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STAT5 Is Essential for Akt/p70S6 Kinase Activity during IL-2-Induced Lymphocyte Proliferation

Heather M. Lockyer,2* Eric Tran,2*† and Brad H. Nelson3*†

IL-2R activates two distinct signaling pathways mediated by the adaptor protein Shc and the transcription factor STAT5. Prior mutagenesis studies of the IL-2R have indicated that the Shc and STAT5 pathways are redundant in the ability to induce lymphocyte proliferation. Yet paradoxically, T cells from STAT5-deficient mice fail to proliferate in response to IL-2, suggesting that the Shc pathway is unable to promote mitogenesis in the genetic absence of STAT5. Here we show in the murine lymphocyte cell line Ba/F3 that low levels of STAT5 activity are essential for Shc signaling. In the absence of STAT5 activity, Shc was unable to sustain activation of the Akt/p70S6 kinase pathway or promote lymphocyte proliferation and viability. Restoring STAT5 activity via a heterologous receptor rescued Shc-induced Akt/p70S6 kinase activity and cell proliferation with kinetics consistent with a transcriptional mechanism. Thus, STAT5 appears to regulate the expression of one or more unidentified components of the Akt pathway. Our results not only explain the severe proliferative defect in STAT5-deficient T cells but also provide mechanistic insight into the oncogenic properties of STAT5 in various leukemias and lymphomas. The Journal of Immunology, 2007, 179: 5301–5308.

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4 Abbreviations used in this paper: PIP3, phosphatidylinositol 3,4,5-triphosphate; PDK1, phosphoinositide-dependent kinase 1; caSTAT5, constitutively active mutant of STAT5; QPCR, quantitative PCR; wsSTAT5A, wild-type STAT5A.

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the PI3K/Akt and Ras/Erk pathways and induce lymphocyte proliferation (39). Altogether, these results indicate that STAT5 is capable of at least two mechanistically distinct modes of signaling.

In addition to having a major role in survival and proliferative signaling, activated STAT5 can also promote apoptosis under some conditions (38, 42). IL-2 plays a major role in sensitizing T cells to activation induced cell death, and this was found to depend on STAT5 signaling (42). Furthermore, in a lymphocyte cell line, caSTAT5 was shown to promote apoptosis by inducing expression of the growth-inhibitory protein JAB (JAK-binding) (38). Finally, naturally occurring isoforms of STAT5 can be produced by alternative splicing or proteolytic cleavage by enzymes such as cathepsin G or calpain (43–45). Although the exact physiological significance of these isoforms remains to be determined (45, 46), overexpression of isoforms lacking the C-terminal trans activation domain can exert a dominant-negative effect on STAT5 signaling and induce apoptosis in certain cell types (43, 47–50).

Prior mutagenesis studies, in which the Shc or STAT5 docking sites on IL-2Rβ were selectively removed, indicated that Shc and STAT5 are redundant in the ability to induce lymphocyte proliferation (6, 7, 13, 24, 35, 51, 52). However, T cells rendered genetically deficient in STAT5 are completely nonproliferative upon TCR and IL-2 stimulation, suggesting that STAT5 is absolutely required for mitogenesis irrespective of the Shc pathway (36). This could mean that STAT5 contributes to proliferative signaling even when not activated by the IL-2R, as has been shown for STAT1 (53). Alternatively, IL-2Rβ mutants reported to activate Shc alone might also activate STAT5 to low but functionally significant levels.

To distinguish these possibilities, we expressed mutant cytokine receptors that selectively activate Shc or STAT5, either alone or in combination, in subclones of the lymphoid cell line Ba/F3. We find that, unexpectedly, a low level of STAT5 activity is essential for sustained activation of the Akt/p70S6 kinase pathway by Shc. Our results demonstrate a novel, essential connection between the Shc and STAT5 pathways, explain the severe proliferative defective in STAT5-deficient lymphocytes, and provide insight into the oncogenic role of STAT5 in various leukemias and lymphomas.

