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Immunology

Evidence for a role for the phosphotyrosine-binding domain of Shc in interleukin 2 signaling

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ABSTRACT **Stimulation via the T-cell growth factor interleukin 2 (IL-2) leads to tyrosine phosphorylation of Shc, the interaction of Shc with Grb2, and the Ras GTP/GDP exchange factor, mSOS. Shc also coprecipitates with the IL-2 receptor (IL-2R), and therefore, may link IL-2R to Ras activation. We have further characterized the Shc–IL-2R interaction and have made the following observations. (*i*) Among the two phosphotyrosine-interaction domains present in Shc, the phosphotyrosine-binding (PTB) domain, rather than its SH2 domain, interacts with the tyrosine-phosphorylated IL-2R β chain. Moreover, the Shc-PTB domain binds a phosphopeptide derived from the IL-2R β chain (corresponding to residues surrounding Y338, SCFTNQGpYFF) with high affinity. (*ii*) *In vivo*, mutant IL-2R β chains lacking the acidic region of IL-2Rβ (which contains Y338) fail to phosphorylate Shc. Furthermore, when wild type or mutant Shc proteins that lack the PTB domain were expressed in the IL-2-dependent CTLL-20 cell line, an intact Shc-PTB domain was required for Shc phosphorylation by the IL-2R, which provides further support for a Shc-PTB–IL-2R interaction *in vivo*. (*iii*) PTB and SH2 domains of Shc associate with different proteins in IL-2- and T-cell-receptor-stimulated lysates, suggesting that Shc, through the concurrent use of its two different phosphotyrosine-binding domains, could assemble multiple protein complexes. Taken together, our *in vivo* and *in vitro* observations suggest that the PTB domain of Shc interacts with Y338 of the IL-2R and provide evidence for a functional role for the Shc-PTB domain in IL-2 signaling.**

Growth stimulation by interleukin 2 (IL-2) is mediated via the multisubunit IL-2 receptor (IL-2R). IL-2R is composed of a 55-kDa α chain, a 70- to 75-kDa β chain, and a 64-kDa γ chain (reviewed in ref. 1). Biochemical and genetic studies indicate an essential role for the cytoplasmic domains of the IL-2R β chain (286 aa) and IL-2R γ chain in mediating signals via the IL-2R. Within the cytoplasmic tail of the β chain, a serine-rich region (which is essential for mitogenesis and for interactions with the Syk and JAK1 tyrosine kinases) and an acidic region (necessary for Ras activation and for binding of the Src-family kinase Lck) have been defined (2–5). The cytoplasmic tail of the γ chain has been shown to interact with the JAK3 tyrosine kinase (6–11).

Following IL-2 stimulation, a number of proteins, including the IL-2R β and γ chains, are tyrosine-phosphorylated (2, 9, 12). One of the prominent tyrosine-phosphorylated intracellular substrates is the adapter protein Shc (13–15). Both the 52- and 46-kDa isoforms of Shc (with the 52-kDa isoform being more prominent) are tyrosine-phosphorylated upon IL-2 stimulation. Tyrosine-phosphorylated Shc subsequently interacts with the adapter protein Grb2, which, in turn, interacts with the Ras guanine nucleotide exchange factor, mSOS (16, 17). The interaction of Shc with activated receptors, and the formation of a complex of Shc–Grb2–mSOS has been implicated in events leading to Ras activation for a number of receptors, including growth factor receptors, antigen receptors on T and B cells, and cytokine receptors (reviewed in ref. 18).

Shc contains two domains capable of interacting with tyrosine-phosphorylated proteins: the C-terminal SH2 (Shc-SH2) domain and the recently described N-terminal phosphotyrosine-binding (PTB) domain (19, 20). The Shc SH2 domain has been shown to interact with the receptors for epidermal growth factor (EGF) (13, 21), platelet-derived growth factor (PDGF) (22), and T cell receptor (TCR)-ζ chain (23). On the other hand, the PTB domain has been shown to interact with phosphorylated Trk, EGF receptor, c-ErbB2, polyoma middle T antigen (mT), and a novel 145-kDa tyrosine-phosphorylated protein in TCR, PDGF, and fibroblast growth factor-stimulated cells (19, 20, 24, 25). The solution structure of the Shc-SH2 domain bound to a TCR-ζ chain peptide, as well as peptide library screens, indicated that residues +1 and +3 C terminal to the phosphotyrosine make relevant contacts with the Shc-SH2 domain (26, 27). However, the specificity of the PTB domain appears to be determined by residues which are N terminal to the phosphotyrosine (−1, −3, etc.). A motif that contains a critical asparagine at the −3 position and can form a tight β-bend conformation has been predicted and demonstrated as a preferred sequence for interactions with the PTB domain (28–31).

