Steel Factor Induces Serine Phosphorylation of Stat3 in Human Growth Factor-Dependent Myeloid Cell Lines

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Steel factor (SLF) acts synergistically with various hematopoietic growth factors that use the Jak-Stat pathways in vivo and in vitro, although the contribution by SLF to this pathway is unknown. We show here that SLF induces time- and dose-dependent phosphorylation of Stat3 in the human growth factor-dependent cell lines MO7e and TF-1. This phosphorylation occurs exclusively on serine residues. Simultaneous stimulation with SLF plus other cytokines that induce tyrosine phosphorylation of Stat3, such as interleukin-9 (IL-9) in MO7e cells or IL-6 in TF-1 cells, resulted in tyrosine phosphorylation and enhanced serine phosphorylation of Stat3. Serine phosphorylation alone did not promote nuclear translocation or DNA binding activity to the six-inducible element of Stat3. However, costimulation with SLF plus IL-9 in MO7e cells resulted in the nuclear translocation of serine-hyperphosphorylated Stat3. Serine phosphorylation of Stat3 was also observed by the stimulation of cells with granulocyte-macrophage colony-stimulating factor and IL-3, which do not induce tyrosine phosphorylation of Stat3.

These results suggest that SLF might modulate the Jak-Stat pathway by serine phosphorylation and that the Jak-Stat pathway may be differentially regulated by the combination of a unique set of Stat proteins in a fashion that is determined by the SH2-binding specificity of the receptor and by the SH2-binding specificity of the receptor tyrosine kinases. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

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MATERIALS AND METHODS

Cells, cell culture, and cell proliferation assay. Human growth factor-dependent myeloid cell lines, MO7e and TF-1, were cultured in RPMI 1640 supplemented with 20% fetal bovine serum (FBS) and 100 U/mL GM-CSF. These cell lines have been described elsewhere. Before cytokine stimulation, these cells were washed twice, resuspended in RPMI 1640 supplemented with 0.5% bovine serum albumin (BSA), and then incubated for 18 hours without growth factors (factor starvation). [3H]Thymidine uptake was used to measure cell proliferation by incubation of triplicate aliquots of cells cultured in flat-bottom microtiter plates (1 × 10⁴ cells/150 μL) with or without cytokines for 72 hours at 37°C. Cells were labeled with [3H]thymidine (Amersham, Arlington Heights, IL) at 0.5 μCi/well for the last 4 hours.

Cytokines, antibodies, and reagents. Recombinant human (rh) SLF, rhIL-3, and rhGM-CSF were kindly provided by Dr. D.E. Williams (Immunex Co., Seattle, WA). rhIL-6 and rhIL-9 were obtained from R & D Systems (Minneapolis, MN). Anti-Stat2, Stat3, Stat5, and Stat6 monoclonal antibodies (MoAbs), anti-ISGF3 polyclonal Ab, and anti-phosphotyrosine MoAb (PY20) were purchased from Transduction Laboratories (Lexington, KY). Anti-Stat3 and Stat4 polyclonal Abs and synthetic double-strand oligonucleotide corresponding to a binding site for six-inducible factor (six-inducible element [SIIE]) and its mutant in the DNA binding region were from Santa Cruz Biotechnology Inc (Santa Cruz, CA). Agarose-conjugated antiphosphotyrosine (4G10) was obtained from Upstate Biotechnology, Inc (Lake Placid, NY). Horseradish peroxidase (HRP)-conjugated antimouse and antirabbit IgG and [32P]orthophosphate were purchased from Amersham.

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In vivo labeling with $^{32}$P. After factor starvation, cells were washed three times with Minimum Essential Medium without sodium phosphate (Sigma, St Louis, MO) and incubated for 45 minutes in the same medium supplemented with 10% dialyzed FBS at a cell density of $10^7$/mL. $^{32}$Porthophosphate (100 μCi/mL) was added and cells were further incubated for 3 hours. After stimulation with various cytokines, cells were washed with ice-cold phosphate-buffered saline and then lysed as described below.