Materials and Methods

Plasmid construction

β-wt, β-Y338, β-Y510, and wild-type STAT5a (wtSTAT5a) have been previously described (24, 35). All receptor mutants were generated using standard PCR-based techniques. β-Y338GG was created from β-Y338 by modification of the C terminus to the sequence Y338GGF[stop]. G-Y510 was created by joining the human G-CSF receptor extracellular domain to human gp130 at EcoRI to incorporate the transmembrane and Jak binding domains (box 1 and 2) of gp130. The Shp2- and STAT3-binding sites of gp130 were then replaced with a single STAT5 docking site corresponding to Y510 and flanking residues from human IL-2Rβ (YLSLQELQ[stop]). All receptor mutants were sequenced and cloned into a human GM-CSFR expression vector containing a neomycin resistance gene (54). The caSTAT5A1S expression plasmid has been described elsewhere (55).

Cell culture

Murine proB Ba/F3 cells stably transfected with human GM-CSFRα, designated BAF.GM, were maintained in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, and 10% WEHI-conditioned medium (source of murine IL-3). Upon human GM-CSF stimulation, the GM-CSFRα receptor chain dimerizes with the murine common β-chain and induces strong lymphocyte proliferation mediated by STAT5, Shc, Gab2, ERK, and PI3K, which are signaling intermediaries also used by IL-2R (56). GM-CSF can be purchased at low cost through the hospital pharmacy and therefore represents an inexpensive yet high quality cytokine to serve as a positive control. The murine IL-2-dependent T cell lines CTLL-2 (CD8+) and HT-2 (CD4+) were maintained in RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 25 mM HEPES, 1 mM sodium pyruvate, and 25 μM 2-ME. For the generation of stable transfectants using Ba/F3 lymphocytes, linearized plasmids were electroporated into cells, and stably transfected subclones were selected at limiting dilution for G418 resistance (0.8 μg/ml, Sigma-Aldrich). Receptor expression was assessed by flow cytometry with Abs to human IL-2Rβ or human G-CSFR (BD Biosciences). For all experiments, we used subclones with receptor expression levels between 0.5 and 1.5 log fluorescence units (Fig. 1B).

Western blots

Cytosplastic and nuclear extracts of BAF.GM cells expressing either β-wt, β-Y338, β-Y338GG, or a combination of β-Y338GG and G-Y510 were prepared and immunoblotted as described (57) with the following modifications: cells were washed three times with 1× PBS, and following 4 h of incubation in medium without added cytokine, 20 × 10^6 cells were stimulated with recombinant human GM-CSF (100 ng/ml), IL-2 (100 U/ml), G-CSF (100 ng/ml), or a combination of IL-2 and G-CSF at 37°C for the indicated time points. Extracts from 2 × 10^6 cells were run on 3−8% Tris-acetate gels (Criterion XT; BioRad Laboratories) and transferred to nitrocellulose. Western blotting was performed by blocking membranes in pH 7.5 0.1 M Tris, 0.9% NaCl, 0.05% Tween (TBS-T) containing 1% (w/v) BSA. Membranes were incubated for 3 h in blocking buffer containing Abs to phospho-STAT5 (Tyr694), phospho-Shc (Tyr 317 or Tyr239/240), phospho-Gab2 (Tyr452), phospho-Shp2 (Tyr425), phospho-(Tyr) p85 PI3K, phospho-Akt (Ser 473), phospho-p70S6K (Thr 421/Ser424), phospho-S6 (Ser235/236), or phospho-ERK-p44/42 MAPK (Thr202/Tyr204), all from Cell Signaling Technology. Membranes were washed with TBS-T and incubated with HRP-conjugated goat anti-rabbit Abs (The Jackson Laboratory). Bound Abs were detected by ECL (Amersham). After detection, membranes were stripped for 1 h at 60°C with 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 0.1 M 2-ME before washing with TBS-T, blocking in TBS-T + 1% BSA and reprobing with control rabbit Abs specific for: Gab2 (Upstate Biotechnology), p70S6K (Santa Cruz Biotechnology), STAT5, Shc, Shp2, p85 PI3K, Akt, S6 or ERK-p44/42 MAPK (Cell Signaling Technology).