We have previously reported that upon IL-2 stimulation, Shc forms a complex with the tyrosine-phosphorylated IL-2R β chain (15) and with Grb2 and mSOS and may couple IL-2R activation to Ras activation. However, the nature and requirement for the Shc–IL-2R interaction were not delineated. In this report, we demonstrate a PTB-dependent interaction of Shc with the acidic region of the IL-2R β chain. Through the use of mutant receptors and mutant Shc proteins, we ascribe a functional relevance to this interaction in leading to Shc phosphorylation.

MATERIALS AND METHODS

Cells. Maintenance of the IL-2-dependent CTLL-20 cells and the murine T-cell hybridoma BYDP have been described (15, 32). Human peripheral blood lymphocytes (PBL) were isolated using standard techniques and stimulated with 1 μg/ml phytohemagglutinin (PHA) at a density of 5×10^5

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Abbreviations: IL-2, interleukin 2; IL-2R, IL-2 receptor; PTB, phosphotyrosine binding; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; TCR, T-cell receptor; mT, middle T antigen; PHA, phytohemagglutinin; wt, wild type; FL, full-length Shc; N, N-terminal domain; ΔN, N-terminal domain deleted Shc; CH, collagen homology domain, NCH, N+CH domain; HA-FL, HA-tagged full-length Shc protein; HA-ΔN, N-terminal domain-deleted Shc protein; anti-PTyr, anti-phosphotyrosine.

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cells/ml for 3 days at 37°C. The proliferating T cells (PHA-blasts) were washed four times and starved overnight (10–14 h) in RPMI 1640 medium with 0.5% serum before use. BaF/Bo3 cells expressing wild type (wt) and mutant IL-2R β chains have been described (kindly provided by Tada Taniguchi) (2). The cells were maintained in medium supplemented with 10% WEHI-231 conditioned medium as a source of interleukin (IL-3)]. The cells were washed 4×, resuspended in medium without IL-3, and starved for 6–8 h before use in stimulation.

Generation of CTLL-20 Transfectants. Oligonucleotides encoding two tandem HA-tags were first cloned into pBluescript vector (Stratagene). Full-length Shc (FL) or N-terminal domain deleted Shc (ΔN) were subcloned in-frame into this vector with the HA-tag at the C terminus, then subcloned into the pMHNeo eukaryotic expression vector (33). DNA (20 μg) encoding these proteins was transfected into CTLL-20 cells by electroporation. Transfectants were selected in 2 mg of G418 per ml and positive clones were identified by anti-Shc immunoblotting of total lysates using antibodies directed to the Shc-SH2 domain (Transduction Laboratories, Lexington, KY).

Antibodies. Affinity-purified polyclonal and monoclonal anti-Shc antibody and horseradish peroxidase-linked anti-phosphotyrosine antibody (RC20) were obtained from Transduction Laboratories. Anti-phosphotyrosine antibody 4G10 was obtained from Upstate Biotechnology (Lake Placid, NY). Antibody to the murine IL-2R β chain and human IL-2R β chain were purchased from Biosource (Camarillo, CA) and Endogen (Cambridge, MA) and from Santa Cruz Biotechnology. The anti-ζ antiserum was a gift from L. Samelson (National Institutes of Health).

IL-2 and TCR Stimulation. CTLL-20 cells were starved for 4 h without IL-2 in complete medium and PHA-blasts were starved as described above. Cells ($10–20 \times 10^6$ per sample) were stimulated with 200 units of recombinant human IL-2 per ml (a gift from Hoffman–La Roche) for 5 min at 37°C. The cells were lysed in a buffer containing 1% Nonidet P-40, 50 mM Tris (pH 7.6), 150 mM NaCl, 1 mM Na_3VO_4, 10 mM NaF, 10 mM sodium pyrophosphate, 10 μg/ml each of aprotinin and leupeptin, and 2 mM phenylmethylsulfonyl fluoride (15). BYDP cells (20×10^6) and PHA-blasts (20×10^6) were stimulated with anti-CD3 and anti-CD4 antibodies as described (25). Immunoprecipitations and immunoblotting were performed as described (15). Briefly, anti-Shc or anti-IL-2R β chain antibody was added to the lysates along with protein A/protein G Sepharose beads and incubated for 2 h at 4°C. The beads were washed extensively and the proteins bound to the beads were resolved by SDS/PAGE, transferred to nitrocellulose (Schleichler & Schuell), immunoblotted with the indicated antibody, and developed by the enhanced chemiluminescence (ECL) system (Amersham).