Cell lysis and nuclear extract preparation. Cells were lysed in lysis buffer as described previously. After 20 minutes on ice, insoluble fractions were removed by centrifugation at 14,000g for 20 minutes (whole cell lysate). Nuclear extract was prepared as described previously. Cells were washed with ice-cold buffer A (10 mmol/L HEPES, pH 7.9, 10 mmol/L KCl, 1.5 mmol/L MgCl$_2$, 0.5 mmol/L dithiothreitol [DTT], 0.5 mmol/L phenylmethylsulfonyl fluoride [PMSF]) and were resuspended in buffer A containing 0.1% Nonidet P-40 and incubated for 5 minutes on ice. The nuclear pellet was pelleted by centrifugation. The supernatant was centrifuged for 20 minutes at 14,000g and the resulting supernatant was saved as cytoplasmic extracts. The nuclear pellets were rinsed with buffer A and resuspended in buffer C (20 mmol/L HEPES, pH 7.9, 420 mmol/L NaCl, 1.5 mmol/L MgCl$_2$, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 0.5 mmol/L PMSF, 25% glycerol). After gently rocking for 15 minutes at 4°C, insoluble nuclear material was removed by centrifugation at 14,000g for 15 minutes. The protein concentration of these cell extracts was measured with a Bio-Rad protein assay (Bio-Rad, Hercules, CA). Equal amounts of protein were loaded in each lane or were used for immunoprecipitation and gel-mobility shift assay.

Immunoprecipitation and immunoblotting. Immunoprecipitation and immunoblotting were performed as described previously. Briefly, for immunoprecipitation, cell extracts were mixed with 1 μg of anti-Stat3 polyclonal Ab at 4°C for 2 hours. Antibody-Ab complexes were collected with protein A sepharose beads (Pharmacia Biotech, Uppsala, Sweden). Immunoprecipitates were washed with lysis buffer five to seven times before analysis. Immunoprecipitates and cell extracts were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore Corp, Bedford, MA). Membranes were blocked in Tris-buffered saline containing 0.5% Tween 20 and 1% BSA for 1 hour at room temperature and incubated with appropriate primary Abs for 2 hours. Blots were visualized with horseradish peroxidase-conjugated secondary Abs and enhanced chemiluminescent system (ECL, Amersham). To reprobe with another first Ab, membranes were incubated in stripping buffer (62.5 mmol/L Tris, pH 6.7, 100 mmol/L 2-mercaptoethanol, 2% SDS) for 30 minutes at 50°C, washed, and then used for further study.

Phosphoamino acid analysis. $^{32}$P-labeled anti-Stat3 immunoprecipitates were separated by 7.5% SDS-PAGE, transferred to PVDF membranes, and visualized by autoradiography. The region corresponding to phosphorylated Stat3 on the membrane was excised and hydrolyzed in 6 N HCl. Phosphoamino acid analysis was then performed on nitrocellulose thin-layer chromatography plates (Merck, Darmstadt, Germany) as described previously with 1 μg each of standard phosphoamino acids in pH 3.5 electrophoresis buffer. Electric gel mobility shift assay (EMSA). EMSA was performed as described previously. A double-strand synthetic SIE consensus oligonucleotide (5'-GTG CAT TTC CCG TAA ATC TTG TCT ACA-3') was 5' end-labeled with [γ$^{32}$P]ATP (Amersham) by polynucleotide T4 kinase (Boehringer Mannheim, Indianapolis, IN). Nuclear extract (5 μg) was incubated with 3 μg of poly(dI-dC) (Pharmacia Biotech) for 15 minutes on ice before adding 10,000 cpm of radiolabeled probe in binding buffer (20 mmol/L HEPES, pH 7.9, 20 mmol/L KCl, 5 mmol/L MgCl$_2$, 5 mmol/L DTT, 10% glycerol). For supershift assay, nuclear extract was preincubated with anti-Stat3 Ab or preimmune rabbit serum for 1 hour on ice. Competitions were performed with either the unlabeled homologous probe or a oligonucleotide mutated in the DNA binding region (5'-GTG CAT CCA CCG TAA ATC TTG TCT ACA-3'). After binding reaction for 10 minutes at room temperature, DNA-protein complexes were separated by 5% native polyacrylamide gel in 0.25× TBE buffer.