Proliferative assays

BrdU incorporation was assessed using the Cell Proliferation Biotrak ELISA system (Amersham). Assays were conducted in triplicate with 10^6 transfected BAF.GM cells cultured in 200 μl of medium plus the appropriate stimulus. After 48 h, cells were fixed, permeabilized, and incubated with peroxidase-labeled anti-BrdU (1/100 in Ab dilution solution) for 90 min. Bound Abs were detected by TMB substrate and read at 450 nm on a Molecular Devices plate reader.

FIGURE 1. A, Schematic diagram of wild-type (β-wt) and mutant IL-2 receptors with key tyrosine (Y) residues highlighted. Upon IL-2 stimulation, β-wt delivers a wild-type IL-2 signal (Shc + STAT5); β-Y338 activates Shc and modest levels of STAT5; and β-Y338GG exclusively activates Shc. B, Flow cytometric analysis of IL-2Rβ expression on BAF.GM cells expressing β-wt, β-Y338, or β-Y338GG. Subclones displaying IL-2Rβ expression within 0.5−1.5 log fluorescent units were selected for use in subsequent experiments. A representative clone for each receptor construct is shown. Max, Maximum.

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RESULTS

Transient transfections

Baf.GM lymphocytes stably expressing β-Y338GG were resuspended at 12.5 × 10⁶ cells/ml in 10 mM MgCl₂ PBS solution with 100 μg total of plasmid DNA (wtSTAT5A or caSTAT5A in combination with a GFP vector) and electroporated (350 V, 975 μF) with a GenePulser Xcell (BioRad). Cells were then rested overnight in RPMI 1640 supplemented with 10% FBS, 2 mM l-glutamine, 50 μM penicillin, 50 μg/ml streptomycin, and 10% WEHI-conditioned medium (a source of murine IL-3). After recovery, cells were washed three times with 1× PBS, starved of cytokines for 4 h, and then stimulated with 100 U/ml IL-2 or medium alone. At t = 1 and 10 h, cells were harvested for intracellular flow cytometry as described below. Successful transfectants, as demarcated by GFP expression, were analyzed for phospho-S6 content (see Intracellular flow cytometry). Transfection efficiency typically ranged between 2 and 5%.

Intracellular flow cytometry

Cells were stimulated for the indicated time points with the appropriate cytokine(s), and fixed with formaldehyde (2% v/v final concentration) for 10 min at 37°C. Fixed cells were then spun at 500 × g, and cell pellets were permeabilized with 100% ice cold methanol and incubated on ice for 20 min to achieve complete permeabilization. Cells were rehydrated by washing twice with >10 volumes of 1× PBS + 0.5% BSA. Cells were then stained with Abs to phospho-STAT5 or phospho-S6 (1/200; Cell Signaling Technology) for 30–60 min at room temperature, washed twice with 1× PBS + 0.5% BSA and then stained with anti-rabbit IgG (conjugated to PE at 1/100) for 30–60 min at room temperature in the dark (CalTag Laboratories). Events were collected with a BD FACSCalibur flow cytometer and CellQuest Pro software. Data analysis was performed using FlowJo software (Tree Star).

