Expression of Signal Transducers and Activators of Transcription Proteins in Acute Myeloid Leukemia Blasts

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ABSTRACT

Hematopoietic cytokine receptor signaling pathways involve activation of signal transducers and activators of transcription (STAT) proteins, which are postulated to be involved in cellular differentiation. Aberrant STAT isoforms (β forms rather than the normal α forms) have been described and have been found to block the normal signaling pathway from the receptor. Bcr/Abl proteins have been suggested to directly activate STATs, without exposure to growth factors. We asked whether STATs play a role in leukemogenesis. We analyzed constitutive and induced patterns of STAT activity in pretreatment blasts from 36 newly diagnosed acute myeloid leukemia (AML) patients and studied protein tyrosine kinases (PTKs) that may be involved in STAT activity, using in vitro and in-gel kinase assays. The β forms were expressed in 21 of 27 samples (78%). Constitutive STAT3 and STAT5 activity was found in samples from 28 and 22% of patients, respectively. Response to exogenous cytokines identified two groups. STAT activity in one group was modulated by exogenous cytokines: constitutive STAT activity increased in some patients but decreased or disappeared in response to cytokines in others. The second group was cytokine insensitive. Additionally, we found constitutive PTK activity in two patients whose blasts demonstrated constitutive STAT activity, suggesting that PTKs use cytokine receptor signal pathways to activate STATs in AML blasts without exposure to exogenous cytokines. Our data suggest that (a) constitutive expression of aberrant STATs may be involved in blocking differentiation of AML blasts, (b) exogenous cytokines may activate STAT-inhibitory pathways, and (c) STATs may be activated by PTKs in some AML blasts.

INTRODUCTION

Members of the JAK family of protein kinases and the STAT DNA-binding proteins are activated by tyrosine phosphorylation and are involved in many growth factor and cytokine receptor signaling pathways (reviewed in Refs. 1 and 2). The existence of different functional isoforms (alternate splice products) of several STAT proteins has been documented. For example, STAT3 (now STAT3α) has been described to have an isoform (STAT3β) that is truncated by 55 amino acid residues at the COOH-terminal end (3). STAT3β was found to be more commonly expressed in some AML blasts (4, 5). Similarly, STAT5A and STAT5B, originating from two separate genes, are described to have at least two isoforms (α and β) each (6–8). The β isoforms were shown to have a transdominant negative effect on gene induction in the STAT pathway (9, 10).

Recently, several groups have demonstrated that, in addition to causing STAT activation, cytokines may also indirectly induce STAT inhibition. A new family of JAK inhibitors have been discovered (11–14). Among them are three isoforms of one family termed cytokine-inducible SH2 protein (11), JAK-binding protein (12), STAT-induced STAT inhibitor-1 (13), or suppressor of cytokine signaling-1 (14). These molecules inhibit the catalytic activity of the JAKs and, thus, the activation of signaling intermediates such as the STATs. In addition, immediately acting inducible proteins that down-regulate JAKs and STAT proteins following cytokine induction include the protein tyrosine phosphatase SHP-1 (15). Moreover, phosphatases such as MKP-1 (3CH134) and CL100 specifically inactivate the MAPK pathway (16–18). Although it remains unclear whether the activity of any phosphatases is involved in signaling by the hematopoietic receptors on the action of STATs, they offer another mechanism to counter the effect of receptor-recruited kinases and signaling molecules such as STATs. This may then allow for other pathways (e.g., MAPK) to be activated and lead to inhibition of differentiation and induction of proliferation.

Finally, non-Jak PTKs such as other family members of Src, Fes, and Tec may be activated within the cell, without cytokines and growth factors, and cause STAT activation. For example, it has been suggested that STAT3 is the v-Src target responsible for transformation, based on the observation that it is activated in v-Src-transformed cells (19). Similarly, the products of the t(9;22), p210BCR/ABL, and p190BCR/ABL have been suggested to activate directly STAT family members, without exposure to any growth factors (20–23). This may explain why growth factor-dependent cells become growth factor independent upon transfection of p210BCR/ABL or p190BCR/ABL.

We asked whether STAT proteins, besides being sensitive indicators of hematopoietic cytokine receptor signaling, might also play a role in leukemogenesis. We hypothesized that any of the following mechanisms could apply: (a) expression of alternate STATβ forms that would inhibit the differentiation normally induced by the α forms and, hence, also inhibit apoptosis; (b) induction of STAT inhibition by cytokines, blocking signal transduction toward differentiation; and (c) constitutive PTKs activity, inducing MAPK pathway as well as STATs without the requirement of exogenous growth factor stimulation.