RESULTS

Tyrosine phosphorylation of Stat3 family proteins in factor-dependent cell lines. Tyrosine phosphorylation of Stat family proteins, presumably by Jak family tyrosine kinases, has been shown to be essential for their DNA binding and transcriptional activation. We first examined the effect of various hematopoietic growth factors on tyrosine phosphorylation of Stat family proteins in human factor-dependent cell lines, M07e and TF-1. Factor-starved M07e cells were stimulated with 100 ng/mL SLF, 200 U/mL GM-CSF, 200 U/mL IL-3, or 5 ng/mL IL-9 for 10 minutes at 37°C. Whole cell lysates of cytokine-stimulated cells were immunoprecipitated with agarose-conjugated antiphosphotyrosine MoAb and immunoprecipitates were separated by SDS-PAGE followed by immunoblotting with specific antibodies against Stat family proteins. As shown in Fig IA, in M07e cells, Stat5 was found to be tyrosine-phosphorylated in response to GM-CSF, IL-3, and IL-9. Stat3 was tyrosine-phosphorylated by IL-9 (Fig 1B). Tyrosine phosphorylation of Stat3 reached a plateau at 5 ng/mL of IL-9 (data not shown). Tyrosine phosphorylation of either Stat5 or Stat3 was detected after 100 ng/mL of SLF stimulation. Tyrosine phosphorylation of other Stat family proteins tested (ISGF3, Stat2, Stat4, and Stat6) was not detectable or was very weak after stimulation with IL-9, GM-CSF, IL-3, or SLF in these conditions (data not shown).

In TF-1 cells, which respond to the proliferative stimulation effect of IL-3, IL-6, GM-CSF, and SLF, but not to IL-9, tyrosine phosphorylation of Stat5 by IL-3 and GM-CSF (Fig 1C) and tyrosine phosphorylation of Stat3 by IL-6 (Fig 1D) was observed. Maximal tyrosine phosphorylation of Stat3 was obtained by 20 ng/mL of IL-6 (data not shown). Thus, SLF had no effect on the tyrosine phosphorylation of Stat5 family proteins in TF-1 cells (Fig 1C and D) or in M07e cells (Fig 1A and B). Regarding GM-CSF, IL-3, and IL-6, these results confirm reports by others, while these data show that IL-9 uses both Stat3 and Stat5 as downstream signaling pathways, probably as a result of activation of Jak family tyrosine kinases (Jak1, Jak2, and Tyk2) in M07e cells.

Slower mobility of Stat3 in SDS-PAGE by SLF is caused by hyperphosphorylation. Immunoblotting of whole cell lysates with anti-Stat5 MoAb showed mobility shift of Stat5 in response to tyrosine phosphorylation in M07e cells (Fig 2A). SLF did not modify mobility of Stat5 in SDS-PAGE (Fig 2A). Although SLF also did not induce tyrosine phosphorylation of Stat3 (Fig 1B), slightly slower mobility of Stat3 in SDS-PAGE was observed in M07e cells treated with SLF compared with control medium-treated cells (Fig 2B). It has been reported by others that IL-6 and epidermal growth factor (EGF) induce two tyrosine-phosphorylated forms of Stat3 and that the slower band is serine phosphorylated. Unlike the results found in those previous reports, Stat3 in cells treated with SLF did not form doublet bands, but rather formed one slower band (Fig 2B). To investigate
if this slower mobility was caused by phosphorylation of Stat3 on amino acids other than tyrosine, M07e cells were labeled with $[{^{32}}P]$orthophosphate and then stimulated with various cytokines. As shown in Fig 3, immunoprecipitation with anti-Stat3 Ab of $[{^{32}}P]$-labeled cell lysates showed that SLF can induce time- and dose-dependent phosphorylation of Stat3.

**Effect of SLF alone and in combination on phosphorylation of Stat3.** Proliferative effects of SLF and cytokines that induced tyrosine phosphorylation of Stat3 were examined by $[^{3}H]$thymidine uptake. It was confirmed that SLF and IL-9 in M07e cells (Fig 4A) and SLF and IL-6 in TF-1 cells (Fig 4B) had significant proliferative effects at the concentrations used in this study. Moreover, simultaneous stimulation with SLF plus these cytokines induced synergistic proliferation (Fig 4).