Gluthathione *S*-Transferase (GST)-Shc Fusion Proteins and Precipitations. GST-Shc fusion protein encoding Shc N+CH (denoted NCH), CH, and SH2 domains have been described (25). GST-Shc N (1–232) was generated by polymerase chain reaction (PCR) using primers 5′ and 3′ of the desired regions on Shc cDNA and was cloned into pGEX2T (Pharmacia) and expressed in *Escherichia coli*. Precipitations with different Shc domains, GST alone, or GST-Shc fusion proteins (2–4 μg) bound to glutathione Sepharose beads were performed as described (25).

Peptides and Inhibition Studies. Tyrosine phosphorylated 10-mer IL-2R peptide (SCFTNQG**pY**FF) and the control 9-mer Shc peptide (PS**pY**VNVQNL) were synthesized as described (34). For inhibition studies, 25 μg of peptides were incubated with 2–4 μg of fusion proteins in a 50 μl volume of lysis buffer for 30 min on ice before the addition of unstimulated or stimulated lysates and were analyzed as described above. The affinity of the IL-2R peptide for Shc-PTB was measured by its ability to inhibit the interaction of purified Shc-PTB to labeled middle T peptide (25).

RESULTS

IL-2 stimulation of CTLL-20 cells leads to tyrosine-phosphorylation of Shc and also to the coprecipitation of Shc with the IL-2R β chain (15). To determine if these events occur in primary human T cells, we examined PHA-blasts following IL-2 activation. Both tyrosine-phosphorylation of Shc and the coprecipitation of the tyrosine-phosphorylated IL-2R β chain with Shc were observed in PHA-blasts (Fig. 1). Thus, in both cell lines and primary lymphocytes, stimulation via the IL-2R leads to tyrosine phosphorylation of Shc and its association with the tyrosine-phosphorylated IL-2R β chain. Shc immunoprecipitates also contained the p145 protein that has been seen in Shc immunoprecipitates after stimulation via a number of receptors (20, 23, 35, 36).

Shc Interacts Via Its N-Terminal Domain with the IL-2R β Chain. Shc is composed of an N-terminal domain (N), a central collagen-homology (CH) domain, and a C-terminal SH2 domain. To determine which domain of Shc interacts with the IL-2R β chain, we incubated lysates from IL-2-stimulated CTLL-20 cells with GST-fusion proteins encoding different domains of Shc. Interestingly, fusion proteins with the N+CH domains (NCH) precipitated a band corresponding to the IL-2R β chain, while the SH2 domain did not (Fig. 2*a*). Further experiments indicated that the N-terminal domain, and not the CH domain, binds to the IL-2R β chain (Fig. 2*b Upper*; data not shown).

Since the N-terminal region of Shc contains the recently described PTB domain (19, 20, 24, 28), we determined if the Shc-NCH interaction with IL-2R β chain can be inhibited by a mT phosphopeptide that binds to the Shc-PTB domain. The precipitation of IL-2R β chain was inhibited by the mT peptide but not by a control phosphopeptide, suggesting a PTB-dependent interaction with specific tyrosine(s) within the IL-2R β chain (Fig. 2*b Upper*). Although Shc N alone can precipitate IL-2Rβ, it is intriguing that we routinely found that GST-NCH fusion protein precipitated the IL-2R β chain better than GST-N alone (Fig. 2*b Upper*). However, we did not observe this difference between N and NCH with respect to their interaction with p145 (data not shown). Thus, although the Shc–IL-2R interaction is PTB-dependent, a contribution