MATERIALS AND METHODS

Patient Population. Pretreatment cells were studied from 36 newly diagnosed AML patients who were referred to Roswell Park Cancer Institute between December 1993 and January 1997. Twenty-five had de novo AML; 11 patients had secondary AML, including 9 who had an antecedent hematologic disorder and 2 who had received chemotherapy for a prior malignancy. Ages of the patients ranged from 18 to 84 years (median 65 years). Nineteen were male. Samples from normal bone marrow donors were used as controls. Studies were approved by the Roswell Park Cancer Institute Institutional Review Board. Informed consent was obtained from all patients.

Cytogenetic Analysis. Cytogenetic analyses were performed on all but one. For cytogenetic analyses, bone marrow samples were processed using short-term unstimulated cultures (24–72 h). Clonality criteria and descriptions of chromosome aberrations were according to the International System for Human Cytogenetic Nomenclature (1995; Ref. 24). Three patients had t(15;17), 3 had t(8;21), 13 had normal cytogenetics, 6 had one or two other aberrations [1 with del(5q), 1 with +8, 1 with t(9;22), 1 with +11, 1 with...
+mar as the sole abnormality, and 1 with +8,+13], and 10 had three or more cytogenetic aberrations including, among others, 3q— (one patient), —5 or 5q— (three patients), —7 (three patients), +8 (one patient), and 11q23 (two patients).

Materials. All chemicals were purchased from Sigma Immunochemicals (St. Louis, MO) unless otherwise specified, and all cytokines and growth factors were kindly provided by Amgen (Thousand Oaks, CA) unless otherwise specified.

Cell Collection. Light-density bone marrow cells were isolated by 1.077 g/mm² Ficoll-Hypaque density gradient centrifugation and were either used immediately or were cryopreserved under viable conditions, using standard methodology. Cell viability was verified by trypan blue dye exclusion. Cells from three patients were studied both fresh (on the day of collection) and following cryopreservation and thawing.

EMSA to Study STAT Activation. The DNA-binding activity of STAT proteins was assessed by EMSA. Whole cell extracts prepared as described (25) were incubated with 32P-labeled oligomers corresponding to the binding elements for STAT1 and STAT3 (SIE; Ref. 26), STAT5 (TB2; Ref. 27), and STAT6 (GAS; Ref. 28). The reaction products were analyzed by 5% PAGE and autoradiography. Extracts of MO7E cell line treated with TPO (40 ng/ml) and displaying activated STAT3a, STAT5A«, and STAT5Ba served as internal references for these analyses. To account for background activity, the lowest level of detection was set as <2%. Band intensity of >2% was defined as constitutive activity.

The identity of the STAT bands was determined by antibody supershift with the anti-STAT3 (C-20), anti-STAT5 (C-17), or anti-STAT6 (S-20) monoclonal antibodies (all obtained from Santa Cruz Biotechnology, Santa Cruz, CA).

Exposure to Cytokines. To study whether blasts that constitutively express activated STAT proteins are still cytokine responsive through STAT signaling, cells were exposed to G-CSF (10 ng/ml), GM-CSF (10 ng/ml), or TPO (40 ng/ml) for varying periods of time, ranging from 15 min to 3 h. These concentrations were chosen based on our previous work demonstrating induction of AML blast proliferation in vitro by these cytokines (29). Blasts were then harvested, and cell extracts were prepared as described above for the various assays.

In Vitro Phosphorylation Assay for Any Constitutive Kinase Activity. To study constitutive expression of protein kinases, identical amounts of cell extracts, prepared as described above, were incubated in kinase buffer with 20 μM ATP and 10 μCi of [γ-32P]ATP (3000 Ci/mmol; Amersham Life Science, Arlington Heights, IL) at 30°C for 10 min and then boiled in 2X SDS buffer. The reaction products were analyzed by 10% SDS gel electrophoresis, transferred onto a 0.1-μm nitrocellulose membrane, and subjected to autoradiography (30).

Western Blotting. To quantitate STAT3 and STAT5 proteins expressed by the leukemic cells and to study the expression of phosphotyrosine kinase proteins, Western blot analysis was performed as described previously (31). Briefly, the phosphotyrosine membrane, from the in vitro kinase assay described above, was incubated with anti-STAT3 or anti-STAT5 antibodies (both obtained from Transduction Laboratories, Lexington, KY) or antiphosphotyrosine antibody (PY-20; Santa Cruz Biotechnology). STAT3, STAT5, and PTKs were detected using the ECL Western blotting detection system (Amersham).