Quantitative PCR (QPCR) analysis

Cells were snap frozen in an ethanol-dry ice bath and stored at −80°C. Total RNA was isolated using the RNeasy Mini kit (Qiagen) following the manufacturer’s protocol and quantified using a NanoDrop ND-1000 spectrophotometer. RNA was reverse transcribed to cDNA using the iScript cDNA synthesis kit (BioRad). Cis expression was measured by QPCR using the intron-spanning primer set: Cis forward 5'-CGATGGTCCTCGG GACATGGTC-3'; Cis reverse 5'-CAATTTGCTCCAAGCCAGGCAGC-3'. Cys expression was determined with the intron-spanning primer set: c-myc forward TTTTGCTATTGGGGGACATGGTT; c-myc reverse CATCGT C GTGGCGTCTCG. GAPDH was used as a reference gene, and transcript levels were assessed using the primers: GAPDH forward 5'-AAC TTTGG CATTGTTGAAGG-3'; GAPDH reverse 5'-ACACATTGGGGGTAGGA ACA-3'. QPCR was performed using the iCycler MyIQ real-time PCR detection system (Bio-Rad) with the following two-step protocol: initial denaturation at 95°C for 90 s, followed by 40 cycles of denaturation at 95°C for 10 s and 30 s extension at 55°C. After final denaturation at 95°C for 1 min, a melt curve analysis was performed starting at 55°C and increasing by increments of 1°C up to 95°C. Relative gene expression was calculated using the Bio-Rad Gene Expression Macro version 1.1 software. Expected product sizes were verified by standard agarose gel electrophoresis.

An IL-2Rβ mutant reported to exclusively activate Shc induces low levels of STAT5 activity

To assess the possibility that IL-2R mutants thought to exclusively activate Shc might also activate low levels of STAT5, we first re-evaluated a previously described IL-2Rβ truncation mutant designated β-Y338 (also known as ββΔ355) which contains the Shc docking site at Y338 but lacks all other cytoplasmic tyrosine residues, including all previously defined STAT5 activation sites (Fig. 1 and Refs. 8 and 13). In accord with prior reports, β-Y338 induced robust activation of the Shc pathway, as evidenced by IL-2-induced phosphorylation of Shc, p70S6K, S6, and ERK (Fig. 2A), and this was associated with strong proliferation in the lymphocyte cell line BAF.GM, a derivative of Ba/F3 cells (Fig. 2B and Materials and Methods). Sensitive immunoblotting with phospho-specific Abs revealed that the β-Y338 mutant also induced low-level tyrosine phosphorylation of the full-length isoform of STAT5, despite lacking all known STAT5 docking sites (Fig. 2A). Furthermore, QPCR analysis revealed low but reproducible induction of the STAT5-specific target gene Cis by β-Y338 (Fig. 2C). Thus, previous studies concluding that the Shc pathway alone could induce lymphocyte proliferation may be confounded by low-level STAT5 activation by receptor mutants such as β-Y338 (6, 13, 24, 42).
Activation of the Shc pathway alone does not sustain long-term Akt/p70S6K activity or lymphocyte proliferation and viability

To further reduce STAT5 activation while sparing the Shc pathway, a second IL-2R mutant was constructed, designated /H9252-Y338GG, in which two phenylalanine residues immediately C-terminal to the Shc docking site at Y338 were mutated to glycine and the C terminus was further truncated to residue 341 to eliminate any potential binding to Y338 by the SH2 domain of STAT5 (Fig. 1). We expected the phosphotyrosine binding domain of Shc to still bind to /H9252-Y338GG, because the phosphotyrosine binding domain recognizes residues N-terminal to Y338 (58). As intended, the /H9252-Y338GG mutant showed reduced STAT5 tyrosine phosphorylation and CIS induction in response to IL-2, whereas tyrosine phosphorylation of Shc still occurred (Fig. 2, A and C). Remarkably, this was associated with a major reduction in cell proliferation and viability, suggesting that the Shc signal, when isolated from STAT5, is not sufficient for mitogenesis and cell survival (Fig. 2B and data not shown). To understand the biochemical basis of this proliferative impairment, we evaluated key signaling events associated with the Shc pathway. At 1 h poststimulation, the /H9252-Y338GG mutant showed normal phosphorylation of Shc, ERK, p70S6K, and its substrate S6, suggesting that early Shc signaling was intact (Fig. 2A). However, by 12 h poststimulation, the /H9252-Y338GG mutant showed greatly impaired phosphorylation of p70S6K and S6, whereas Shc and ERK phosphorylation were only modestly diminished (Fig. 2A). A more refined time course revealed that p70S6K and S6 phosphorylation began to diminish 3–6 h after stimulation of /H9252-Y338GG, despite normal phosphorylation of Shc and ERK at these time points (Fig. 2D).