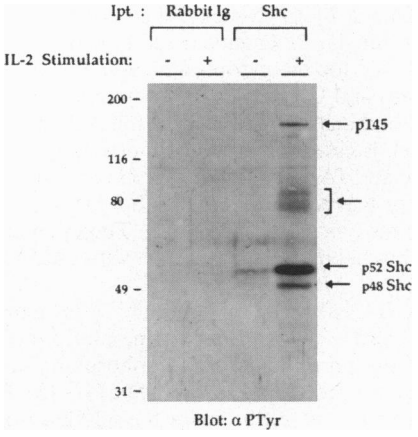

FIG. 1. Coprecipitation of Shc and IL-2R β chain upon IL-2 stimulation. PHA-blasts (20×10^6) were stimulated with or without IL-2 and immunoprecipitated with control rabbit Ig or anti-Shc antibodies. The proteins were resolved by SDS/8% PAGE, and analyzed by anti-phosphotyrosine (anti-PTyr) immunoblotting using RC20H antibody. The band indicated by the unmarked arrow indicates the IL-2R β chain (based on comigration with IL-2R β chain, data not shown).

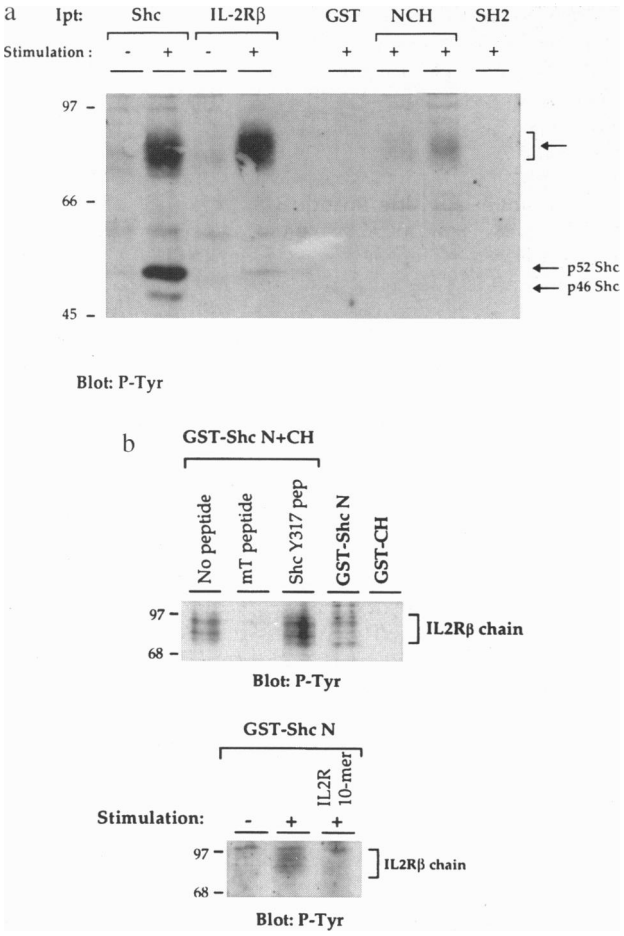

FIG. 2. Shc interacts via its PTB domain with the IL-2R β chain. (*a*) Lysates from IL-2 stimulated CTLL-20 cells (15×10^6) were incubated with GST alone, two different preparations of GST-NCH, and GST-SH2 fusion proteins. The bound proteins were analyzed by anti-PTyr immunoblotting (RC20H). Immunoprecipitation with antibodies to Shc and IL-2R β chain from the same experiment are also shown. The band indicated as the IL-2Rβ was identified as such by anti-IL-2Rβ immunoblotting (data not shown). The bands indicated as Shc were identified by anti-Shc immunoblotting (data not shown and ref 15). (*b*) GST-NCH fusion proteins were incubated with mT or a control phosphopeptide for 30 min on ice and subsequently mixed with activated CTLL-20 lysates. (*Upper*) The precipitation of IL-2R β chain was assessed by anti-PTyr immunoblotting. (*Lower*) A similar experiment using GST-N fusion protein and the 10-mer IL-2R phosphopeptide is shown.

by the CH domain (such as stabilizing the PTB interaction with IL-2Rβ) cannot be excluded.

A number of studies have indicated that sequences which contain a −3 asparagine and can make a tight β-bend conformation interact with Shc-PTB (28–31, 37). IL-2R β chain contains a SCFTNQGYFF sequence surrounding Y338 within the acidic region that may provide a potential Shc-PTB binding site. We addressed this possibility by synthesizing a 10-mer phosphopeptide (SCFTNQG**pY**FF, denoted IL-2R peptide), and several lines of evidence indicated that this peptide interacted specifically with the Shc-PTB. (*i*) *In vitro* binding studies indicated that the IL-2R peptide bound to the Shc-PTB with a relative affinity of 0.8 ± 0.05 μM (compared to 0.6 ± 0.06 μM for mT) (data not shown and ref. 38). (*ii*) The IL-2R peptide was able to inhibit the binding of Shc N with the IL-2R β chain (Fig. 2*b Lower*). (*iii*) The 10-mer IL-2R peptide was also able to inhibit the interaction of the Shc-PTB with p145 (data not shown).

