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The IL-2 Receptor Promotes Proliferation, \( bcl-2 \) and \( bcl-x \) Induction, But Not Cell Viability Through the Adapter Molecule Shc

James D. Lord,†‡ Bryan C. McIntosh,* Philip D. Greenberg,†‡§ and Brad H. Nelson‡‡

IL-2, the principal mitogenic factor for activated T cells, delivers a proliferative signal through ligation of the heterotrimeric IL-2R. This proliferative signal is critically dependent upon cytoplasmic tyrosines on the \( \beta \)-chain of this receptor (IL-2R\( \beta \)) becoming phosphorylated in response to ligand. We found that at least one of these tyrosines (Y338) also mediates cell survival and induction of \( bcl-2 \), \( bcl-x \), and \( c-my c \) in the murine T cell line CTLL-2. Since the adapter molecule Shc binds to phosphorylated Y338, the specific contribution of Shc to these events was evaluated. An IL-2R\( \beta \)/Shc fusion protein, in which Shc was covalently tethered to a truncated version of IL-2R\( \beta \) lacking all cytoplasmic tyrosines, revealed a robust proliferative signal mediated through Shc. This Shc-mediated signal induced expression of \( c-my c \) as well as the antiapoptotic genes \( bcl-2 \) and \( bcl-x \) with normal magnitude and kinetics. Nonetheless, signals from this fusion protein failed to sustain the long-term viability of CTLL-2 cells. Thus, induction of \( bcl \) family genes and delivery of a competent proliferative signal are not sufficient to promote cell survival and mediate the antiapoptotic effects associated with a complete IL-2 signal. The Journal of Immunology, 1998, 161: 4627–4633.

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est T cells become metabolically active following recognition of cognate Ag by the TCR, but do not commence DNA synthesis (1) and instead rapidly undergo apoptosis (2–4), unless additionally stimulated by IL-2 or other mitogenic cytokines. The proliferation and survival of activated T cells have been associated with the expression of \( c-my c \) (5) and \( bcl \)-family proto-oncogenes (6–9), respectively. However, the biochemical pathways linking the IL-2R to these genes and the regulation of proliferation and survival remain largely undefined.

IL-2 binds to a cell surface receptor complex composed of three distinct chains, IL-2R\( \alpha \), IL-2R\( \beta \), and \( \gamma \) (10), inducing catalytic activation of the Janus kinases Jak1 and Jak3 (11–13). Activation of these tyrosine kinases requires the presence of a serine-rich, or S, region within the membrane-proximal 86 cytoplasmic residues of IL-2R\( \beta \) (14). These 86 amino acids contain Box1 and Box2 motifs found in many members of the hemopoietic receptor superfamily (15). Although Jak1 appears to be dispensable for IL-2-mediated mitogenesis (16), Jak3 activation is essential, since 1) IL-2 fails to mediate a proliferative signal in Jak3-deficient mice (17, 18), and 2) overexpression of a catalytically inactive version of Jak3 in the pro-B cell line BA/F3 inhibits the induction of \( c-my c \) and subsequent cell proliferation promoted by IL-2 (19).

In addition to catalytic activation of Jak3, tyrosine phosphorylation of IL-2R\( \beta \) is necessary for proliferation, since point mutation of three specific cytoplasmic tyrosine residues of IL-2R\( \beta \) to phenylalanine abrogates both phosphorylation of this receptor subunit and mitogenic signaling (20). By contrast, the cytoplasmic tyrosine residues of \( \gamma \) are dispensable for mitogenic signaling (21, 22). IL-2R\( \beta \) contains six cytoplasmic tyrosines (Y338, Y355, Y358, Y361, Y392, and Y510), all of which are distal to the S region. Y338, Y355, Y358, and Y361 are located in a segment referred to as the acidic or A region, whereas Y392 and Y510 are in the C-terminal H region (Fig. 1). Although Y355, Y358, and Y361 are completely dispensable for mitogenesis, the presence of at least one of the other three tyrosine residues on IL-2R\( \beta \) (Y338, Y392, or Y510) is necessary for tyrosine phosphorylation of this chain and the generation of a proliferative signal (20).

