pro-B cell line (F7) that lacks detectable p56^{lck}. F7 cells are a subclone of the interleukin 3 (IL-3)-dependent BAF-B03 cell line that were rendered IL-2 responsive by stable introduction of a p75/IL-2Rβ-expression plasmid (8). When deprived of IL-3, these cells exhibit vigorous proliferative responses to IL-2 and can grow long term in IL-2-supplemented media. By using F7 cells as a model of an lck-deficient, IL-2-responsive lymphocyte, we show here that IL-2 can specifically regulate the activity of another member of the src family of kinases, p53/56^{lyn}, and that at least a small proportion of the lyn kinase in F7 cells can be coimmunoprecipitated when using antibodies specific for p75/IL-2Rβ. Since *lyn* is normally expressed in B cells but not in T cells (9), our findings raise the possibility that p53/56^{lyn} may mediate some of the intracellular effects of IL-2 in B-lineage cells, in contrast to p56^{lck}, which acts in T cells. Furthermore, these results demonstrate some flexibility in the ability of various src-like PTKs to couple to IL-2 signaling pathways and imply that cell lineage-specific effects of IL-2 may be determined, at least in part, by the repertoire of src family kinases expressed in T and B cells.

MATERIALS AND METHODS

Cell Culture and Stimulation. F7 cells were a kind gift of M. Hatakeyama and T. Taniguchi (Osaka University, Japan) (8). These cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (HyClone) and 10% conditioned medium from the IL-3-producing cell line WEHI-3B (10). Cells were used for experiments at the end of their usual 3- to 4-day culture cycle, when most of the IL-3 had been consumed. F7 cells were washed three times in Hanks' buffered salt solution and returned to culture in medium lacking IL-3 for 24–48 h. Resting F7 cells were then restimulated with purified recombinant human IL-2 (Cetus) or mouse IL-3 (Genzyme) for various times prior to cell lysis for immunoprecipitations.

Antibodies. For these investigations, we prepared a rabbit antiserum with reactivity for the lyn kinase by using a synthetic peptide corresponding to the last 10 amino acids of the human lyn kinase. This peptide had the sequence [C]ATEGQYQQQP and was conjugated to maleimide-activated bovine serum albumin (Pierce) by the method of Partis *et al.* (11). The specificity of this antiserum for detection of the p53/56^{lyn} kinase in F7 cells was demonstrated by immunoprecipitation, *in vitro* kinase assays, and immunoblotting

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Abbreviations: IL, interleukin; IL-2R, IL-2 receptor; PTK, protein-tyrosine kinase; mAb, monoclonal antibody.

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assays, as well as by peptide competition experiments and by comparisons to anti-lyn antibodies from other sources.

Antibodies to the eight known members of the src family of PTKs were generously provided by other investigators. The anti-src mouse monoclonal antibody (mAb) 327 was provided by J. Brugge (University of Pennsylvania) (12). Rabbit polyclonal antisera raised against synthetic peptides included anti-fyn [amino acids (aa) 29–48] provided by R. Abraham (Mayo Clinic) as well as anti-lck (aa 39–58), anti-yes (aa 5–71), anti-lyn (aa 18–62), anti-fgr (aa 16–58), and anti-blk (aa 2–50) provided by J. Bolen (Bristol-Myers Squibb, Syracuse, NY) (7, 13). Polyclonal rabbit antisera, raised against *Escherichia coli*-produced TrpE–lck and TrpE–hck fusion proteins, were provided by B. Sefton (Salk Institute) and S. Ziegler (Immunex, Seattle), respectively (14, 15).

The mouse anti-human p75/IL-2Rβ mAb 20G6 [IgM(κ)] was developed by immunization of mice with NIH 3T3 cells that had been transfected with a human IL-2Rβ cDNA. Immunoprecipitation, immunoblotting, and deletional analysis have established that this mAb specifically recognizes the extracellular domain of human p75/IL-2Rβ (E. Park, T.T., J.C.R., and H.U.S., unpublished results).