Acidic Region of IL-2R β Chain Is Essential for Shc Phosphorylation. To determine if the acidic region (which contains Y338) influences Shc-mediated signaling, we examined BaF/Bo3 cells expressing either the wt, acidic region-deleted (A^-) or serine-rich region deleted (S^-) IL-2R β chains. Tyrosine phosphorylation of Shc was detected in cells expressing the wt IL-2R β chain, but not in cells expressing S^- or A^- receptors (Fig. 3 *Top*). Immunoblotting for the level of Shc indicated that the same level of Shc protein was precipitated in all lanes (Fig. 3 *Middle*). Due to low levels of expression of the IL-2R in these cells it was difficult to demonstrate coprecipitation of Shc and IL-2R from these cells.

While there are no tyrosines in the serine-rich region, the acidic region contains four potential tyrosines (Y338, Y355, Y358, and Y361). When we examined the tyrosine phosphorylation of the IL-2R β chain itself (by anti-IL-2Rβ immunoprecipitations), the A^- receptor was still phosphorylated (albeit less well compared to wt, possibly due to the lack of the four tyrosines within the acidic region) (Fig. 3 *Bottom*). The lack of phosphorylation of the S^- receptor was not surprising because the serine-rich region is essential for initiating phosphorylation via the IL-2R (2, 5). Interestingly, phosphorylation of tyrosine-residues outside of the acidic region was still occurring in the A^- receptor, yet was insufficient for Shc phosphorylation. A simple model to explain these results was that Shc phosphorylation may occur in sequential steps: the initial phosphorylation of the IL-2R β chain on critical tyrosines within the acidic region, followed by docking of Shc to the phosphorylated receptor and the subsequent tyrosine-phosphorylation of Shc.

The Y355/Y358 receptor, which has dual Tyr → Phe point mutations of the two sites in the IL-2R β chain was comparable to the wt receptor in phosphorylating Shc (Fig. 3 *Top*), thereby ruling out a critical role for these two tyrosines. This suggested that one of the other two tyrosines (Y338 or Y361) may be involved in the interaction with Shc. Examination of residues surrounding Y361 indicated a lack of the critical −3 asparagine shown to be necessary for Shc-PTB binding (28–31). Taken together with our phosphopeptide studies using the Y338 IL-2R peptide, it is compelling that Y338 of IL-2Rβ most likely serves as the Shc interaction site *in vivo*.

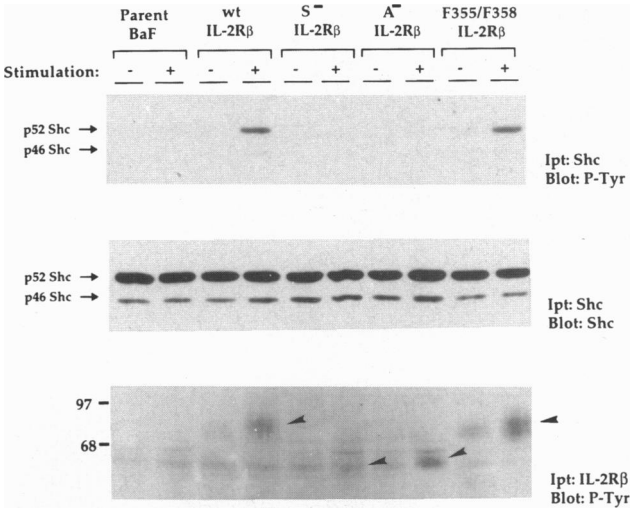

FIG. 3. Acidic region of the IL-2 β chain influences Shc phosphorylation. BaF/Bo3 cells expressing wt, serine-rich region-deleted (S^-), acidic-region deleted (A^-), or Tyr → Phe mutated (Y355/358) IL-2R β chains were stimulated with IL-2, immunoprecipitated with anti-Shc (*Top*) or anti-IL-2R β chain antibodies (*Bottom*) and immunoblotted with anti-PTyr antibody 4G10. The blot from the *Top* panel was reprobed with monoclonal anti-Shc antibody (*Middle*). The arrows in the *Bottom* panel point to IL-2R β chain. The S^- and A^- receptors are ≈10 kDa lower in molecular weight. The migration of wt and mutant IL-2R β chains were identified by direct anti-IL-2Rβ immunoblotting (data not shown).