The most membrane-proximal cytoplasmic tyrosine (Y338) of IL-2R\( \beta \), when phosphorylated in response to IL-2, serves as a binding site for Shc (23, 24), an adapter molecule that associates with Grb2 to mediate activation of ras and the MAP kinase pathway (25–27). The ability of Y338 to both interact with Shc and mediate proliferation has led to the hypothesis that Y338 mediates a proliferative signal specifically through Shc (20). However, Y338 may interact with other molecules in addition to Shc that might instead be responsible for the proliferative signal. For example, the tyrosine kinase Lck, which has been proposed as a component of the IL-2 proliferative signal (28), has been shown to interact with the A region of IL-2R\( \beta \), which contains Y338 (29), although this interaction has not been mapped specifically to Y338. Similarly, the phosphatase SHP-2 is dependent upon the A region for tyrosine phosphorylation (30) and has been suggested to play a key role in IL-6- and EGF-mediated mitogenesis (31, 32). Additionally, the adapter molecules IRS-1 and IRS-2, which appear to mediate the IL-4 proliferative signal (33), are phosphorylated in response to IL-2 (34) and contain phosphotyrosine binding domains with a phosphopeptide-binding specificity similar to that of Shc (35). Thus, while interactions of IRS-1 or IRS-2 with IL-2R\( \beta \)
have also not been mapped specifically to Y338, these apparently mitogenic adapter molecules potentially share the Y338 docking site with Shc. Therefore, a number of molecules could potentially mediate proliferation from Y338 of IL-2Rβ, and the evidence specifically linking Shc to the proliferative signal is currently only correlative.

In addition to promoting proliferation, the IL-2R prevents the apoptosis of activated T cell (3, 4), an event that, unlike proliferation, has not previously been specifically linked to IL-2Rβ cytoplasmic tyrosines. Although it is possible that cell viability is simply a default consequence of proper cell cycle progression, there is also evidence that cytokine receptors such as the IL-2R generate antiapoptotic signals that are distinct from proliferative signals. Indeed, resting T cells can receive a nonmitogenic survival signal from IL-2 that, unlike the IL-2 proliferative signal, is not dependent upon Jak3 activation, and instead requires activation of the lipid kinase phosphatidylinositol-3 kinase (36). Moreover, the transcription factor STAT3 appears to mediate a signal for cell survival, but not proliferation, from the IL-6R chain gp130 by inducing the proto-oncogene bcl-2 (32). bcl-2 and the related gene bcl-x, both of which are induced by IL-2 (2, 9, 28), have been proposed to promote cell viability because constitutive overexpression of either gene in cytokine-dependent cell lines can significantly delay the onset of cytokine starvation-mediated apoptosis in the absence of proliferation (6, 7, 9). However, it is not known whether physiologic expression of these genes is sufficient to account for the ability of cytokines to prevent apoptosis.

In this study, we have analyzed the contribution of Shc to proliferation and survival signals mediated by the IL-2R. We demonstrate that covalently linking Shc to a truncated version of IL-2Rβ lacking all cytoplasmic tyrosines restores the ability of the receptor to promote c-myc induction and cell proliferation, thereby directly demonstrating a role for Shc in IL-2-mediated mitogenesis. Additionally, the IL-2Rβ/Shc fusion protein mediates induction of the antiapoptotic genes bcl-2 and bcl-x with normal kinetics. Nonetheless, this Shc-mediated signal is insufficient to maintain cell survival for more than a few days. Thus, IL-2R-induced proliferation and bcl-family gene expression are not sufficient to maintain the long-term survival of activated T cells. Rather, Y338 of IL-2Rβ appears to activate an unknown pathway independent of Shc that leads to downstream events that are necessary for long-term cell viability.

