Biochemical analysis of interleukin-2 receptor β chain phosphorylation by p56^{lck}

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Abstract Tyrosine phosphorylation of multiple proteins, including the receptor itself, is an initial event in IL-2 signaling and leads to recruitment of SH2 or PTB domain-containing proteins to the receptor. In this study, we have used subdomains of the IL-2 receptor β chain (IL-2Rβ) expressed in *Escherichia coli* as GST fusion proteins to identify the tyrosine residues that could be phosphorylated by p56^{lck}, one of the critical tyrosine kinases activated by IL-2. We report that recombinant p56^{lck} phosphorylates in vitro tyrosine residues within the IL-2Rβ chain but not those within the IL-2Rγ chain. p56^{lck} phosphorylates tyrosine residues 355, 358 and 361 but not 338 of the IL-2Rβ chain acidic subdomain. Interestingly, phosphorylation of Tyr-358 appears to require the presence of either Tyr-355 or Tyr-361. p56^{lck} also phosphorylates very efficiently the two tyrosines present in the IL-2Rβ chain C-terminal region, Tyr-392 and Tyr-510. We also investigated the association of p56^{lck} with the IL-2Rβ chain which was found to depend on a short stretch of the IL-2Rβ chain acidic subdomain, and to be independent of the presence of its tyrosine residues.

Key words: Interleukin-2 receptor; Tyrosine phosphorylation

1. Introduction

Recent studies have uncovered the complexity of the signaling events induced by interleukin-2 (IL-2), a cytokine that plays a central role in lymphocyte differentiation, proliferation and apoptosis [1,2]. The IL-2 receptor (IL-2R) comprises three polypeptide subunits, the α, β and γ chains, of which only β and γ are critical for signal transduction. IL-2Rβ deletion analyses have delineated three functional cytoplasmic subdomains, respectively named proximal (or serine-rich), acidic and C-terminal [3], that cooperate for a full proliferative response to IL-2 [4–7]. IL-2 binding induces oligomerization of its receptor, which results in activation of several protein tyrosine kinases (PTK): p56^{lck} of the src family [8], the PTK Syk of the Syk/Zap 70 family [9], and JAK1 and JAK3 of the Janus kinase family [10]. These kinases phosphorylate multiple cellular substrates [11–14] including the IL-2R β and γ chains themselves [15,16]. Phosphorylated tyrosine residues in the IL-2R complex serve as docking sites for signaling or adaptor proteins that contain Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains.

The first non-receptor PTK found in association with the IL-2R was p56^{lck} [17]. p56^{lck} is expressed predominantly in T lymphocytes and plays an essential role in T lymphocyte maturation and proliferation. p56^{lck} has been reported to associate constitutively with the IL-2Rβ chain, and IL-2 stimulation increases its kinase activity [18,19]. Based on experiments using IL-2Rβ deletion mutants transfected into COS cells, it was observed that this interaction involves the catalytic domain of p56^{lck} and the IL-2Rβ acidic region and that p56^{lck} may phosphorylate several tyrosine residues of the IL-2Rβ chain [17]. However, direct biochemical evidence for this interaction or identification of p56^{lck} target tyrosine residues in the IL-2Rβ chain are still missing.

In this work, we have used IL-2Rβ subdomain GST fusion proteins and recombinant p56^{lck} to directly identify the tyrosine residues that can be phosphorylated by p56^{lck}. The interactions between the cytoplasmic subdomains of IL-2Rβ and p56^{lck} have been studied and we provide biochemical evidence for p56^{lck} association with a short stretch of the IL-2Rβ chain acidic subdomain.