Immune Complex Kinase Assays and Immunoblotting. Aliquots of F7 cells were normalized for cell counts ($5–10 \times 10^6$), and the cells were washed in phosphate-buffered saline and lysed in ice-cold lysis buffer [1% Nonidet P-40/10 mM Tris, pH 7.6/50 mM NaCl/30 mM sodium pyrophosphate/50 mM NaF/1 mM phenylmethylsulfonyl fluoride/aprotinin (0.23 units/ml)/leupeptin (10 μg/ml)/10 μM pepstatin/1 mM sodium orthovanadate] at 4°C for 10 min. Nuclei and cellular debris were removed by centrifugation at $16,000 \times g$ for 15 min at 4°C. In many cases, lysates were further normalized for total protein content with similar results. The supernatants were precleared by incubation for 30 min with formalin-fixed *Staphyloccous aureus* (Pansorbin, Calbiochem) that had been presaturated with normal rabbit serum and then incubated with 3 μl of antisera for 1 h at 4°C. In some cases, competing peptide (10 μg) was included to verify the specificity of antibodies. Immune complexes were collected with fixed *S. aureus* that had been preincubated with lysis buffer containing 1% nonfat dry milk. Immunoprecipitates were washed two times with lysis buffer and once with buffer containing 10 mM Tris (pH 7.2), 100 mM NaCl, and 100 μM sodium orthovanadate.

Kinase assays were performed by resuspending immunoprecipitates in 30 μl of kinase reaction mixture [10 mM Tris, pH 7.2/10 mM $MnCl_2$/10 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP (Amersham)/1 μM unlabeled ATP] for 2 min at 20°C. Preliminary experiments determined that all kinase reactions proceeded linearly through the first 3 min at 20°C. Enolase (5 μg; Boehringer Mannheim) was denatured with 50 mM acetic acid at 37°C for 15 min and was added to some reactions as an exogenous substrate. The reaction was terminated by the addition of 30 μl of SDS gel loading buffer. Samples were analyzed by electrophoresis in 7.5% or 8% polyacrylamide gels. The gels were treated with 1 M KOH at 56°C for 1 h to remove phosphoserine and phosphothreonine before autoradiography, using XRP or XAR film (Eastman Kodak) with intensifying screens at −80°C.

For immunoblots, immunoprecipitated proteins were separated by SDS/PAGE and transferred to nitrocellulose filters. Blots were then incubated with affinity-purified antibody specific for the lyn kinase, followed by ^{125}I-labeled protein A (0.25 μCi/ml; Amersham). The affinity-purified anti-lyn antibody was prepared by using the C-terminal lyn peptide immobilized to an agarose support (Sulfolink; Pierce), as described (16).

For kinase reimmunoprecipitation experiments, the precleared lysates prepared from 5×10^7 F7 cells were incubated with 1 μl of mAb 20G6 ascites for 2 h at 4°C, and the immune complexes were collected by using 25 μl of goat anti-mouse IgM antibody immobilized to agarose beads (Sigma). The immunoprecipitates were washed five times with lysis buffer and incubated with 50 μl of kinase buffer (10 mM Tris, pH 7.2/10 mM $MgCl_2$/3 mM $MnCl_2$/0.1% Nonidet P-40/30 μM Na_3VO_4/20 μCi of [γ-^{32}P]ATP) for 15 min at 20°C to allow autophosphorylation of PTKs associated with p75/IL-2R. Immune complexes were then dissociated by boiling in 100 μl of 1% SDS elution buffer containing 10 mM Tris (pH 7.2) and 1 mM Na_3VO_4 for 5 min. After collecting supernatants, diluting 5-fold with Nonidet P-40 lysis buffer (final SDS concentration of 0.2%), and preclearing with Pansorbin to remove remaining immunoglobulin, samples were incubated with antibodies against lyn or fyn kinase. These kinase-containing immune complexes were recovered with Pansorbin and analyzed by SDS/PAGE (8% gels).