In-Gel Kinase Assay for Strong Constitutive Kinase Activity. To study the presence of protein kinases with strong autocatalytic activity, cell lysates were resolved on an SDS gel. SDS was removed by washing in HEPES (40 mM), followed by urea (8 M), and the gel was incubated in in-gel kinase buffer containing 20 μM ATP and 75 μCi of [γ-32P]ATP for 1 h at 25°C. Excess [γ-32P]ATP was removed from the gel, and the proteins were fixed by incubation in 20 ml of 15% methanol and 7% acetic acid. The gel was then dried and exposed to film for autoradiography (30).

RESULTS

Constitutive STAT Activation

Constitutive STAT3 Activity. To study constitutive STAT3 activity in leukemic blasts, we analyzed bone marrow samples from 36 newly diagnosed AML patients by EMSA. Ten samples (28%) had constitutive activation of STAT3, as shown in Fig. 1 (filled bars).

Constitutive STAT5 Activity. To study constitutive STAT5 activity by leukemic blasts, we analyzed bone marrow samples from the same 36 AML patients. Eight samples (22%) had constitutive STAT5 activity. As shown in Fig. 2 (filled bars), all had a signal intensity that was lower than that of the control cell line. Two of the STAT3-high expressor samples and two of the STAT3-low expressors had constitutively active STAT5 as well (Table 1). Four samples that had constitutively active STAT5 did not have constitutive STAT3 activity.

Constitutive STAT6 Activity. Samples from 10 of the patients were studied for constitutive STAT6 activity. As a positive control,
we used the acute lymphoblastic leukemia cell line ALL-1 exposed to interleukin 4. No samples showed constitutive DNA-binding activity of STAT6. We, therefore, decided not to pursue further STAT6 activation in samples from AML patients.

Expression of STATα versus STATβ

STAT3α versus STAT3β. As shown in Fig. 3A, all but one sample (patient 960453) had a slower migrating band than that of the control cell line. This suggests that the sample derived from patient 960453 expressed STAT3α, whereas all other samples expressed STAT3β. The presence of the β isoform was verified by supershift assay using an antibody against the COOH-terminal epitope of STAT3. As shown in Fig. 3B, the addition of the antibody supershifted the SIE band in samples containing extract from patient 960453 only. No supershift was detected in any other sample. Thus, the data indicate that cells from all AML patients with constitutively active STAT3 with the exception of patient 960453, expressed primarily STAT3β. The relative concentration of STAT3 among AML samples was determined by immunoblotting with anti-STAT3 antibody, which is directed against the NH2-terminal domain, and, therefore, recognizes both STAT3β and STAT3α. As shown in Fig. 3C, the sample from patient 960453 primarily expressed STAT3α, whereas the other samples expressed STAT 3β. Each sample contained roughly equivalent amounts of STAT3. Therefore, the differences in intensity of the shifted SIE bands between samples (Fig. 3A) are probably due to differences in the level of STAT3 activity. In a separate analysis of cell lysates prepared under immediate denaturing conditions, we demonstrated that the presence of STAT3β was not a proteolytic product generated during extraction. These data suggest that the expression of STAT3β is probably the result of alternatively spliced STAT3 mRNA (32).

Table 1 Clinical characteristics of AML patients whose blasts had constitutively active STAT proteins

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<th>Patient no.</th>
<th>Age (yr)</th>
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*2nd, secondary AML; ND, not done; FAB, French-American-British classification; + + + +, samples with STAT activity exceeding that of M0E cells exposed to thrombopoietin (control); + + + , samples with STAT activity between 50 and 100% of the control; + +, samples with STAT activity between 25 and 49% of the control; +, samples with STAT activity between 2 and 24% of the control; −, samples with activity <2% of the control.

Table 1 Clinical characteristics of AML patients whose blasts had constitutively active STAT proteins

Fig. 2. Constitutive and induced STAT5 activity in AML blasts. Cells were cultured in the presence of control medium alone or in the presence of the indicated cytokines [G-CSF (10 ng/ml), GM-CSF (10 ng/ml), or TPO (40 ng/ml)] for 15 min. Data are expressed in comparison to the internal reference (M0E cell line exposed to TPO). To account for the background activity, a lowest level of detection was set as <2%. * experiment was not done. Patient numbers are shown on the bottom.
STATα versus STATβ. To demonstrate which of the STAT5 isoforms bound to TB2 in these cell extracts, we examined the effect of antibodies against STAT5A and STAT5B on the electrophoretic mobility of TB2. As shown by the representative data in Fig. 4A, the addition of the pan-STAT5 antibodies supershifted the major portion of the TB2 band duplex in samples containing extract from patients 960421 and 950495 only. No supershift was detected in any of the other samples. Failure of antibody recognition was attributed to the presence of STAT5β. This could be verified by the specific binding of STAT5β but not STAT5α to the TB1 oligomer that has been developed as a substrate that is specific to the β-form of STAT5A and STAT5B. As shown in Fig. 4B, the samples that did not demonstrate supershift with anti-STAT5 antibody had TB1 complex on EMSA. Analysis of whole cell extracts by immunoblotting with anti-STAT5 antibody that is directed against a central epitope demonstrated that all patient samples express similar levels of STAT5 (A and B combined). As shown in Fig. 4C, the STAT5 isoform was readily apparent in sample 960421, which migrated as the full-length STAT5α, whereas samples 950110 and 950375 contained only the smaller STAT5β form. These results suggest that, in some samples, STATβ is expressed. As with STAT3, we determined that the STAT5β form most likely represents a product of alternatively spliced mRNA (33) rather than a product of proteolysis.