N-Terminal Domain of Shc Is Required for Its Phosphorylation. If the PTB-dependent interaction of Shc with IL-2R is necessary for tyrosine phosphorylation of Shc, we speculated that Shc proteins lacking the PTB domain will fail to be phosphorylated. To address this possibility, we generated stable CTLL-20 transfectants expressing either the HA-tagged full-length (HA-FL) or N-terminal domain-deleted (HA-ΔN) Shc protein (Fig. 4*a*). It is important to note that the ΔN protein still contained an intact Y317, the major tyrosine-phosphorylation site on Shc. Following IL-2 stimulation, the HA-FL Shc protein was efficiently phosphorylated, whereas the ΔN mutant failed to be tyrosine-phosphorylated (Fig. 4*b Lower*). Phosphorylation of endogenous p52 Shc was not affected by the coexpression of transfected ΔN Shc. Anti-Shc immunoblotting (Fig. 4*b Lower*) and anti-HA blotting (data not shown) demonstrated the presence of ΔN Shc protein. Moreover, only the HA-FL Shc, but not the ΔN mutant, coprecipitated with the IL-2R β chain (Fig. 4c). It is noteworthy that a similar ΔN mutant when expressed in NIH 3T3 cells was tyrosine-phosphorylated upon EGF stimulation (39). Thus, the failure of phosphorylation of ΔN Shc appears to be specific for the IL-2R and further supports the model that binding of Shc to the IL-2R precedes phosphorylation of Shc.

PTB and SH2 Domains of Shc Interact with Different Phosphoproteins in TCR-Stimulated Lysates. We have previously demonstrated that Shc, via its SH2 domain, can interact with the tyrosine-phosphorylated TCR-ζ chain (23, 27). The recent description of the PTB domain of Shc prompted us to examine which phosphoproteins interact with the PTB domain of Shc following TCR stimulation. We incubated lysates from a murine TCR-stimulated cell line (BYDP 4.3) and human PHA-lymphoblasts with GST-NCH, GST-CH, or GST-SH2 fusion proteins (Fig. 5). Both from lysates of BYDP cells and PHA-blasts, GST-NCH precipitated the p145 protein. On the other hand, the SH2 domain did not interact with p145, but instead precipitated a prominent band corresponding to the TCR-ζ chain. The PTB-dependent precipitation of p145 from these lysates was inhibited by the mT phosphopeptide, and the precipitation of TCR-ζ by Shc-SH2 was inhibited by a TCR-ζ phosphopeptide (data not shown and ref. 23). These data suggest that PTB and SH2 domains of Shc can interact with different proteins during T-cell activation. It is also noteworthy that the PTB domain of Shc did not precipitate any of the chains of the TCR/CD3 complex, suggesting that recruitment of Shc to the TCR occurs primarily via the SH2 domain.

DISCUSSION

In this report we have characterized the interaction of Shc with the IL-2R β chain and make the following observations. (*i*) The interaction of Shc with the IL-2R β chain is mediated by the PTB domain, rather than the SH2 domain. (*ii*) BaF/Bo3 cells expressing mutant IL-2R β chains revealed that the acidic region of the IL-2R β chain (comprising Y338) is essential for Shc phosphorylation. Experiments using receptors carrying point mutations within the acidic region of the IL-2R β chain, in conjunction with the use of phosphopeptides derived from the acidic region of the receptor, implicate Y338 as the site of