2. Materials and methods

2.1. Construction of GST fusion proteins

A plasmid, pDKCRβ, containing the full length cDNA for human IL-2Rβ chain was kindly provided by Dr. T. Kono [20]. A *Bcl*I-*Bal*I fragment was inserted into *Bam*HI-*Sma*I-digested PGEX-3X plasmid (Pharmacia) to yield a GST fusion protein containing the full length IL-2Rβ chain cytoplasmic domain (GST-βFL, amino acids 240–525). This plasmid was then used as a template to amplify cDNA fragments by PCR and construct PGEX plasmids encoding the following GST fusion IL-2Rβ chain subdomains: GST-βProx (amino acids 240–307), GST-βAci (amino acids 306–383) and GST-βCter (amino acids 382–525). In addition, for the purpose of some of the experiments described in this report, the GST-βAci protein was further expressed as three shorter constructs GST-βAci1, GST-βAci2 and GST-βAci3 corresponding to amino acids 306–337, 335–365 and 362–383 respectively. For numbering amino acids of the IL-2Rβ chain as shown here, the initial methionine residue of the precursor polypeptide is taken as number 1. Mutations of tyrosine residues into phenylalanine were achieved by PCR using appropriate oligonucleotides containing a TAC to TTC codon change. For GST-IL-2Rγ construction, a fragment of the IL-2Rγ cDNA, corresponding to the intracytoplasmic domain (amino acids 288–369), was amplified by RT-PCR from peripheral blood lymphocytes mRNA (sense primer: 5′-GAA CGG ACG ATG CCC C; antisense primer: 5′-GTT CAG GTT TCA GGC) and inserted into PGEX-2T using the IL-2Rγ cDNA endogenous *Eco*RI site. All constructs, whether wild type or mutated, were verified by DNA sequencing. GST fusion proteins were produced in *Escherichia coli* strain BL21(DE3) by induction for 4 h with 1 mM IPTG and purified by adsorption onto glutathione (GSH)-Sepharose beads as previously described [21].

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Abbreviations: IL-2, interleukin-2; IL-2R, interleukin-2 receptor; GST, glutathione *S*-transferase; SH2, Src homology 2 domain; PTB, phosphotyrosine binding domain; PTK, protein tyrosine kinase

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2.2. Baculovirus-expressed p56^{lck} and in vitro phosphorylation

The cDNA for human p56^{lck} was inserted in AcNMPV and the selected recombinant baculovirus was used to infect Sf9 insect cells. 48 h after infection, Sf9 cells were harvested, washed once with PBS and lysed. The cell lysate was centrifuged at 100 000×*g* for 30 min, the supernatant was recovered, supplemented with 10% glycerol and stored at −80°C. p56^{lck} represented up to 1% of the total proteins in the crude Sf9 cell lysate as judged by scanning densitometry of Coomassie blue-stained SDS-polyacrylamide gel.

To study phosphorylation of GST-IL-2Rβ by p56^{lck}, 100 pmol of purified GST fusion proteins was adsorbed onto GSH-Sepharose beads and washed in kinase reaction buffer (20 mM HEPES pH 7.5, 10 mM $MnCl_2$, 1.5 μM ATP). Recombinant p56^{lck}-containing Sf9 cell lysate (0.7 μl) was then added in a final volume of 30 μl kinase buffer supplemented with 10 μCi [γ-^{32}P]ATP. After 15 min at 30°C the reaction was stopped by adding 1 ml of cold lysis buffer, and following four washes the proteins bound to the beads were recovered by boiling in Laemmli sample buffer and separated by SDS-PAGE. Gels were treated by bathing in 1 M KOH for 1 h at 55°C, stained with Coomassie brilliant blue and dried before autoradiography.

2.3. Cell culture

The human T-cell chronic lymphocytic leukemia-derived, IL-2-dependent Kit 225 cell line was kindly provided by Dr. T. Hori (Kyoto University, Japan) [22]. Cells were maintained in culture medium (RPMI 1640, 10% fetal calf serum and antibiotics) supplemented with 0.5 nM recombinant human IL-2 (generously provided by P. Ferrara, Sanofi, France). Kit 225 cells were deprived of IL-2 by washing three times and resuspending the cells at 5×10^5/ml in culture medium without IL-2 for 48 h. For stimulation, IL-2-deprived Kit 225 cells were resuspended at 2×10^7/ml in culture medium and incubated at 37°C without (control) or with 1 nM recombinant IL-2 for 5 min. Cells were harvested by centrifugation and incubated for 30 min on ice in cold lysis buffer (50 mM Tris pH 8, 150 mM NaCl, 0.5% NonidetP40, 10 mM NaF, 1 mM EDTA, 1 mM PMSF, 1 mM vanadate and 2 μg/ml each leupeptin and aprotinin). Lysates were clarified by centrifugation at 15 000×*g* for 20 min at 4°C.