Proliferation Assays. Short-term proliferative responses to IL-2 were measured by [^{3}H]thymidine incorporation assays. Approximately 5×10^3 F7 cells in 100 μl of medium containing various concentrations of IL-2 were cultured in triplicate in 96-well flat-bottom plates (Falcon). After 20 h, 1 μCi of [^{3}H]thymidine was added to each well, and the cells were harvested 16 h later onto glass filters for scintillation counting. All data were presented as the percentage of the maximal [^{3}H]thymidine incorporation (mean ± SD) for the triplicate determinations from three experiments.

RESULTS

Determination of the Repertoire of src-Related PTKs in F7 Cells. To determine which of the eight known src-like PTKs are present in proliferating F7 cells, we used specific antibodies for immunoprecipitations and then detected these PTKs by radiolabeling with [γ-^{32}P]ATP based on the ability of PTKs to autophosphorylate during *in vitro* kinase assays. As shown in Fig. 1, F7 cells contained easily detectable amounts of the p53/56^{lyn} and p59^{fyn} PTKs. Barely detectable levels of p62^{yes} kinase were also found in these pro-B cells. No p60^{src}, p59/64^{hck}, or p55^{fgr} was detected in F7 cells by this assay, consistent with previous reports of the src family gene expression in B-lineage cells (13). Control immune complex kinase assays performed with cell lysates derived from various myeloid cell lines confirmed the ability of these antibodies to detect the src, hck, and fgr proteins, thus excluding technical reasons for the failure to detect these particular PTKs in F7 cells (data not shown). Though most B cells express *blk* and some contain low levels of *lck* (7), no p55^{blk} or p56^{lck} was detected in F7 cells. Parallel kinase reactions using a T-lymphoblastic leukemia cell line TALL103/2 (Fig.

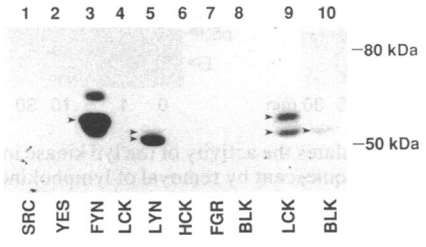

FIG. 1. Determination of the repertoire of the src family of kinases in F7 cells. The presence or absence of various members of the src family of PTKs was determined in proliferating F7 cells (5×10^6 cells per assay) through the use of specific antibodies for immunoprecipitation of individual PTKs. Kinases were radiolabeled with [γ-^{32}P]ATP for 15 min at 20°C by virtue of their ability to autophosphorylate during *in vitro* kinase assays and analyzed by SDS/PAGE (8% gels). Cell lysates were prepared from F7 cells (lanes 1–8), the human T-cell line TALL103/2 (lane 9), and the murine B-cell line WEHI-231 (lane 10). Arrowheads indicate the gel positions of the p59^{fyn} (lane 3), p53/56^{lyn} (lane 5), p56/59^{lck} (lane 9), and p55^{blk} (lane 10) kinases.

1, lane 9) and a B-cell line WEHI-231 (Fig. 1, lane 10) confirmed the validity of these immune complex kinase assays for detection of $p56^{lck}$ and $p55^{blk}$, respectively. A 59-kDa form of lck was also found in the TALL103/2 cells, which has previously been attributed to serine phosphorylation of $p56^{lck}$ (4, 17). Thus, of the eight known src-like PTKs, only lyn and fyn were present in F7 cells at significant levels.