We next determined the nature of SLF-induced Stat3 phosphorylation in more detail. $^{32}$P-labeled factor-starved M07e cells were stimulated with cytokines, and Stat3 immunoprecipitates were analyzed by immunoblotting and autoradiography. Medium-treated cells showed basal phosphorylation of Stat3 (Fig 5C, lane 2) as compared with immunoprecipitation with preimmune serum (Fig 5C, lane 1). Simultaneous stimulation with SLF plus IL-9, which induced synergistic proliferation of M07e cells (Fig 4A), resulted in enhanced phosphorylation of Stat3 (Fig 5C, lane 5). Enhancement of overall phosphorylation was 3.5-fold by SLF, 2.5-fold by IL-9, and 4.2-fold by SLF plus IL-9 compared with medium-treated cells. In SLF plus IL-9–treated cells, tyrosine-phosphorylated Stat3 appeared as a single slightly slower migrating band in SDS-PAGE (Fig 5B, lane 5), whereas that of IL-9–treated cells appeared as a doublet band (Fig 5B, lane 4). To define amino acid residues phosphorylated by the stimulation with SLF, phosphoamino acid analysis was used. Regions corresponding to the mobility of Stat3 of the membrane were cut and hydrolyzed at the same time. Afterwards, all of the hydrolyzed amino acids were loaded in each lane and were separated on the same TLC plate. Figure 5D shows that SLF induces phosphorylation of Stat3 exclusively on serine residues. IL-9 induces tyrosine phosphorylation of Stat3.
phosphorylation and, to a lesser extent than does SLF, serine phosphorylation (Fig 5D). Densitometric analysis showed that serine phosphorylation was approximately 3.5 times higher after stimulation with 100 ng/mL of SLF-treated than in nontreated cells. Notably, simultaneous stimulation with SLF plus IL-9 induced phosphorylation of Stat3 on both serine and tyrosine residues (Fig 5D). Serine phosphorylation of Stat3 by the stimulation with SLF plus IL-9 was approximately 1.7 times higher than that of IL-9 alone as determined by densitometric analysis.

Phosphorylation of Stat3 was also detected after stimulation with GM-CSF and IL-3 (Fig 5C, lanes 6 and 7), which were confirmed not to induce tyrosine phosphorylation of Stat3 (Fig 5B, lanes 6 and 7). Using densitometric analysis, phosphorylation of Stat3 was 3.5-fold and 2.7-fold enhanced, respectively, in GM-CSF- and IL-3–treated cells compared with medium-treated cells. GM-CSF and IL-3, which induced tyrosine phosphorylation of Stat5 but not Stat3, induced serine phosphorylation of Stat3 (Fig 5D).

To confirm that serine phosphorylation of Stat3 by SLF is induced not only in MO7e cells but also in other myeloid cells, TF-1 cells were labeled with [32P]orthophosphate and stimulated with SLF, IL-6, SLF plus IL-6, and GM-CSF. Phosphorylation of Stat3 was also shown in TF-1 cells by the stimulation with SLF and GM-CSF (Fig 6C, lanes 3 and 6) that did not induce tyrosine phosphorylation of Stat3 (Fig 6B, lanes 3 and 6). Simultaneous stimulation with IL-6 plus SLF that induced synergistic proliferation in TF-1 cells (Fig 4B) resulted in enhanced overall phosphorylation (Fig 6C, lane 5) and serine phosphorylation (Fig 6D) of Stat3 compared with that with IL-6 alone (Fig 6C, lane 4 and Fig 6D). After stimulation of cells with SLF plus IL-6, tyrosine-phosphorylated Stat3 migrated as a slower single band in SDS-PAGE (Fig 6B, lane 5) compared with cells stimulated with IL-6 alone (Fig 6B, lane 4). Also, stimulation with 100 nmol/L 12-O-tetradecanoyl phorbol-13-acetate (TPA) for 10 minutes induced serine phosphorylation but not tyrosine phosphorylation of Stat3 both in MO7e and TF-1 cells (data not shown).

Translocation of hyper-phosphorylated Stat3 to the nucleus. For transcriptional activity of Stat3, translocation from the cytoplasm to the nucleus is a crucial event. In this context, we examined the localization of Stat3. Cytoplasmic and nuclear extracts were prepared from MO7e cells stimulated with SLF and/or IL-9. Immunoblotting of nuclear extracts with anti-Stat3 MoAb showed translocation of Stat3 to the nucleus in response to IL-9 and IL-9 plus SLF in MO7e cells (Fig 7A). SLF alone had no effect on this translocation of Stat3, and GM-CSF did not induce translocation (Fig 7A). Stat3 was observed in the cytoplasm and was...
synergistic proliferation of M07e and TF-1 cells by the stimulation with SLF plus cytokines that induce Stat3 tyrosine phosphorylation. Factor-starved M07e (A) or TF-1 (B) cells were plated at 1 x 10^6 per well in RPMI 1640 containing 5% FBS with indicated cytokines. The concentrations of cytokines used were as follows: 100 ng/mL SLF, 5 ng/mL IL-9, and 20 ng/mL IL-6. Cells were labeled with [3H]thymidine for the last 4 hours of 72 hours of culture and then the amount of incorporated [3H]thymidine was calculated using a scintillation counter. Data are the mean of triplicate culture SD. P values were measured using the unpaired Student’s t-test. *P < .0005 v control medium; **P < .005 v control medium; #P < .0001 for greater than additive effects.