**Materials and Methods**

**Plasmid construction**

Expression vectors encoding the chimeric αγ- and ββ-chains (formerly denoted Gμα/2γ and GMββ2/2β) under the control of the human β-actin promoter have been described previously (37). The signal peptide of ββ was replaced with that of the GM-CSFα-chain to improve expression of ββ (B.H.N., unpublished results). Truncations of ββ were generated by PCR with oligonucleotides encoding premature stop codons, and PCR products were cloned between a unique AflI site in the cytoplasmic domain of ββ and a unique XbaI site immediately 3′ to the stop codon of ββ. Point mutations were generated by the splice-overlap extension PCR technique (38), and the PCR products were similarly inserted between the unique AflI and XbaI sites. To generate ββ325-Shc, the murine Shc cDNA was first crudely ligated C-terminal to ββ. A specific oligonucleotide encoding an AflI site separated from the N-terminal methionine of p52 Shc by four glycine residues was then used to synthesize, by PCR, an in-frame fusion between Ser253 of ββ and the N-terminal methionine of Shc. This PCR product was ligated between the unique AflI site of ββ and a unique BamHI site in Shc. All regions of IL-2Rβ generated by PCR were sequenced with the Applied Biosystems Prism dye terminator cycle sequencing kit (Perkin-Elmer, Norwalk, CT).

**Cell culture**

The murine IL-2-dependent T cell line CTLL-2 was obtained from American Type Culture Collection (Manassas, VA) and maintained in 10% FCS, 45% RPMI (Life Technologies, Gaithersburg, MD). 45% Click’s media (Altick Enterprises, River Falls, WI), supplemented with 2 mM l-glutamine, 50 U/ml penicillin, 50 mg/ml streptomycin, 1 mM sodium pyruvate, 0.07% sodium bicarbonate, 5 mM HEPES, 25 mM β-mercaptoethanol, and, unless otherwise specified, 50 U/ml human rIL-2 (Chiron, Emeryville, CA). Linearized plasmids were introduced into cells by electroporation, and stable transfectants were selected for resistance to G418 in 96-well plates at limiting dilution to isolate independent subclones. Receptor expression was assessed by flow cytometry with Abs to human GM-CSFRα or βc (Santa Cruz Biotechnology, Santa Cruz, CA). Subclones with comparable receptor expression were chosen for further analyses.

**Proliferative assays**

Thymidine incorporation assays were conducted in triplicate wells with 104 cells/well exposed to titrated doses of GM-CSF or IL-2 for 20 h, followed by a 4-h [3H]thymidine pulse (2.5 μCi/well). Cells were harvested onto glass fiber filters, and DNA synthesis was quantitated by liquid scintillation counting. The data presented in Figures 1 and 3 were based on a dose of GM-CSF (100 ng/ml) that was found to elicit a maximal response from all functional receptor mutants.

**Immunoprecipitations**

Cells that had been stimulated as indicated were washed once with PBS and then lysed on ice in lysis buffer (0.05 M, pH 7.4, Tris base, 0.5% Nonidet P-40, 0.15 M sodium chloride, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM PMSF or AEBSF-4-(2-aminoethyl)benzene sulfonyl fluoride, 10 mg/ml leupeptin, and 10 mg/ml aprotinin). Nuclei were removed by centrifugation, and cytoplasmic fractions were precleared with rabbit anti-rat Ig antisemum (Boehringer Mannheim, Indianapolis, IN) and protein A-agarose (Santa Cruz Biotechnology). Shc protein was then immunoprecipitated with polyclonal rabbit antisemum (Transduction Laboratories, Lexington, KY) and protein A-agarose (Santa Cruz Biotechnology). Agarose-bound immune complexes were washed twice with lysis buffer and then boiled for 3 min in SDS sample buffer.

**Western blots**

Immunoprecipitated proteins were run on acrylamide gels and transferred to nitrocellulose. Nitrocellulose blots were blocked with TTBS (0.1 M, pH 7.5, Tris base, 0.9% sodium chloride, 0.05% Tween 20) containing 5% powdered skim milk (Carnation, Glendale, CA) or 1% BSA (for antiphosphotyrosine probes), and probed with murine mAbs to Shc (Transduction Labs) or phosphotyrosine (Upstate Biotechnology, Lake Placid, NY). Blots were then washed with TTBS, probed with peroxidase-conjugated goat anti-rabbit or anti-mouse Abs (Life Technologies, Grand Island, NY), and washed again with TTBS. Bound Abs were detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL). ERLK1/ERLK2 phosphorylation was assayed per the protocol of the Phospho-MAPK Antibody Kit (New England Biolabs, Beverly, MA). Blots were stripped with a 30-min, 50°C incubation in 62.6 mM Tris-HCl (pH 6.7), 0.1 M β-mercaptoethanol, and 2% SDS.