2.4. GST pull down and kinase assay

To study phosphorylation mediated by kinases present in Kit 225 cells, IL-2-stimulated cell lysates (5 mg proteins) were precleared with GSH-Sepharose beads. Precleared lysates were rocked for 2 h at 4°C with 50 μg of purified GST fusion proteins adsorbed onto GSH-Sepharose beads. The beads were then washed in kinase buffer and incubated for 15 min at 30°C in kinase buffer supplemented with 10 μCi [γ-^{32}P]ATP. The reaction was stopped by boiling in sample buffer, and proteins were separated and analyzed as above.

2.5. Antibodies and immunoblotting

Polyclonal antibodies against IL-2Rβ and p56^{lck} proteins were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-GST monoclonal antibody was raised and purified in the laboratory.

For immunoblotting the membranes were blocked for 2 h at room temperature with 5% non-fat dry milk in Tris-buffered saline (TBS), 0.2% Tween (TBS-T). Membranes were washed four times in TBS-T and incubated for 1 h with optimal concentrations of primary antibodies diluted in TBS-0.05% Tween. Following four additional washes in TBS-T, the membranes were further incubated for 45 min with horseradish peroxidase-conjugated secondary antibodies (sheep anti-mouse Ig from Amersham). The bands were revealed using Amersham ECL reagents and autoradiographic films.

3. Results

3.1. Expression of IL-2R and subdomains as GST-fusion proteins in E. coli

The full length IL-2Rβ (βFL, amino acids 240–525) and γ (amino acids 288–369) cytoplasmic regions as well as IL-2Rβ proximal (βProx, amino acids 240–307), acidic (βAci, amino acids 306–383) and C-terminal (βCter, amino acids 382–525) subdomains were expressed as GST fusion proteins in *E. coli* (Fig. 1). These proteins could be expressed and purified in soluble form at reasonable levels, although, as shown in Fig. 1B, some level of degradation could not be avoided, particularly in the full length protein. In contrast, the acidic as well as the C-terminal subdomains could be routinely purified as single intact proteins. In addition, the full length GST-IL-2Rβ as well as the GST-βCter proteins were recognized in Western blot by two different antibodies raised against a C-terminal IL-2Rβ peptide (not shown).

3.2. In vitro phosphorylation of the IL-2Rβ chain by p56^{lck}

Although transient expression of p56^{lck} together with the IL-2Rβ chain in COS cells [17] clearly indicated that p56^{lck} was involved in the phosphorylation of the IL-2Rβ chain, no detailed study of the p56^{lck} target tyrosine residues in the IL-2Rβ chain has been reported. We have used the GST fusion proteins described above to determine which residues were phosphorylated in vitro by baculovirus-expressed p56^{lck}. As shown in Fig. 2A, p56^{lck} phosphorylated the full GST-IL-2Rβ as well as the acidic and C-terminal subdomains but neither the Prox subdomain nor the control GST protein. In addition, a similar GST-IL-2Rγ cytoplasmic domain con-