IL-2 Specifically Regulates the Activity of the lyn Kinase in F7 Cells. To determine whether IL-2 regulates the activity of the lyn or fyn kinase in F7 cells, we deprived these pro-B cells of lymphokines for 24–48 h to achieve quiescence and then restimulated them with saturating amounts of IL-2 (50 units/ml). At various times thereafter, aliquots of cells were removed from culture, and lysates were prepared for immunoprecipitation and *in vitro* kinase assay. As shown in Fig. 2*A*, IL-2 induced a rapid and transient increase in the activity of the lyn kinase. Phosphorylation of the exogenous substrate, enolase, increased within 1 min after addition of IL-2 to F7 cells, peaked at 15 min (about 6-fold increase by densitometric analysis), and began declining within 30 min. Increased autophosphorylation of both the 53-kDa and 56-kDa forms of this enzyme [which have been attributed to alternative splicing (18)] was also maximal at 15 min after IL-2 addition to quiescent F7 cells. Similar results were obtained with two different anti-lyn antibodies [one directed against the NH_2 end (13) and the other the COOH end of the protein (see *Methods*)].

Fig. 2*B* demonstrates that, over the same time course of IL-2 stimulation, activity of the $p59^{fyn}$ kinase did not change. Though the low levels of $p62^{yes}$ kinase activity in F7 cells made accurate measurements difficult, the activity of this PTK was also unaltered by IL-2 stimulation (data not shown). Thus, in these pro-B cells, regulation of the lyn kinase appears to be specifically associated with IL-2 signaling.

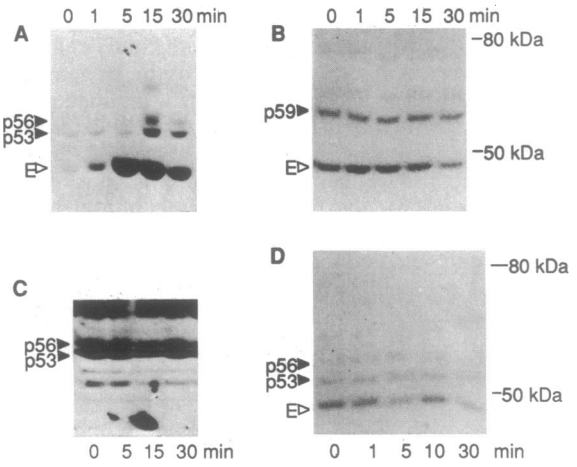

FIG. 2. IL-2 regulates the activity of the lyn kinase in F7 cells. F7 cells were rendered quiescent by removal of lymphokines from their medium and then restimulated with recombinant IL-2 (*A–C*) or IL-3 (*D*) at 50 units/ml for the various times. (*A*) lyn immune complex kinase assays after addition of IL-2. Kinase reactions were performed by incubating the immunoprecipitates with [γ-^{32}P]ATP for 2 min at 20°C, under which conditions all kinase reactions proceeded linearly. (*B*) fyn immune complex kinase assays after addition of IL-2. (*C*) Anti-lyn immunoprecipitates were subjected directly to SDS/PAGE (without performing *in vitro* kinase reactions), transferred to nitrocellulose filters, and then analyzed for relative levels of $p53/56^{lyn}$ proteins by immunoblot assay using immunoaffinity-purified anti-lyn antiserum followed by ^{125}I-labeled protein A. (*D*) lyn immune complex kinase assays after addition of IL-3. Closed arrowheads indicate the positions of the $p53/56^{lyn}$ kinases (*A* and *D*) and the $p59^{fyn}$ kinase (*B*). Open arrowheads indicate the exogenous substrate enolase (E).

Immunoblot determination of the relative levels of $p53/56^{lyn}$ indicated that the observed IL-2-inducible increases in lyn kinase activity reflect a real change in the specific activity of the enzyme, since levels of the lyn protein were unaltered by the IL-2 treatments (Fig. 2*C*). Furthermore, the IL-2-mediated increase in the specific activity of $p53/56^{lyn}$ could not be attributed to a generalized increase in cellular metabolism caused by addition of growth factor to quiescent cells, because restimulation of resting F7 cells with IL-3 induced maximal proliferation (data not shown) but produced only a modest increase (about 2-fold) in lyn kinase activity (Fig. 2*D*). Thus, under the conditions employed here, IL-2 was a relatively specific regulator of lyn kinase activity in F7 cells. It deserves mention, however, that despite the minor effect of IL-3 on lyn kinase activity in F7 cells, we have found that IL-3 can markedly influence $p53/56^{lyn}$ activity in some myeloid cell lines (T.T., R. O'Connor, D. Santoli, and J.C.R., unpublished data).