**Electrophoretic mobility shift assays**

Cells that had been stimulated as indicated in the Figure 4 legend were washed once with buffer H (20 mM, pH 7.9, HEPES, 1 mM EDTA, 0.1 mM EGTA, 2 mM magnesium chloride, 1 mM sodium orthovanadate, 20 mM sodium fluoride, 1 mM DTT, 0.1 mM AEBSF-4-(2-aminoethyl)benzene sulfonyl fluoride, and 1 mg/ml leupeptin) and lysed in buffer H plus 0.2% Nonidet P-40 at 0°C. Nuclei were pelleted by centrifugation and then extracted with buffer K (buffer H plus 0.42 M sodium chloride and 20% v/v glycerol). A probe for STAT activity was generated with incompletely digested Fc graft DNA element from the FcγRI promoter, which were annealed, radiolabeled with [α-32P]dCTP by an end-filling T4 polymerase reaction, and purified with a MicroSpin G-25 column (Pharmacia, Piscataway, NJ). The probe was added to nuclear extracts in 50 mM potassium chloride, 10 mM HEPES (pH 7.9), 10% glycerol, 1 mM DTT, and 87.5 mg/ml dITP/dCTP at room temperature for 30 min, and the reaction mixture was run on an acrylamide gel, followed by autoradiography.

**Northern blots**

Cells that had been stimulated as indicated were pelleted by centrifugation and flash frozen in a dry ice/ethanol bath. RNA was harvested from thawed
To perform structure/function analyses of IL-2Rβ adapter molecule Shc with a PstI 1.2-kb bcl-2 fragment of murine expressed, adherent murine 3T3 cells were cultured without cytokine for 4 h and then stimulated for 10 min (A and B) or the indicated times (C) with 100 ng/ml GM-CSF (GM), 100 U/ml IL-2 (IL2), or media alone (0). A. Shc phosphorylation. Shc was immunoprecipitated from cells and probed by Western blot with an Ab to phosphotyrosine (pTyr). Blots were stripped and reprobed with an Ab to Shc to demonstrate even loading. Arrows indicate the ββ355-Shc protein (filled arrowheads) or endogenous p52 Shc (open arrowheads). B. MAP kinase activation. Whole cell lysates from indicated cells were probed by Western blot with antiserum recognizing phosphorylated ERK1 and ERK2. Blots were stripped and reprobed with antisera recognizing all forms of ERK1 and ERK2. Blots were stripped and reprobed with antisera recognizing all forms of ERK1 and ERK2 to demonstrate even loading. C. Northern blots showing c-fos expression in cells expressing the indicated versions of ββ. Blots were stripped and reprobed for GAPDH expression to demonstrate even loading.

Results

**IL-2Rβ induces c-myc expression and proliferation through the adapter molecule Shc**

To perform structure/function analyses of IL-2Rβ in a T cell line that normally responds to IL-2 and hence expresses a functional endogenous IL-2R, we utilized a previously described chimeric GM-CSF/IL-2R. The chimeric receptor consists of two chains, αγ and ββ, containing the extracellular domains of the human GM-CSF receptor α- and β-chains fused, respectively, to the transmembrane and intracellular regions of γc and IL-2Rβ. When co-expressed, αγ and ββ deliver in response to human GM-CSF a signal that is biochemically and physiologically indistinguishable from that induced in the same cell by the wild-type IL-2R (21, 37, 39). The murine cytotoxic T lymphocyte line CTLL-2 was cotransfected with αγ and either full-length ββ (ββwt) or mutated derivatives of ββ (Fig. 1). G418-resistant subclones of transfectedants were analyzed for receptor expression by flow cytometry (data not shown), and those with comparable expression of both αγ- and the various ββ-chains were chosen for further study.