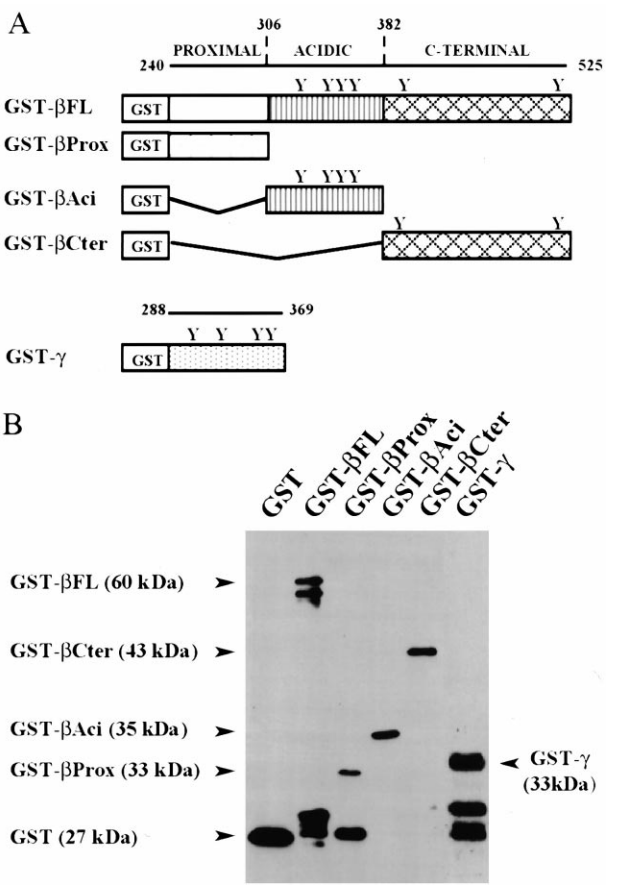

Fig. 1. Expression of GST-IL2R fusion proteins. A: Schematic representation of the IL-2R cytoplasmic subdomains and PGEX constructs encoding GST-βFL (amino acids 240–525), GST-βProx (amino acids 240–307), GST-βAci (amino acids 306–383), GST-βCter (amino acids 382–525) and GST-γ (amino acids 288–369). B: 100 ng of GST-IL-2R fusion proteins were purified on GSH-Sepharose beads, separated by SDS-PAGE on a 10% acrylamide gel, transferred onto a nitrocellulose membrane and analyzed by Western blot with anti-GST antibodies. The apparent molecular mass of each GST-fusion protein is indicated.

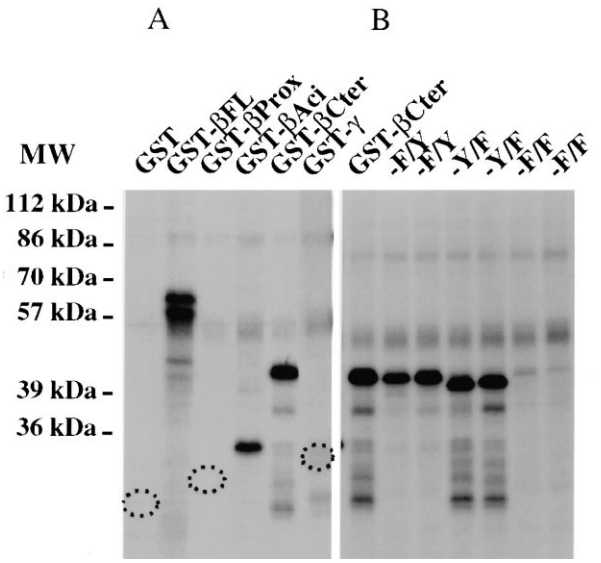

Fig. 2. p56[lck] phosphorylates the acidic and C-terminal subdomains of IL-2Rβ. A: Purified GST fusion proteins (100 pmol) were incubated with p56[lck]-containing Sf9 cell lysates (0.7 μl per point) in 30 μl of kinase buffer supplemented with 10 μCi [γ[32]P]ATP, then separated by SDS-PAGE. Following treatment with 1M KOH, the gel was dried and autoradiographed. Dotted circles indicate the position of GST, GST-βProx and GST-γ proteins as verified on the Coomassie blue-stained gel. B: The same experiment as in A was performed with GST-βCter proteins containing Tyr to Phe mutations at position 392 (noted F/Y), 510 (Y/F) or both (F/F). For each mutation two proteins obtained from independent *E. coli* colonies are shown.

struct, although containing four tyrosine residues, was not phosphorylated by p56[lck].