Serine-phosphorylated after stimulation of cells with SLF (data not shown). These results suggest that serine phosphorylation occurs in the cytoplasm and phosphorylation on tyrosine but not on serine residues is crucial for nuclear translocation of Stat3.

Although the protein amount and tyrosine phosphorylation state of nuclear Stat3 were almost equal between IL-9 stimulation and IL-9 plus SLF stimulation (Fig 7B and C), overall phosphorylation was enhanced approximately 1.6-fold by stimulation with IL-9 plus SLF (Fig 7D). Furthermore, nuclear Stat3 formed a doublet band in SDS-PAGE after stimulation of the cells with IL-9 alone, whereas Stat3 formed a single slower migrating band after stimulation with IL-9 plus SLF (Fig 7B and C). Phosphoamino acid analysis of nuclear-translocated Stat3 showed that Stat3 in the nucleus was phosphorylated both on serine and tyrosine residues (data not shown), and the ratio of [3P] incorporated into serine/tyrosine was increased from 5.3 to 8.6 by simultaneous stimulation of cells with IL-9 plus SLF (Fig 7E).

Activation of DNA binding activity containing Stat3 in response to IL-9 or simultaneous stimulation with IL-9 plus SLF. Stat3 is known to bind SIE DNA consensus site upon activation. Therefore, SIE binding activity was assessed by EMSA with nuclear extracts of SLF and/or IL-9–treated M07e cells. As shown in Fig 8, IL-9 activated a specific DNA-binding protein (Fig 8, lane 3) whose binding could be competed with excess unlabeled probe itself but not by the unlabelled probe mutated in the DNA binding region (Fig 8, lanes 5 and 7). This DNA binding activity was not observed in nontreated cells or after SLF treatment alone (Fig
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**Fig 6.** Phosphorylation and phosphoamino acid analysis of Stat3 in TF-1 cells. Factor-starved TF-1 cells labeled with \(^{32}\)P were stimulated with SLF (100 ng/mL), IL-6 (20 ng/mL), SLF + IL-6, and GM-CSF (200 U/mL) for 10 minutes at 37°C. Anti-Stat3 immunoprecipitates were separated by 7.5% SDS-PAGE and immunoblotted with anti-Stat3 MoAb (A) or with antiphosphotyrosine MoAb (B) and visualized by autoradiography (C). Lane 1, mock; lane 2, control medium; lane 3, SLF; lane 4, IL-6; lane 5, SLF + IL-6; lane 6, GM-CSF. The corresponding region of Stat3 was excised and subjected to phosphoamino acid analysis (D). Similar results were obtained from three independent experiments.

**Fig 7.** Phosphorylation of nuclear translocated Stat3 by the simultaneous stimulation with SLF and IL-9. Factor-starved MO7e cells were labeled with \(^{32}\)P and stimulated with SLF (100 ng/mL), IL-9 (5 ng/mL), SLF + IL-9, and GM-CSF (200 U/mL) for 10 minutes at 37°C. Nuclear extracts were prepared as described in the Materials and Methods and then separated by 7.5% SDS-PAGE and immunoblotted with anti-Stat3 MoAb (A). Anti-Stat3 immunoprecipitates of nuclear extracts stimulated with IL-9 alone or SLF + IL-9 were separated by 7.5% SDS-PAGE and immunoblotted with anti-Stat3 MoAb (B) or immunoblotted with antiphosphotyrosine MoAb (C) and visualized by autoradiography (D). These results are representative of four independent experiments. The corresponding region of Stat3 was excised and subjected to phosphoamino acid analysis, and then corresponding regions of tyrosine phosphorylation and serine phosphorylation were quantitated by densitometry. The ratio of \(^{32}\)P incorporation to serine/tyrosine was calculated. *P < .01 (E).

**DISCUSSION**

The role of tyrosine phosphorylation for Stat family proteins are well delineated. We describe here for the first time that cytokines that do not induce tyrosine phosphorylation of Stat3, such as SLF, GM-CSF, and IL-3, can induce serine phosphorylation of Stat3 in human growth factor-dependent myeloid cell lines. It has been reported by others that the PMSP sequence, conserved in the C-terminal region of Stat1α, Stat3, and Stat4, is similar to the MAP kinase recognition consensus sites.\(^{23}\) We have previously reported that MAP kinase is activated after stimulation with SLF and GM-CSF but not with IL-9.\(^{18,25}\) Moreover, it has recently been reported by others that dominant negative MAP kinase inhibits interferon-β-induced transcription in human fibroblasts.\(^{26}\) These lines of evidence suggest that MAP kinase might play a role in the serine phosphorylation of Stat3 by SLF. The serine-threonine kinase cascade activated by SLF and GM-CSF involves not only MAP kinase but also raf-1 and other serine kinases that are downstream of MAP kinase and protein kinase C that might be activated as a result of PLC-γ activation.\(^{18,27}\) In the future, it should be determined which serine kinase is responsible for serine phosphorylation.