STAT5 and Shc cooperate to sustain Akt/p70S6K pathway activation and lymphocyte proliferation

The failure of /H9252-Y338GG to sustain p70S6K and S6 phosphorylation could result from reduced STAT5 activation by this mutant. Alternatively, the /H9252-Y338GG mutation could disrupt the interaction of Y338 with other unidentified signaling proteins that regulate p70S6K and S6. To distinguish these possibilities, we attempted to rescue p70S6K and S6 phosphorylation by restoring STAT5 activation through a second receptor. We chose a receptor that could be stimulated independent of /H9252-Y338GG and was structurally
distinct from the IL-2R, such that STAT5 represented one of the few shared signaling elements. Specifically, we made a chimeric receptor that placed STAT5 under the control of a second cytokine, G-CSF. Several groups have described a chimeric G-CSF/gp130 receptor that generates an IL-6-like signal in response to G-CSF that is mediated by Jak1, Jak2, Tyk2, Shp2, and STAT3 (59, 60). We replaced the Shp2 and STAT3 activation sites of G-CSF/gp130 with the STAT5 activation site from IL-2Rβ (Y510) to generate a chimeric receptor designated G-Y510 (Fig. 3A). As expected, G-Y510 induced STAT5 phosphorylation and CIS expression in response to G-CSF, without inducing phosphorylation of STAT3 and Shp2 (components of the IL-6 signal), or Jak3 and Shc (components of the IL-2 signal; Fig. 3B, Fig. 5B, and data not shown). The level of STAT5 phosphorylation was modest compared with the wild-type IL-2R, and consequently G-CSF-induced cell proliferation was weak (Fig. 3C). Nevertheless, when G-Y510 was coexpressed with β-Y338GG, the combination of IL-2 + G-CSF induced a proliferative response equivalent to that of the wild-type IL-2R (Fig. 3C). Thus, the Shc and STAT5 pathways exhibit strong cooperativity even when triggered by heterologous receptors.

We next evaluated G-Y510 and β-Y338GG for synergistic effects on p70S6K and S6. As before, β-Y338GG alone induced strong phosphorylation of p70S6K and S6 at 1 h, and this was greatly reduced by 12 h (Fig. 3B). By contrast, G-Y510 alone induced little or no p70S6K/S6 phosphorylation at 1 or 12 h (Fig. 3B). Importantly, when cells were costimulated with IL-2 + G-CSF, strong phosphorylation of p70S6K and S6 was observed at both 1 and 12 h (Fig. 3B). To quantify these results, we measured S6 phosphorylation by flow cytometry using the same cell cultures. Consistent with the immunoblotting results, the combination of IL-2 + G-CSF induced at least a 4-fold increase in the mean fluorescence intensity at 12 h relative to IL-2 alone (Fig. 3D). Thus, Shc and STAT5 cooperatively regulate p70S6K and S6, even when activated through heterologous receptors. The quantitative data obtained by flow cytometry demonstrate that this was a synergistic rather than an additive effect of the two cytokines.