While the IL-2 proliferative signal can be delivered through three functionally redundant intracellular tyrosines on IL-2Rβ (Y338, Y392, and Y510) (20), the adapter molecule Shc is recruited to IL-2Rβ exclusively through Y338 (24). Therefore, to focus our analysis upon the role of Shc in IL-2-mediated proliferation in the absence of redundant signals from Y392 or Y510, residue Y355 of ββ was replaced with a premature stop codon to generate a mutant (ββΔ355) that lacks all intracellular tyrosine residues except for Y338 (Fig. 1). Consistent with Y338 serving as a binding site for Shc, the ββΔ355 receptor was able to induce Shc phosphorylation in response to GM-CSF unless Y338 was point mutated to phenylalanine (ββΔ355Y338→F) (Fig. 2A). This point mutation also abrogated the ability of ββΔ355 to induce MAP kinase phosphorylation (Fig. 2B) and subsequent induction of the proto-oncogene c-fos (Fig. 2C), events that are mediated through Shc (25, 26).

Consistent with studies using other cell lines (20, 22, 40), ββΔ355 generated a robust proliferative signal in CTLL-2 cells (Fig. 3A). ββΔ355 also induced expression of the proto-oncogene c-myc (Fig. 3B), an event that is critical for T cell proliferation (5). The point mutation of Y338 to phenylalanine present in the ββΔ355Y338→F receptor chain, however, abrogated both c-myc induction (Fig. 3B) and proliferation (Fig. 3A). Nonetheless,
acts with multiple molecules, raising the distinct possibility that Y338 delivers a mitogenic signal through molecules other than Shc. Indeed, STAT5 itself has been implicated in the proliferative signal delivered by other cytokine receptors (42).

To specifically test the hypothesis that Y338 delivers a mitogenic signal through association with Shc, a fusion protein (ββ325-Shc) was constructed to force the association of Shc with IL-2Rβ in the absence of Y338. The N terminus of Shc was covalently attached, through a flexible tetraglycine linker, to the C terminus of a truncated version of ββ lacking all cytoplasmic tyrosines normally necessary for proliferation (Fig. 1). The S region of IL-2Rβ was retained, as this region is necessary for Jak3 activation (14), which in turn is necessary for Shc-mediated signaling (19, 39). We have previously employed a similar fusion protein strategy to reconstitute the signaling function of a truncated γc-chain by covalent attachment of Jak3 (39). The ββ325-Shc chain itself underwent inducible tyrosine phosphorylation (Fig. 2A), presumably at sites within the Shc sequence, which contains the only cytoplasmic tyrosines in ββ325-Shc. Moreover, ββ325-Shc activated the MAP kinase pathway in response to GM-CSF, as indicated by phosphorylation of MAP kinase (Fig. 2B) and induction of c-fos (Fig. 2C). Thus, ββ325-Shc can reconstitute Shc functions and mediate several of the biochemical events normally associated with Y338 of IL-2Rβ. ββ325-Shc also induced Jak3 phosphorylation (data not shown). However, ββ325-Shc did not induce any STAT DNA-binding activity beyond the trace amount induced by the ββΔ355,γγ388→F receptor chain (Fig. 4), indicating that the STAT activation mediated through Y338 in CTLL-2 cells occurs by a Shc-independent mechanism.

Analysis of mitogenic signaling revealed that ββ325-Shc promoted DNA synthesis in response to GM-CSF to a similar extent as did ββΔ355 (Fig. 3A). ββ325-Shc also induced c-myc expression with the same kinetics and magnitude as did ββΔ355 (Fig. 3B), thereby directly demonstrating a biochemical pathway from Shc to this proto-oncogene. The signal from ββ325-Shc was competent for progression through the entire cell cycle, as evidenced by the majority of subclones increasing in number by two- to threefold after 24 h of culture with GM-CSF (Fig. 3C).
The ability of cytokines to regulate cell survival has been ascribed to their ability to regulate expression of bcl-family genes (2, 6, 7, 9). Although the A region of IL-2R\(\beta\)-chain must have redundant sites through which it induces these genes, as the \(\beta\)325 receptor induced both bcl-2 and bcl-x through a Y338-dependent mechanism (Fig. 5). Such a requirement for either Y338 or more distal residues of IL-2R\(\beta\) was obviated by covalent attachment of Shc, as \(\beta\)325-Shc induced normal expression of both bcl-2 and bcl-x genes (Fig. 5). Therefore, these antiapoptotic bcl-family genes, like c-myc, are targets of a Shc-dependent signal.