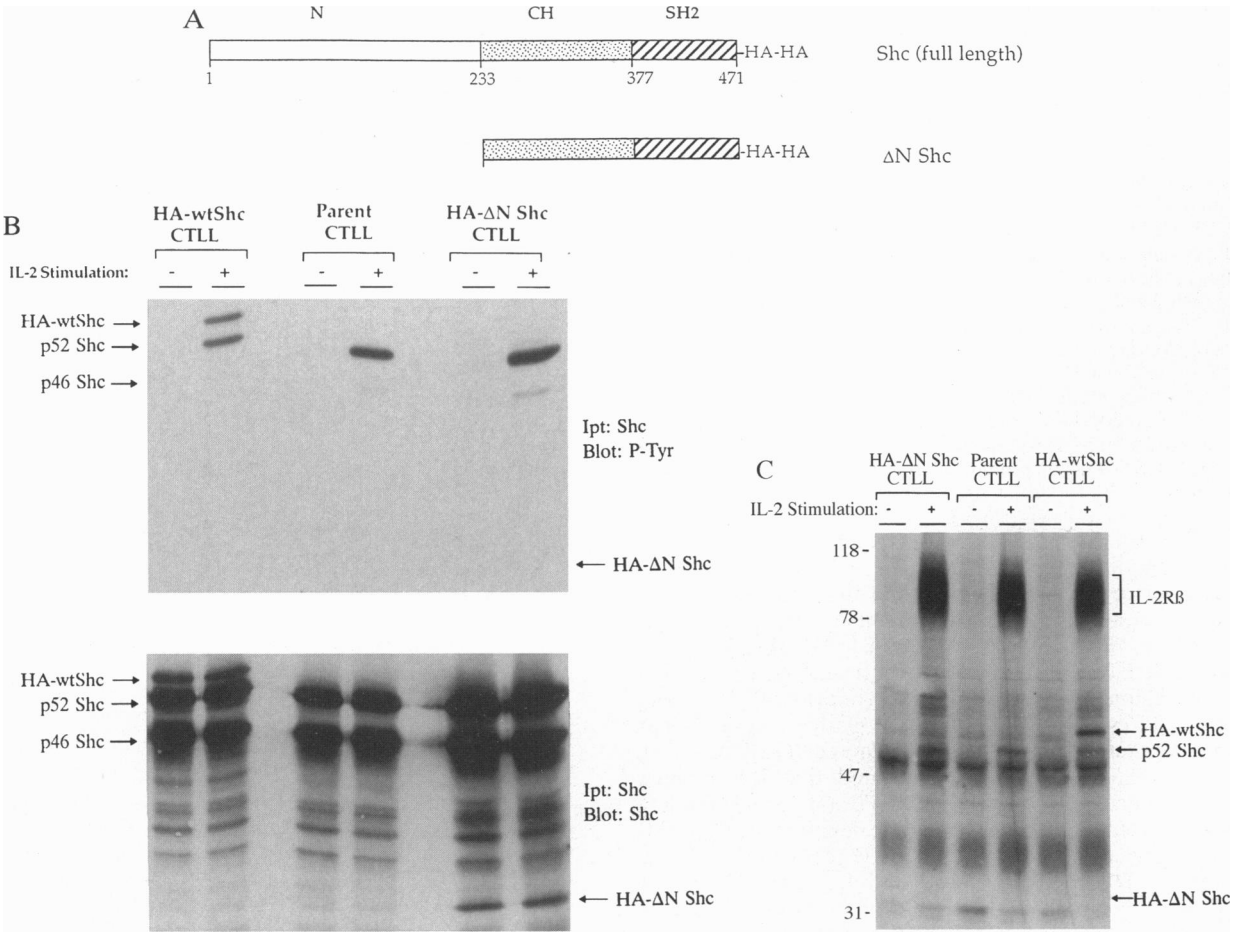

FIG. 4. PTB-domain deleted Shc proteins fail to be phosphorylated upon IL-2 stimulation. (*a*) Schematic representation of HA-tagged full length and ΔN Shc proteins. (*b*) Transfectants and parent CTLL-20 cells were stimulated with IL-2, immunoprecipitated with polyclonal anti-Shc, and immunoblotted with anti-PTyr (*Upper*) or anti-Shc mAb (*Lower*). (*C*) Transfectants and parent CTLL-20 were stimulated with IL-2, immunoprecipitated with anti-IL-2R β chain antibody, and immunoblotted with anti-PTyr antibody.