To further demonstrate that STAT5 was the factor synergizing with the Shc pathway, we generated BAF.GM cells stably coexpressing β-Y338GG and the IL-2 receptor mutant β-Y510 (formerly known as ββA325 + Y510). This mutant has undergone extensive investigation to show that it signals exclusively through the activation of STAT5 by Y510(27, 35, 61). Similar to the results observed with β-Y338GG + G-Y510, IL-2-induced coactivation of β-Y338GG and β-Y510 also led to sustained phosphorylation of S6 at late time points, as assessed by intracellular flow cytometry (Fig. 3E). Together, these results imply an essential role for STAT5 in sustaining the Akt/p70S6K pathway.

c/STAT5 can independently induce S6 phosphorylation
In theory, the G-Y510 chimeric receptor, and even the well-characterized β-Y510 IL-2 receptor, should exclusively activate STAT5. However, the possibility remained that these receptors might activate cellular proteins in addition to STAT5 which may in turn have effects on the Akt/p70S6K pathway. To ensure that
STAT5 was indeed the factor responsible for sustaining the Akt/p70S6K pathway, we took advantage of a well-characterized, constitutively active form of STAT5, caSTAT5A1*6 (55). caSTAT5A1*6 harbors two amino acid substitutions that confer constitutive tyrosine phosphorylation, nuclear localization, and transcriptional activity (55). As expected, when transiently expressed in BAF.GM lymphocytes, caSTAT5A1*6 demonstrated strong, constitutive tyrosine phosphorylation (data not shown). This was associated with potent phosphorylation of S6 to levels exceeding that induced by β-Y338GG or β-wt (Fig. 4 and data not shown). Expression of wtSTAT5A did not affect S6 phosphorylation (Fig. 4); therefore, activated STAT5 is required for this process. Transient expression of caSTAT5A1*6 in the murine T cell lines CTLL-2 and HT-2 also induced phosphorylation of S6 (data not shown); therefore, the link between the STAT5 and Akt/p70S6K pathways is operant in multiple cell types.

STAT5 cooperates with the Shc pathway at the level of Akt

We examined other signaling events downstream of Shc to determine the point at which STAT5 synergizes with the Shc pathway. In cells coexpressing β-Y338GG and G-Y510, IL-2 induced the phosphorylation of Shc, Gab2, Shp2, p85, and ERK, and these events were not enhanced by G-CSF at any time point (Fig. 5A). By contrast, G-CSF enhanced the phosphorylation of Akt and p70S6K at 6 and 12 h, and an even greater effect was seen on the downstream effector protein S6 (Fig. 5A). These results indicate that STAT5 acts at or near the level of Akt. Intriguingly, in addition to STAT5, facilitating Shc signaling, activation of the Shc pathway by β-Y338GG enhanced the tyrosine phosphorylation of STAT5 as well as induction of the STAT5 target genes CIS and c-myc (Fig. 5). Thus, there appears to be bidirectional cooperative signaling between the Shc and STAT5 pathways.

Temporal dissociation of Shc and STAT5 signaling impairs S6 phosphorylation and cell proliferation

STAT5 has been reported to weakly activate the PI3K pathway through its conventional role as a transcription factor (26, 27). In addition, STAT5 can serve as an adaptor protein, forming a complex with Gab2 and the p85 regulatory subunit of PI3K (39). To investigate which of these mechanisms underlies the synergy between STAT5 and Shc, we determined how rapidly STAT5 could rescue the Shc signal, reasoning that this should occur immediately if STAT5 served as an adaptor protein or more slowly if STAT5 served as a transcription factor. Cells coexpressing β-Y338GG and G-Y510 were stimulated with IL-2 alone for 9 h to allow the Shc signal to initiate and then decay. When G-CSF was added, STAT5 phosphorylation increased within 15 min whereas S6 phosphorylation increased over a 1- to 6-h period (Fig. 6). These results are consistent with STAT5 sustaining S6 phosphorylation by serving as a transcription factor rather than an adaptor protein. If cells were washed before the addition of G-CSF, such that the IL-2-induced Shc signal was extinguished before the initiation of the STAT5 signal, there was only negligible rescue of S6 phosphorylation (data not shown). This demonstrates that S6 phosphorylation is not regulated through a simple biphasic mechanism involving an initial Shc phase followed by a STAT5 phase. Rather, both pathways need to be simultaneously active to achieve maximal S6 phosphorylation. Similar to the results for S6 phosphorylation, cell proliferation was maximal only when the Shc and STAT5 pathways were both activated throughout the entire stimulation cycle (data not shown). Thus, continuous, simultaneous Shc and STAT5 signaling is required to achieve maximal S6 phosphorylation and cell proliferation, demonstrating an essential synergy between these pathways.