Concentration Dependence of IL-2-Mediated Increases in lyn Kinase Activity. To further confirm that IL-2 regulates the activity of $p53/56^{lyn}$ in F7 cells, we examined the concentration dependence of IL-2-inducible elevations in the specific activity of this kinase and correlated them with IL-2-stimulated proliferation of these pro-B cells. For these experiments, resting F7 cells were cultured with various concentrations of IL-2. Relative levels of lyn kinase activity and DNA synthesis were then measured in these cells at 10 min and 20 h, respectively.

As shown in Fig. 3, IL-2-mediated increases in lyn kinase activity (as measured by phosphorylation of enolase) occurred in a concentration-dependent and saturable manner. Furthermore, these IL-2-induced elevations in lyn kinase activity roughly correlated with IL-2-stimulated proliferation of F7 cells. On the basis of these observations, IL-2 appears to be a direct regulator of the activity of the lyn kinase in these pro-B cells.

Evidence for Physical Association of $p53/56^{lyn}$ Kinase with the IL-2R Complex. To determine whether the lyn kinase can be found associated with the IL-2R complex, we immuno-

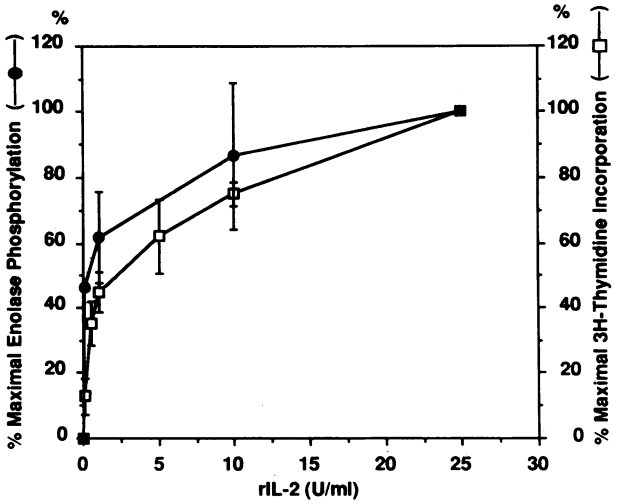

FIG. 3. Concentration dependence of lyn kinase activation by IL-2 and correlation with IL-2-induced proliferation. Resting F7 cells were restimulated with various concentrations of IL-2 and analyzed 15 min later for *in vitro* kinase assays or 20 h later for DNA synthesis. Kinase reactions were performed for 2 min at 20°C with the exogenous substrate enolase, and the results were derived by excising the enolase band from gels and Cerenkov counting. Cellular proliferation data represent percentage of maximal [^{3}H]thymidine incorporation into DNA after IL-2 stimulation. All data are reported as the mean ± SD of three determinations from three experiments. rIL-2, recombinant IL-2.

precipitated p75/IL-2Rβ by using mAb 20G6 and then incubated these immunoprecipitates with [γ-^{32}P]ATP under *in vitro* kinase assay conditions that allowed for kinase autophosphorylation. As shown in Fig. 4*A*, SDS/PAGE analysis of these immune complexes revealed the presence of radiolabeled 53-kDa and 56-kDa proteins (lane 4) that comigrated precisely in gels with the p53 and p56 bands immunoprecipitated by our lyn-specific antiserum (lanes 2 and 3). Peptide competition experiments confirmed the specificity of this anti-lyn antiserum for immunoprecipitation of the p53/56^{lyn} kinase under the conditions of these assays (Fig. 4*A*, lane 1). The ^{32}P-labeled 53-kDa and 56-kDa proteins immunoprecipitated by anti-lyn antibodies (Fig. 4*A*, lanes 2 and 3) and those coimmunoprecipitated with the anti-p75/IL-2Rβ mAb 20G6 (Fig. 4*A*, lane 4) completely withstood treatment with alkaline (1 M KOH for 1 h at 56°C), suggesting that the majority of the *in vitro* phosphorylation occurred on tyrosines.