To identify the tyrosine residues which were phosphorylated by p56[lck], we generated IL-2Rβ GST fusion proteins in which each tyrosine residue was replaced by phenylalanine, individually or together as described in Section 2. A GST-βCter protein mutated on both Tyr-392 and Tyr-510 was not phosphorylated by p56[lck] (Fig. 2B). When Tyr-392 or Tyr-510 was individually mutated to phenylalanine, both proteins were phosphorylated to a somewhat reduced level as compared to the wild type protein (Fig. 2B). Thus both Tyr-392 and Tyr-510 are efficiently phosphorylated by p56[lck] in vitro.

Similar experiments were then undertaken with the acidic domain that contains four tyrosine residues at positions 338, 355, 358 and 361. For the purpose of these experiments, a shorter GST-βAci2 protein encoding residues 335–365 was constructed. It was then used to generate mutants in which a single Tyr, or all four Tyr were replaced by Phe. In addition, each tyrosine residue was individually reintroduced in the 4F construct. As shown in Fig. 3A, the GST-βAci2 construct was heavily phosphorylated, and mutation of all four tyrosines into phenylalanines (−4F, lane 8) reduced its phosphorylation to near background level. Analysis of the results of phosphorylation of the mutant proteins showed that replacing Tyr-338 by Phe did not reduce [32]P incorporation, indicating that Tyr-338 is probably not phosphorylated by p56[lck] (Fig. 3A, lane 4). Consistent with this hypothesis, the GST-βAci2 mutant containing Tyr-338 alone was not phosphorylated by recombinant p56[lck] (Fig. 3B, lane 1). In addition, replacement of either Tyr-355, -358 or -361 by Phe reduced phosphorylation significantly (Fig. 3A) and Tyr-355 as well as Tyr-361 were found to behave as efficient substrates when expressed alone,

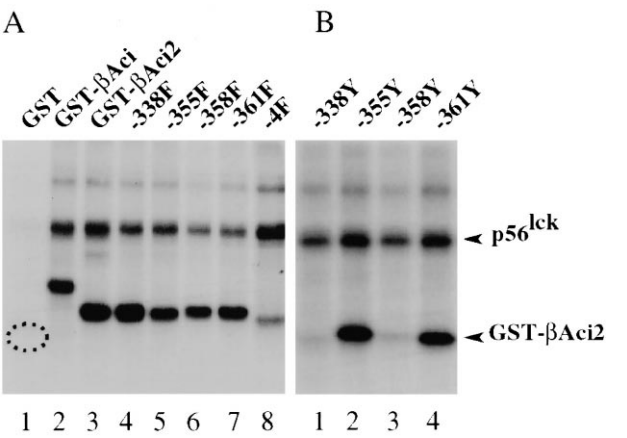

Fig. 3. Analysis of the phosphorylation of the IL-2Rβ acidic subdomain by recombinant p56[lck]. Phosphorylation by p56[lck] was performed as in Fig. 2, using the following GST fusion proteins as substrate: (A) wild type GST-βAci, GST-βAci2 (amino acids 334–365), GST-βAci2 proteins containing single Tyr to Phe mutations indicated by their positions, and GST-βAci2 protein where all Tyr have been mutated to Phe (−4F), and (B) GST-β4F proteins in which single tyrosine residues have been reintroduced at their original position. The autoradiograph shown here is from one representative experiment out of three performed.

whereas Tyr-358 was not phosphorylated (Fig. 3B). In these experiments, equal loading in each lane was verified on the Coomassie blue-stained gel (not shown).