Discussion

We show here that STAT5 is essential for sustained activation of the Akt/p70S6K pathway, which was previously thought to be an exclusive function of Shc. At early time points after IL-2 stimulation, our results are consistent with the conventional model in which a complex containing Shc, Grb2, Gab2, Shp2, and p85 mediates activation of the Akt/p70S6K pathway (14–16). However, when concurrent STAT5 activity was absent, the Akt/p70S6K signal waned after 3–6 h of IL-2 stimulation, and this was associated with drastically impaired lymphocyte proliferation and viability. Restoration of STAT5 activity through a heterologous receptor rescued maximal Akt/p70S6K activity and lymphocyte proliferation. The Shc pathway further promoted these events by enhancing the transcriptional activity of STAT5. This bidirectional, cooperative signaling by Shc and STAT5 appears to operate throughout the proliferative cycle, as temporal dissociation of the Shc and STAT5 signals lead to suboptimal S6 phosphorylation and lymphocyte proliferation. Thus, the Shc and STAT5 pathways do not operate as independent signaling modules, as prior studies have suggested, but instead are intimately linked at the level of the Akt/p70S6K pathway and possibly other regulatory nodes as well. These findings likely explain the severe mitogenic defect seen in STAT5-deficient T cells (36), given that lack of STAT5 signaling would also impair the ability of Shc to activate the Akt/p70S6K pathway.

It has been repeatedly demonstrated that STAT5 can serve as an adaptor protein in the Gab2/Shp2/p85 complex, which could potentially explain the observations reported here (39–41). However, the physiological relevance of this mode of signaling by STAT5 has yet to be fully established. In fact, constitutively active STAT5 mutants that are defective in trans-activating STAT5 target genes, but are theoretically capable of acting as adaptor proteins, were found incapable of inducing leukemia in mice (62, 63). By contrast, constitutively active STAT5 mutants capable of tetramerization and strong DNA binding efficiently induced leukemogenesis, which implicates an important role for STAT5 transcriptional activity, and not adaptor function, in oncogenesis (62, 63).

In this study, we provide four lines of evidence that STAT5 regulates the Akt pathway through a transcriptional mechanism: 1) time-course experiments showed a delayed (1- to 3-h) rescue of the
Akt pathway by STAT5 (Fig. 6); 2) the cooperation between the Shc and STAT5 pathways occurred even in the context of heterologous receptors, suggesting that it involved signaling intermediaries rather than direct complex formation between Shc and STAT5; 3) short term (1-h) activation of the Akt pathway by Shc occurred in the absence of STAT5 activity, indicating that any adaptor function of STAT5 is not essential for formation of the Shc/Grb2/Gab2/p85 complex; and 4) expression of casSTAT5A*6 alone (i.e., without coactivation of the Shc/Grb2/Gab2/p85 complex) strongly activated the Akt/p70S6K pathway (Fig. 4) and indeed promotes factor-independent proliferation of Ba/F3 cells (38, 55). Because casSTAT5A*6 predominantly localizes to the nucleus, possesses strong transcriptional activity, and can bind DNA in the absence of cytokines (55), this is also consistent with a transcriptional mechanism. Attempts to directly confirm the transcriptional activity of STAT5 using the protein translation inhibitor cycloheximide were confounded by direct effects of this agent on p70S6K (data not shown), consistent with a prior report (64). Nevertheless, even though STAT5 can form a complex with Gab2, Shp2, and p85, the collective evidence strongly suggests that it regulates the Akt/p70S6K pathway predominantly by a transcriptional mechanism.