To further test the possibility that the 53-kDa and 56-kDa proteins coimmunoprecipitating with p75/IL-2Rβ represent the lyn kinase, p75/IL-2Rβ immune complexes were prepared and subjected to *in vitro* kinase assay as described for Fig. 4*A* to allow for kinase autophosphorylation. After the kinase reactions, the samples were boiled in 1% SDS buffer to disrupt protein interactions and then diluted with Nonidet P-40 lysis buffer to achieve a final concentration of 0.2% SDS (wt/vol); immunoprecipitations were performed with antibodies specific for p53/56^{lyn}. As a control, antisera specific for the p59^{fyn} kinase were also employed for some samples. The elution method employed here was verified to recover all of the immunoprecipitated p53/56^{lyn} kinase for reimmunoprecipitations (Fig. 4*A*, lanes 2 and 3).

As shown in Fig. 4*B*, immunoprecipitation with anti-p75/IL-2Rβ mAb followed by *in vitro* kinase assay and reimmunoprecipitation with anti-lyn antibody revealed the presence of ^{32}P-labeled 53-kDa and 56-kDa bands, consistent with the lyn kinase (lanes 1 and 2). When anti-fyn antibodies were used, no detectable radiolabeled proteins were reimmunoprecipitated from p75/IL-2Rβ immune complexes (Fig. 4*B*, lanes 3 and 4). p53/56^{lyn} kinase could be reimmunoprecipitated from p75/IL-2Rβ immune complexes even when resting, lymphokine-deprived F7 cells were employed (Fig. 4*B*, lane 1), suggesting that p53/56^{lyn}–p75/IL-2Rβ complexes form in these cells prior to IL-2 stimulation. In addition, stimulation of F7 cells with IL-2 did not induce detectable alterations in the relative amounts of p53/56^{lyn} kinase coimmunoprecipitated with p75/IL-2Rβ (Fig. 4*B*, lane 1 and 2). As shown in Fig. 4*A*, only a small proportion (1–2%) of the total lyn kinase in F7 cells was coimmunoprecipitated with p75/IL-2Rβ under the cell lysis conditions employed here, based on comparisons with the amounts of p53/56^{lyn} kinase that was immunoprecipitated by anti-lyn specific antisera (Fig. 4*A*, lane 4 versus lane 3).