Although the \(\beta\)325-Shc and \(\beta\)325 receptors mediated equivalent bcl-family gene induction, \(\beta\)325-Shc differed from \(\beta\)325 in that it did not support the long-term culture of CTLL-2 cells in the presence of GM-CSF. This appears to be because the \(\beta\)325-Shc receptor fails to prevent apoptosis, for while CTLL-2 subclones could be expanded for weeks with little cell death evident when stimulated through the \(\beta\)325 receptor (data not shown), all subclones stimulated through \(\beta\)325-Shc demonstrated markedly reduced viability by 48 h relative to cells expressing \(\beta\)325, and viable cells were rare or undetectable after 6 days (Fig. 6). Cells expressing \(\beta\)325-Shc and cultured with GM-CSF showed a brief survival advantage over cells expressing the \(\beta\)325 receptor, or cells cultured with media alone. However, this survival advantage was transient, being evident primarily in the first 24 h of culture (Fig. 6). Thus, Shc mediates several aspects of IL-2 signaling, such as c-myc, bcl-2, and bcl-x induction and proliferation, but is not competent to deliver anti-apoptotic signals necessary for the long-term expansion of T cells. Indeed, by promoting proliferation in the absence of survival, \(\beta\)325-Shc demonstrates that the IL-2R promotes mitogenesis and prevents apoptosis by discrete intracellular signals.

**Discussion**

Many growth factor receptors contain tyrosine residues within their cytoplasmic domains that become phosphorylated in response to receptor ligation, and thus provide docking sites for cytoplasmic signaling molecules (43). One such tyrosine residue (Y338) in the cytoplasmic domain of IL-2R\(\beta\) recruits the adapter molecule Shc to the activated receptor complex (23, 24). In the absence of other cytoplasmic IL-2R\(\beta\) tyrosine residues, Y338 is also required for the proliferation (Fig. 3A) (20) and survival (Fig. 6) of IL-2-dependent cells. While this finding suggests that Y338 may therefore deliver proliferation and survival signals through Shc, the fact that Y338 can also lead to activation of an alternative molecule such as STAT5 (41) (Fig. 4) suggests that other signaling pathways may be involved. To specifically identify the consequences of IL-2 signaling that are mediated through Shc, a novel fusion protein (\(\beta\)325-Shc) was designed to physically associate IL-2R\(\beta\) with Shc while precluding the association of other phosphotyrosine-binding proteins with IL-2R\(\beta\). Through this construct, we demonstrated that the \(\beta\)325 receptor, or cells cultured with media alone.
Shc could indeed induce a proliferative signal in the IL-2-dependent murine T cell line CTLL-2 (Fig. 3, A and C). Shc also mediated expression of the promitogenic gene c-myc and the antiapoptotic genes bcl-2 and bcl-x (Figs. 3B and 5). However, these Shc-mediated signals were insufficient to prevent the apoptosis of CTLL-2 cells, which occurs by default upon removal of IL-2 (Fig. 6). Thus, this study demonstrates that the IL-2R delivers distinct signals for cell proliferation and survival through Y338, and that only the former is mediated through Shc.

The strategy of covalently linking a cytoplasmic signaling molecule to a truncated receptor has been successfully applied in the past to determine the roles of Lck in CD4 signaling (44), Jak2 in IFN-γ signaling (45), and Jak3 in IL-2 signaling (39). Such a strategy has the inherent risk that molecules may adopt a conformation or orientation that compromises their signaling capabilities. Therefore, we attempted to reconstitute the normal orientation of Shc relative to IL-2Rβ by linking the N-terminal region of Shc, which normally binds IL-2Rβ (23), to the C terminus of ββ325, which is only 13 residues from the tyrosine (Y338) to which Shc normally binds (Fig. 1). Several indices of normal Shc function, including Shc and MAP kinase phosphorylation and c-fos induction, were fully reconstituted with the ββ325-Shc receptor chain (Fig. 2), while the Shc-independent ability of Y338 to promote STAT activation was not (Fig. 4).