We found that STAT5 does not affect the IL-2-induced phosphorylation of Shc, Grb2, Gab2, Shp2, or p85. Rather, the effect of STAT5 is seen at the level of Akt and the downstream effectors p70S6K and S6 (Fig. 5A). Although Akt plays a central role in cell survival, growth, and proliferation, its mechanism of regulation remains unresolved (21). Maximal activation of Akt requires both translocation to the plasma membrane and phosphorylation of Thr308 and Ser473 (18, 21, 65). Akt is recruited to the plasma membrane by the PI3K product PIP3, which is bound by the pleckstrin homology domains of Akt and PDK1. Subsequently, PDK1 phosphorylates Thr308 and PDK2 phosphorylates Ser473. The precise identity of PDK2 remains controversial, although recent evidence suggests that it may consist of the Rictor/mTOR complex (66). Therefore, STAT5 could potentially promote Akt activation by up-regulating the activity of p110α (the catalytic subunit of PI3K), PDK1, PDK2, or other components of the mTOR pathway. Alternatively, STAT5 could activate Akt by transcriptionally repressing a negative regulator(s). To date, no Akt-specific phosphatase has been found (21). However, the C-terminal modulator protein negatively regulates Akt by preventing its phosphorylation (67). Finally, because Akt is downstream of PI3K, negative regulators of PI3K, such as the tumor suppressors PTEN and p53, could also oppose Akt activity (20). With these candidates in mind, we are currently attempting to identify the STAT5 target gene(s) that regulate the PI3K/Akt pathway.

In addition to showing that STAT5 is essential for sustaining the Akt/p70S6K pathway, we also found that optimal STAT5 transcriptional activity depends on concurrent Shc signaling. This suggests that the Shc pathway is regulating a kinase or phosphatase that controls the phosphorylation of STAT5. Time-course experiments revealed that the Shc pathway enhances STAT5 phosphorylation with delayed kinetics, which suggests a transcriptional mechanism (data not shown); however, the molecular basis of this finding remains to be elucidated.

Prior studies with the IL-2R mutant ββΔ325 + Y510 and the wild-type IL-7R have shown that STAT5 can activate Akt to a low level in the absence of Shc signaling (26, 27). Together with our current results, this might suggest a biphasic mode of Akt regulation, wherein initial activation of the PI3K/Akt pathway is mediated by the well-characterized Shc/Grb2/Gab2/p85 complex followed by a second phase of Akt activity mediated by STAT5. Indeed, the platelet-derived growth factor receptor activates the PI3K pathway through a biphasic mechanism (68). However, we do not believe the data support such a model for the IL-2R, simply because STAT5 alone is a very weak activator of Akt (26, 27). It is only when STAT5 is combined with Shc that strong phosphorylation of Akt, p70S6K, and S6 occurs. Instead, we propose a model in which Akt is predominantly regulated by the Shc/Grb2/Gab2/p85 complex, as indicated by prior studies, but that one or more unidentified factors downstream of this complex depend on STAT5 transcriptional activity for continued expression. Thus, further study of STAT5 target genes will shed new light on the mechanism of Akt regulation by cytokine receptors.

Activated STAT5 has been observed in a number of human cancers, including various leukemias and lymphomas as well as prostate, uterine, ovarian, breast, and head and neck cancers (29–31). STAT5 is traditionally thought to contribute to oncogenesis by trans-activating genes involved in cell cycle progression and survival, such as c-myc, pim-1, bcl-xL, mcl-1, and D-type cyclins (29, 30, 55). Our findings, together with others (39–41), indicate that the oncogenic properties of STAT5 may also be attributable to its ability to promote activation of the PI3K/Akt pathway, which is also widely implicated in oncogenesis (17, 18, 20). If so, then strategies aimed at inhibiting STAT5 activity may have the additional benefit of disrupting the PI3K/Akt pathway, thereby further promoting tumor cell apoptosis. Likewise, inhibitors of the PI3K/Akt pathway might prove efficacious against tumors with dysregulated STAT5 activity.

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Disclosures

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