3.3. p56[lck] association with GST-IL-2Rβ

The experiments described above showed that p56[lck] could

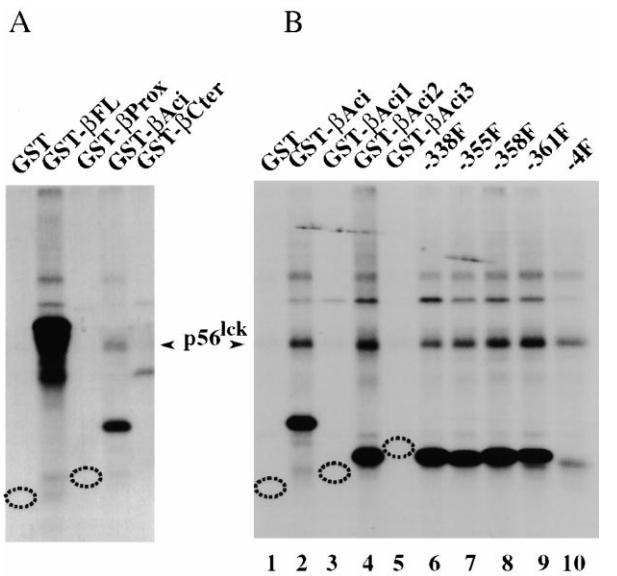

Fig. 4. Baculovirus-expressed recombinant p56[lck] associates with the IL-2Rβ acidic subdomain. A: Purified GST fusion proteins (100 pmol) were pre-incubated for 1 h at 4°C with 4 μl of p56[lck]-containing Sf9 cell lysate and the beads were washed four times in lysis buffer before the kinase reaction was allowed to proceed in the presence of [γ[32]P]ATP. Dotted circles indicate the position of GST and GST-βProx proteins as verified on the Coomassie blue-stained gel. B: The same experiment was performed with the shorter proteins GST-βAci1, 2 and 3, the GST-βAci4F and the single Tyr to Phe mutants of GST-βAci2 as indicated. Dotted circles indicate the position of GST and GST-βAci1 and 3 proteins. Time of exposure for this autoradiograph was 2 days.

phosphorylate not only tyrosine residues present in the acidic subdomain but also those in the C-terminal region. Furthermore, the presence of autophosphorylated p56^{lck}, particularly in Fig. 3, suggested that p56^{lck} remained bound to the GST fusion proteins. Indeed, previous reports have demonstrated that p56^{lck} associated with the acidic subdomain of the IL-2Rβ chain [17,18]. It was therefore of interest to directly address this question. For this purpose, in vitro phosphorylation experiments were performed under conditions slightly different from above. Specifically, recombinant baculovirus-expressed p56^{lck} was first incubated with the GST fusion proteins adsorbed onto GSH-Sepharose beads. Following several washes, the phosphorylation reaction was performed in the presence of [γ-^{32}P]ATP in kinase buffer. The rationale for this procedure was that under these conditions the GST fusion proteins could become phosphorylated by p56^{lck} only if they associated with p56^{lck} during the preincubation step. Results of a representative experiment in this series are shown in Fig. 4. The phosphorylation achieved under these conditions was indeed much lower than in the previous experiments (Fig. 2A) and films had to be exposed for a significantly longer period of time to obtain signals of similar intensity. However, the results clearly showed that the full length IL-2Rβ as well as the GST-βAci fusion proteins were efficiently phosphorylated whereas phosphorylation of GST-βCter was barely detectable (Fig. 4A). In addition, a faint band migrating at 56 kDa was detectable in the GST-βAci lane which probably represents associated p56^{lck} that had undergone autophosphorylation. This phosphorylated band could not be identified in association with the full length IL-2Rβ due to the high signal intensity generated by the 60 kDa GST-βFL itself, and was indeed absent in the GST, GST-βProx and GST-βCter lanes. To investigate the possible role of the tyrosine residues in this association, the experiments were repeated using the shorter GST-βAci subdomains as well as the Tyr to Phe mutants. The following observations were made in this experiment (Fig. 4B): first, the short βAci2 protein (lane 4) as well as the single Tyr to Phe mutants (lanes 6–9) were efficiently phosphorylated under these conditions, indicating that residues other than the tyrosines within this region (amino acids 335–365) were involved in binding p56^{lck}. This was confirmed by the observation that the 4F mutant (lane 10), although not phosphorylated, was still able to bind p56^{lck}, as seen by the presence of the 56 kDa autophosphorylated signal. Finally autophosphorylated p56^{lck} was not detectable in the presence of either GST-βAci1 or GST-βAci3 (lanes 3 and 5).