ββ325-Shc induced both c-myc expression and cell proliferation (Fig. 3), indicating that Shc can mediate a mitogenic signal from the IL-2R, similar to the role Shc has been proposed to play in EGF receptor signaling (46). ββ325-Shc also induced bcl-2 and bcl-x expression (Fig. 5), demonstrating that signals from Shc can activate these antiproliferative genes. However, IL-2R signaling must lead to induction of the bcl-2 gene through multiple pathways, since a dominant negative Jak3 molecule, which abrogates Shc-mediated signals, failed to block induction of bcl-2 (19). Moreover, a full-length form of IL-2Rβ, in which all six cytoplasmic tyrosines were mutated to phenylalanine, induced bcl-2 expression in the absence of the Shc binding site (41). The molecular nature of this alternative pathway to bcl-2 remains undefined.

As an adapter molecule, Shc can interact with multiple downstream signaling molecules that potentially mediate the proliferative and gene-induction signals described in this work. For example, Shc contains an N-terminal phosphotyrosine binding domain that interacts with SH2-containing 5′-inositol phosphatase and a collagen homology domain that interacts with the adapter molecule Grb2 (27, 47, 48). The ras protein is normally activated downstream of Grb2, and constitutively active versions of ras have been shown to mediate bcl-2 and bcl-x induction and, when coexpressed with c-myc, promote proliferation (49, 50). Additionally, overexpression of a dominant negative version of Shc bearing point mutations in the collagen homology domain inhibits c-myc induction by IL-3 and EGF (46, 51). Shc also contains a C-terminal SH2 domain capable of interacting with tyrosine-phosphorylated proteins, although no such interactions have yet been described in the context of the IL-2R. Future structure/function analyses of ββ325-Shc should allow determination of the domains of Shc that mediate proliferation and induction of the c-myc, bcl-2, and bcl-x genes by IL-2.

Although ββ325-Shc induced the antiproliferotic genes bcl-2 and bcl-x (Fig. 5), it was unable to support the long-term culture of CTLL-2 cells because it failed to prevent apoptosis (Fig. 6), indicating that the induction of bcl-2 and bcl-x by IL-2 is not sufficient to support the long-term viability of cells. Indeed, the ability of constitutively overexpressed bcl-2 to prevent cytokine starvation-mediated apoptosis in factor-dependent cells is only transient (6, 9). Similarly, since ββ325-Shc delivered a competent proliferative signal (Fig. 3, A and C), cell cycle progression is likewise not sufficient to prevent apoptosis. Although bcl-2 or bcl-x induction or cell cycle progression may account for the slight survival advantage that the ββ325-Shc receptor confers over media alone (Fig. 6), there must be an additional, presumably Shc-independent, mechanism by which the wild-type IL-2R promotes long-term survival. This could involve the regulation of genes not examined in this study, for example inducing the antiproliferotic bcl-family member A1 (52) or suppressing such proapoptotic members as Bax or Bad (53, 54). Alternatively, it may involve cytoplasmic events implicated in antiproliferotic signaling, such as phosphorylation, and consequent cytoplasmic sequestration, of the proapoptotic bcl-family protein Bad (55) mediated by the kinase c-Akt (56, 57). However, a defect in the latter antiproliferative pathway is unlikely to account for the inability of ββ325-Shc to support cell viability, as ββ325-Shc promoted the phosphorylation of Akt on Ser1173 (J.D.L., unpublished results), which is involved in the activation of this kinase.

The ability of ββ325-Shc to induce robust proliferation (Fig. 3, A and C) without supporting cell survival (Fig. 6) indicates that the IL-2R delivers discrete signals for proliferation and long-term survival, such as have been described in the IL-3/IL-5/GM-CSF and IL-6 receptor systems (32, 58, 59). Because IL-2 is the principal mitogenic cytokine for mature T cells, discrete proliferative and survival signals from the IL-2R may play a decisive role in normal T cell physiology in vivo. For example, through the selective attenuation of either a proliferative or survival signal from the IL-2R, a T cell could be induced to proliferate, persist, or apoptosis upon activation, thus dictating the course of an immune response.

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References


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