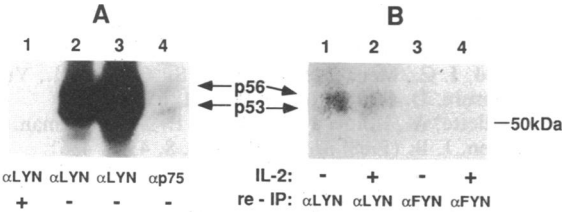

FIG. 4. Coimmunoprecipitation of lyn kinase with the p75/IL-2Rβ subunit. (*A*) p53/56^{lyn} kinase or p75/IL-2Rβ was immunoprecipitated from F7 cell lysates (5×10^7 cells per assay) by using specific antibodies against these proteins indicated under each lane. Immunoprecipitates were then incubated with [γ-^{32}P]ATP for 15 min at 20°C to allow kinase autophosphorylation, and the resulting immune complexes were either boiled directly in Laemmli buffer (lanes 1 and 2) or dissociated by boiling in 1% SDS elution buffer, followed by collecting supernatants and mixing with Laemmli buffer (lanes 3 and 4). Note that equivalent amounts of p53/56^{lyn} kinase were recovered by both elution methods (lane 2 versus lane 3). Samples were analyzed by electrophoresis in 7.5% polyacrylamide gels. In lane 1, 10 μg of competing peptide was added to the cell lysate prior to immunoprecipitation, thus confirming the specificity of the anti-lyn antibodies. (*B*) F7 cells were rendered quiescent by removal of lymphokines from their media and then restimulated with recombinant IL-2 at 50 units/ml for 15 min. The p75/IL-2Rβ molecule was immunoprecipitated from unstimulated (−) or stimulated (+) F7 cell lysates and subjected to immune complex kinase reactions as described for *A*. p75/IL-2R-containing immune complexes were then boiled in 1% SDS elution buffer, diluted 5-fold with lysis buffer, and precleared with Pansorbin. The supernatants were then incubated with antisera specific for lyn or fyn proteins as indicated under each lane. Immunoprecipitates were subjected to electrophoresis in 8% polyacrylamide gels. Arrows indicate the positions of the p53^{lyn} and p56^{lyn} kinases. α, anti; IP, immunoprecipitated; re-IP, reimmunoprecipitated.

DISCUSSION

Here we have shown that IL-2 can regulate the activity of the src family PTK lyn in a pro-B cell line that displays functional human p75/IL-2Rs on its cell surface by virtue of gene transfection. In these F7 cells, which serve as a model of an IL-2-responsive lymphocyte that lacks p56^{lck}, IL-2 induced marked increases in the activity of the lyn kinase, as determined by kinase autophosphorylation and by phosphorylation of the exogenous substrate, enolase. These IL-2-inducible elevations in lyn kinase activity represented an increase in the specific activity of the enzyme, since levels of lyn protein were unaltered by IL-2 stimulations (Fig. 2*C*). Moreover, this effect of IL-2 on the lyn kinase was both concentration-dependent (Fig. 3) and specific, in that (*i*) the activities of the fyn and yes kinases were not increased by IL-2 (Fig. 2*B*), and (*ii*) another growth factor (IL-3) had only a marginal influence on lyn, despite inducing vigorous proliferation (Fig. 2*D*). Thus, the IL-2-mediated increase in lyn kinase activity cannot be attributed to a generalized increase in cellular metabolism caused by restimulation of lymphokine-deprived cells with IL-2.

Because the IL-2-regulated increases in lyn kinase activity were rapid and transient, our findings suggest that lyn kinase activation is an early event associated with IL-2 signaling in F7 cells. Moreover, the finding that at least a small proportion of the p53/56^{lyn} kinase in F7 cells can be coimmunoprecipitated by using a mAb specific for the p75/IL-2Rβ molecule preliminarily suggests that the lyn kinase may receive its activation signals directly from the IL-2R (Fig. 4). Recently it was shown that IL-2 can regulate the activity of the p56^{lck} kinase in T cells (4) and that p75/IL-2Rβ can physically associate with p56^{lck} (3). The region within the lck kinase required for IL-2R association was mapped to the catalytic domain. Since the amino acid sequences of the catalytic domains of the various src-like PTKs are well conserved (7), it is perhaps not surprising that another member of the src family (lyn) is able to functionally and physically couple to the IL-2R complex. It is possible, therefore, that other src-like PTKs besides lck and lyn can be regulated by IL-2. It should be noted in this regard, however, that lyn is the most homologous to lck of the src family of kinases, particularly in its catalytic domain (19).