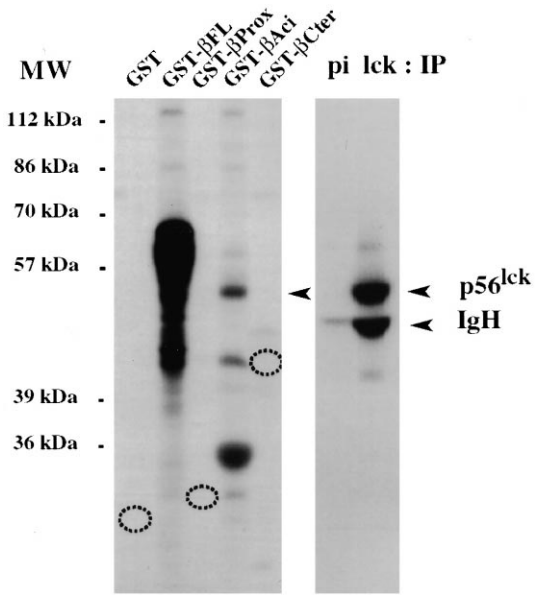

Fig. 5. p56^{lck} from Kit 225 cell lysates associates with the IL-2Rβ acidic subdomain. Purified GST fusion proteins were pre-incubated with cell lysates (5 mg proteins) from IL-2-stimulated Kit 225 cells for 2 h at 4°C, and the beads were washed four times in lysis buffer before the kinase reaction was allowed to proceed in the presence of [γ^{32}P]ATP. In the same experiment, p56^{lck} was immunoprecipitated (IP) from the Kit 225 cell lysate with anti-lck antibodies or preimmune serum (pi), and allowed to autophosphorylate under the same conditions. All samples were run in parallel on a 10% polyacrylamide gel, which was then dried and autoradiographed.

To confirm that an endogenous p56^{lck} could associate with and phosphorylate GST-IL2Rβ proteins, cell lysates from IL-2-stimulated Kit 225 cells were used. Cell lysates were incubated with the various GST-IL-2Rβ proteins and the kinase reaction was allowed to proceed after beads had been washed in lysis buffer. As shown in Fig. 5, lysates from IL-2-stimulated Kit 225 cells did indeed contain a tyrosine kinase activity that was able to bind and phosphorylate the full length GST-IL2Rβ and GST-βAci proteins, but not GST, GST-βCter or GST-βProx (Fig. 5). Furthermore, a 56 kDa phosphorylated band comigrating with p56^{lck} in an anti-p56^{lck} control immunoprecipitate from Kit 225 cell lysates was detectable in association with the GST-βAci protein.

Taken together, the results of the experiments described in Figs. 4 and 5 provide direct biochemical evidence that p56^{lck} physically associates with the IL-2Rβ chain through its acidic subdomain.

4. Discussion

An early and critical step in IL-2 signaling is the activation of several tyrosine kinases and phosphorylation of the IL-2 receptor complex. It was found earlier that p56^{lck} was one of the important kinases activated by IL-2 stimulation. Experiments using COS cells coexpressing p56^{lck} and the IL-2Rβ chain have demonstrated that p56^{lck} associates with the acidic domain of IL-2Rβ chain [17]. The data reported here are well in agreement with these previous findings, and provide additional information. Indeed we have shown that GST-IL2Rβ fusion proteins bound recombinant as well as natural p56^{lck}. This binding is direct, since it is observed in vitro with recombinant proteins, and the binding domain was assigned to a short stretch of 30 amino acid residues of the IL-2Rβ chain (amino acids 335–365). Furthermore, we showed that the association of IL-2Rβ with p56^{lck} does not require phosphorylation of the IL-2Rβ chain and that none of the four tyrosine residues that are present in this subdomain appears to be involved in the interaction with p56^{lck}. These findings clearly exclude a role of the SH2 domain of p56^{lck} in its association with the IL-2Rβ chain.

The IL-2Rβ acidic domain is sufficient for lck binding, but lck activation requires the serine-rich (or proximal) domain [23]. A large body of evidence indicates that the IL-2Rβ-prox region is strictly required for IL-2-induced DNA synthesis [4,8,24,25], but also for prevention of apoptosis [26]. Although necessary, the IL-2Rβ-prox region is not sufficient for proliferation and additional events are required that depend upon the presence of either the acidic or the C-terminal

domain [4,6,7,27]. The IL-2Rβ acidic region seems to be responsible for a pathway leading to c-fos and c-jun induction [28] whereas the C-terminal domain regulates STAT5 activation [4].