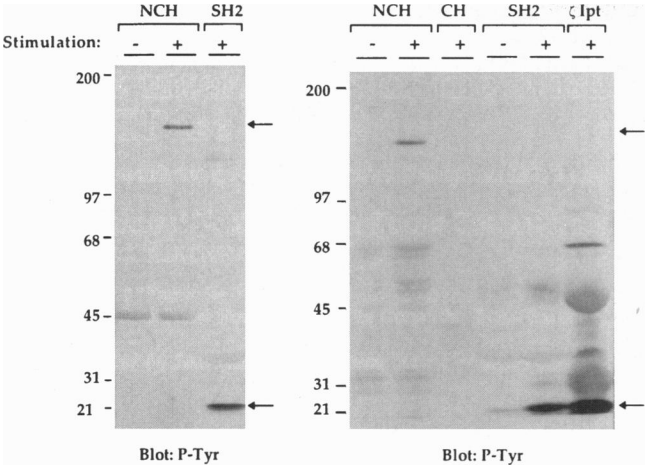

FIG. 5. Shc-PTB and Shc-SH2 domains interact with different phosphoproteins in activated T-cell lysates. GST-NCH or GST-SH2 fusion proteins were incubated with lysates from a murine T-cell hybridoma (BYDP 4.3, *Left*) or PHA-blasts (*Right*) stimulated with anti-CD3 and anti-CD4 antibodies, and the bound proteins were analyzed by anti-PTyr immunoblotting. Anti-TCR-ζ chain immunoprecipitation from PHA-blasts is shown for comparison. p145 and TCR-ζ are indicated by arrows.

interaction with Shc. (*iii*) CTLL-20 transfectants expressing a PTB-deleted form of Shc indicate that an intact N-terminal domain of Shc is necessary for Shc phosphorylation following IL-2 stimulation. This provides a functional role for the PTB domain of Shc in IL-2 signaling.

Our data using GST-fusion proteins indicated that the Shc interaction with the IL-2R is PTB-dependent, but not SH2 domain-dependent. This was further supported by the observation that only the HA-FL proteins but not the PTB-deleted HA-ΔN Shc proteins coprecipitate with the phosphorylated IL-2R β chain. Although we have made several attempts to generate CTLL-20 lines expressing the PTB domain alone to assess a direct PTB–IL-2R interaction *in vivo*, we have failed in our efforts thus far. Whether this reflects dominant negative effects of expressing Shc N alone in CTLL-20 cells needs to be investigated. Our data strongly support a model whereby recruitment of Shc to the IL-2R occurs first, followed by tyrosine-phosphorylation of Shc. It is important to note that while the ΔN Shc failed to be phosphorylated via the IL-2R, this mutant protein is tyrosine-phosphorylated by stimulation via the EGF receptor (39). This may reflect differences between EGFR and IL-2R in recruitment of Shc and a greater dependence of the IL-2R on the PTB domain of Shc.

During TCR signaling, two tyrosine-phosphorylated proteins (Shc and p36) associate with the SH2 domain of Grb2 (23, 40, 41). We did not observe p36 in Grb2 or mSOS immunoprecipitates following IL-2 stimulation of CTLL-20 cells or PHA-blasts and we only observed Shc as the major coprecipitating tyrosine-phosphorylated protein (data not shown). The correlation between the lack of Shc phosphorylation and the failure to activate Ras by the same mutant IL-2Rβ chains, supports the idea that Shc may play an important role in events leading to Ras activation by the IL-2R. However, it should be noted that an interaction between Raf and IL-2R β chain has also been documented (42). The relative importance of Raf-bound to the IL-2R and Shc-mediated Ras activation, which in turn leads to Raf activation, remains to be determined.

In activated T-cell lysates, we find that GST-Shc fusion proteins containing PTB and SH2 domains precipitate different phosphoproteins. Interestingly, while the Shc-SH2 domain precipitated the TCR-ζ chain, the PTB domain did not precipitate any of the chains of the TCR/CD3 complex, but instead interacted with a 145-kDa phosphoprotein. Thus, only the SH2 domain of Shc appears to be involved in binding to the TCR, while the PTB domain may be "free" to recruit other proteins (such as p145) to the proximity of the receptor. Whether a TCR–Shc–p145 complex actually exists in cells remains to be determined. Interestingly, Shc-PTB interacts with both p145 and the IL-2R in IL-2-stimulated cells. Since Shc contains only one PTB domain and currently there is no evidence for dimerization of Shc, the Shc-p145 and Shc-IL-2R most likely exist as separate complexes.

When naive T cells are activated appropriately by antigen, they secrete IL-2 and also express the high affinity receptors for IL-2. The binding of IL-2 to the IL-2R provides an autocrine/paracrine stimulation leading to proliferation of antigen-specific T cells. It is striking that the same adapter protein Shc, through the alternative use of two different phosphotyrosine-binding domains, can participate in the initial stimulatory phase via the TCR and the subsequent proliferative phase via the IL-2R. Along with our earlier observations that Shc can regulate the interaction of Grb2 with mSOS in T cells (25), these data indicate multiple roles for Shc in different stages of T cell activation.

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