8, lanes 1 and 2). Simultaneous stimulation of cells with IL-9 and SLF induced an SIE binding activity that comigrated in EMSA with that induced by IL-9 alone (Fig 8, lanes 4 and 8). These DNA-protein complexes were shown to contain Stat3 by performing a supershift with an anti-Stat3 Ab that resulted in the retarded migration of the complexes (Fig 8, lanes 9 and 10).
of Stat3 for a further understanding of Stat-mediated signaling. However, the fact that TPA can induce serine phosphorylation of Stat3 (data not shown) suggests that, at least in part, activation of protein kinase C might be involved in this process.

The biological significance of phosphorylation on serine residues of Stat family proteins remains to be defined. However, recent reports have begun to clarify the importance of serine phosphorylation of Stats. Serine phosphorylation is reported to be required to form complexes of DNA with Stat3 homo-dimers. Our data showed that serine phosphorylation alone by SLF did not induce nuclear translocation or DNA binding activity of Stat3 (Figs 7A and 8). However, simultaneous stimulation of the cells with SLF plus other cytokines that induce tyrosine phosphorylation of Stat3 resulted in the translocation of the serine-hyperphosphorylated Stat3 to the nucleus (Fig 7). This suggest that SLF might modulate Jak-Stat pathway. Because GM-CSF and IL-3 also induced serine but not tyrosine phosphorylation of Stat3 (Figs 5 and 6), Jak-Stat pathway could be modulated by the hematopoietic progenitor cells that express particular sets of the cytokine receptors in the presence of multiple cytokines. EMSA using an SIE oligonucleotide as probe showed that IL-9 and IL-9 plus SLF induced a DNA binding activity that containing Stat3 in MO7e cells (Fig 8). Although these results did not clarify the role of serine hyperphosphorylation of Stat3, it is possible that transcriptional activity of serine-phosphorylated Stat3 is augmented without affecting its DNA binding or that IL-9 plus SLF can induce distinct DNA binding activity other than SIE.

Mechanisms underlying the synergism of SLF and other cytokines remain largely unknown, although a number of possible candidate intracellular events have been implicated. Considering SLF synergy, our observation is potentially important. First, increased phosphorylation of Stat3 by SLF plus IL-9 in MO7e cells (Fig 5C, lane 5) and by SLF and IL-6 in TF-1 cells (Fig 6C, lane 5) corresponds to increased mitogenesis at the cellular level (Fig 4). Second, translocation of serine-hyperphosphorylated Stat3 to the nucleus can be achieved only by the simultaneous stimulation of SLF and other cytokines that use Stat3 but not by SLF alone (Fig 7). It is noteworthy that others have reported recently that maximal activation of transcription by Stat1α and Stat3 requires both tyrosine and serine phosphorylation. Thus, it is highly possible that concomitant stimulation with SLF and other cytokines that use Stat3 can induce a higher transcriptional activation status of Stat3 in hematopoietic progenitor cells. It has been reported by others that dominant negative Stat5 inhibits IL-3-dependent cell growth and that Stat5 antisense oligodeoxynucleotides inhibit proliferation followed by erythroid differentiation in Friend virus-transformed murine erythroleukemia cells.

Second, it is likely that the Jak-Stat pathway might also contribute to some proliferation processes. In addition, in U937 monocytic cells, during differentiation induced by interferon-γ, Stat1 is reported to be phosphorylated on serine residues but not on tyrosine residues. This finding suggests that serine phosphorylation of Stats might play a role in the differentiation process in some cell types.

The contribution of Jak-Stat pathways to the functional response of hematopoietic progenitor cells requires further investigation. However, taken together with these previous reports and our data presented here, serine phosphorylation of Stat3 by SLF and other cytokines, especially through simultaneous stimulation with hematopoietic growth factors that induce tyrosine phosphorylation of Stat3, might be involved in the regulation of proliferation and/or differentiation of myeloid stem and progenitor cells.

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