The mechanisms responsible for activation of the p56^{lck} and p53/56^{lyn} kinases in IL-2-stimulated lymphocytes remain unknown, but the data suggesting physical association of these kinases with the IL-2R complex permit some speculations. First, our findings in the F7 pro-B cell line and those obtained by others in the human NK-like cell line, YT, and in COS cells cotransfected with p75/IL-2Rβ- and p56^{lck}-encoding expression plasmids (3) indicate that p53/56^{lyn} and p56^{lck} can apparently associate with the IL-2R complex even before cells are stimulated with IL-2. Thus, preformed complexes involving the p75/IL-2Rβ, these src-like PTKs, and

probably other unidentified receptor components provide a situation similar to the classical growth factor receptors (such as those for platelet-derived growth factor and epidermal growth factor), wherein molecules with extracellular ligand-binding domains (p75/IL-2R) and intracellular nonreceptor kinases ($p56^{lck}$, $p53/56^{lyn}$) can function as a single unit. Second, by analogy to the situation with receptors for platelet-derived growth factor and epidermal growth factor (20–22), the association of $p56^{lck}$ and $p53/56^{lyn}$ with IL-2Rs could permit kinase activation through a mechanism involving ligand-inducible receptor dimerization. Thus, association of $p56^{lck}$ and $p53/56^{lyn}$ with the IL-2R may allow for intermolecular phosphorylation reactions among kinases brought into proximity as a result of ligand-induced IL-2R dimerization or clustering, with the end result being IL-2-induced up-regulation of kinase activity (E. Park, T.T., J.C.R., and H.U.S., unpublished results). The recent demonstration of ligand-inducible dimerization of the human growth hormone receptor, which has significant structural similarities to p75/IL-2Rβ (23), lends indirect support to this model. Of course, alternative explanations are possible. For example, IL-2R association could render $p56^{lck}$ and $p53/56^{lyn}$ accessible to protein-tyrosine phosphatases such as CD45 that can activate these src-like PTKs by dephosphorylation at specific tyrosine residues (Tyr-505 in lck) involved in down-regulating the activities of these nonreceptor PTKs (24, 25).

Our finding that IL-2 can regulate the activity of lyn in F7 cells does not prove that naturally IL-2-responsive cells that express *lyn* but not *lck* will exhibit similar behavior. However, these data do have some general implications for mechanisms of src-like PTK participation in IL-2 signaling. Though the IL-2-mediated regulation of $p56^{lck}$ in T cells (4) and of $p53/56^{lyn}$ in a B-lineage cell (F7) has elements of specificity (fyn and yes kinases are not activated in IL-2-stimulated T and B cells), the data presented here indicate that there is also some flexibility in the coupling of src-like kinases to IL-2 signaling pathways. This flexibility suggests that the specific src-like kinase available for IL-2 signaling may be determined at the level of gene expression during lymphocyte differentiation, thus raising the possibility that differences between the substrates of $p56^{lck}$ in T cells and NK cells and those of $p53/56^{lyn}$ in B cells may allow lineage-specific responses to IL-2—for example, cytolytic killing by T cells and NK cells versus antibody production by B cells (1, 26). Though activation of the lyn kinase correlated with IL-2-induced proliferation of F7 cells (Fig. 3) and ample precedence exists for regulation of cellular growth by $p60^{src}$ and its related PTKs (27–29), only future experiments can directly assess the relevance of the lyn kinase for IL-2-mediated proliferation.

In addition to demonstrating that a lymphokine receptor (IL-2R) can regulate the activity of more than one member of the src family of kinases (lck and lyn), our findings also provide another example of a particular src-like PTK physically associating with and functionally coupling to more than one receptor system. In T cells, for example, $p56^{lck}$ is physically associated with and regulated by receptors for IL-2, as well as the CD4 and CD8 molecules, which appear to participate in T-cell antigen receptor signaling mechanisms (6). By analogy to lck in T cells, our finding that IL-2 regulates $p53/56^{lyn}$ in a B-lineage cell line and the previous demonstration that lyn can physically associate with and be regulated by the surface immunoglobulin complex in B cells (13, 30) imply that lyn may function in both antigen- and IL-2-initiated signal transduction pathways in B cells. Further investigations should focus on delineating the molecular basis for both the flexibility and specificity in the association and functional coupling of particular src-like PTKs to immune cell receptors, as well as determining the repercussions of activation of various members of the src family of nonreceptor PTKs for lymphocyte responses.

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