Transfection experiments using mutated or truncated IL-2Rβ constructs clearly demonstrated that activation of the Ras pathway, like activation of $p56^{lck}$, required both the serine-rich and the acidic IL-2Rβ subdomains [28]. One of the critical residues in the acidic subdomain is Tyr-338, which lies within an NPQY environment representing a consensus binding site for the PTB domain of the Shc adaptor protein [7,25]. In parallel experiments (unpublished) we have indeed observed that Shc associates with GST-βAci depending upon phosphorylation of Tyr-338. However, our finding that Tyr-338 is not phosphorylated by $p56^{lck}$ suggests that the primary role of $p56^{lck}$ in activation of the Ras pathway may rather be the phosphorylation of Shc as recently demonstrated [29]. Since Tyr-338 phosphorylation is required for the recruitment of Shc to the IL-2Rβ chain and the activation of the Ras pathway, another kinase must be responsible for Tyr-338 phosphorylation. This kinase could be JAK-1 since it has been reported that, when coexpressed in COS-7 cells with IL-2Rβ chain constructs, JAK-1 was able to phosphorylate Tyr-338 [7].

The acidic domain of the IL-2Rβ chain contains three additional tyrosine residues. Using GST-IL2Rβ fusion proteins and a series of Tyr to Phe mutants, our data clearly demonstrate that the three tyrosine residues Tyr-355, Tyr-358 and Tyr-361 behaved as efficient substrates in vitro for baculovirus-expressed $p56^{lck}$. However, the construct containing Tyr-358 and Phe at all other relevant positions was not phosphorylated by $p56^{lck}$. This result suggests that the presence or the prior phosphorylation of one of these nearby Tyr residues may influence the activity of $p56^{lck}$ towards Tyr-358. Sequential phosphorylation of multiple residues has previously been described in other systems, particularly for serine/threonine or dual specificity kinases but little information is available for src family tyrosine kinases. The precise roles of residues Tyr-355, Tyr-358 and Tyr-361 are not yet elucidated. Experiments using chimeric proteins suggest that they are not critical for IL-2-induced cell proliferation [6,27]. Our data show that $p56^{lck}$ can phosphorylate these residues but it is not known whether these phosphorylated tyrosines serve as a docking site for other signaling molecules, and in parallel experiments, we have not identified additional proteins that could associate with the phosphorylated IL-2Rβ acidic subdomain.

In vitro, $p56^{lck}$ phosphorylates very efficiently Tyr-392 and Tyr-510 in the C-terminal domain of the IL-2Rβ chain. These two tyrosine residues of the IL-2Rβ are known to be involved in the activation of STAT5, acting as docking sites for the STAT5 SH2 domain [30,31]. In particular, phosphorylated peptides derived from the IL-2β chain Tyr-392 or Tyr-510 have been reported to inhibit the DNA binding activity of STAT5 [31]. In stable transfection experiments, it has also been found that Tyr-392 alone could mediate STAT5 activation as well as Tyr-510 [4,32,33]. Thus, by phosphorylating the IL-2Rβ chain STAT5 docking sites, $p56^{lck}$ may play an essential role in STAT5 activation by IL-2, and it has also been recently established that $p56^{lck}$ is involved in STAT5 activation by the T-cell receptor [34].

We have shown here that $p56^{lck}$ phosphorylates five out of the six tyrosine residues within the IL-2Rβ chain. It is of interest to note that a strict respect of consensus src kinase target sequences [35] is not observed for all these residues. In particular Tyr-355 and -358 are not preceded by acidic residues, whereas an aspartate residue is indeed present at position2 of Tyr-392 and of Tyr-510.

These results provide a better insight into the interactions between $p56^{lck}$ and the IL-2Rβ chain. Experiments in progress are using these GST-IL-2Rβ fusion proteins to study their association with relevant signal transducing molecules activated by IL-2.

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