We have analyzed a total of 27 patients’ samples by Western blotting for the expression of STATα versus STATβ forms. This includes the samples that had constitutive active STATs and those that did not. Twenty-one of the 27 samples (78%) expressed the STATβ. Expression of the α/β isoforms was similar whether they were analyzed for STAT3 or STAT5. There was no significant difference in the amount of the resident STATs whether the samples had a relatively high signal activity, as determined by EMSA (samples from patients 960453, 960586, and 960484) or relatively lower signal activity, as determined by EMSA (samples from patients 950495, 950517, 950122, 950521, 950373, 950374, 960447, 960431, 950375, and 950110), or whether the samples did not constitutively express STAT proteins (all others; data not shown).

Modulation of STAT Activity by Exogenous Cytokines

Kinetics of STAT Activation. To study whether blasts with or without constitutively activated STAT proteins are still cytokine responsive through STAT signaling, blasts from five patients were exposed to G-CSF, GM-CSF, and TPO for periods of 15 min to 3 h. Exposure to any of the cytokines for 15 min was optimal for STAT activation in four of five patients, whereas in the fifth patient, 30 min was the optimal time for STAT activation. Furthermore, STAT3 activation could be seen in some samples cultured for 1 h in medium alone (Fig. 5A). This suggests that there may be autocrine stimulation of STAT3 in some samples. We elected to treat all patients’ samples for 15 min.

STAT Activation by G-CSF, GM-CSF, and TPO. The ability of cells in the 36 AML patients samples to respond to G-CSF, GM-CSF, and TPO was determined. Data collected from all patients demonstrated five patterns of STAT activation: (a) no constitutive STAT activity and no activation of STAT by these cytokines (data not shown); (b) no constitutive STAT activity but clear induction following exposure to cytokines (data not shown); (c) constitutive STAT activity and further enhancement of STAT activity following exposure to cytokines (Fig. 1, sample 950495; and Fig. 2, samples 950490, 960431, and 950375); (d) constitutive STAT activity but reduction of STAT activity following exposure to specific cytokines (Fig. 1, samples 950517, 950122, and 950373; Fig. 2, sample 960421); and (e) constitutive STAT activity, with no further modulation following exposure to cytokines (Fig. 1, samples 960453 and 950521; Fig. 2, 950453 and 950110). Expression of the α/β isoforms was not significantly affected by exposure to exogenous cytokines, i.e., samples that contained the β forms prior to exposure to any cytokine (control) contained the β forms after exposure to cytokines (data not shown).

Cryopreservation did not modify the constitutive or induced STAT activation in two of three patients’ samples, but in the sample from the third patient, the enhanced STAT signals detected in the fresh sample were not detected in the cells after the freeze-thawing procedure (data not shown). In no case could we detect the inverse process, that is, the appearance of constitutive active STAT by cryopreservation. This
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Samples without Constitutive STAT Activity. Data on STAT activity and in vitro and in-gel kinase assays, as well as on Western blotting, are available for 29 patient samples (data not shown). Of the 19 samples that demonstrated no constitutive STAT activity, 2 showed enhanced kinase activity in the in vitro kinase assay, as well as in the Western blotting, suggesting that these cells have a discrete PTK but that this kinase is not effective on STAT. Nevertheless, these samples responded to cytokines by substantial STAT activation, indicating the presence of appropriate signal transduction.

Samples with Constitutive STAT5 Activity. Data on in vitro and in-gel kinase assays, as well as on Western blotting, in samples with constitutive STAT5 activity were available on six patient samples. Representative data are shown in Fig. 6, A–C (left). Elevated PTK level was demonstrated in one sample with constitutive STAT5 activity alone (960421). Fig. 6A demonstrates constitutive PTK activity, as evidenced by the in vitro kinase assay. The molecular weight of this kinase is Mr ~89,000. A similar band is depicted in Fig. 6B, suggesting that this molecule is a PTK (arrowhead). This molecule was not detected by the in-gel kinase assay (Fig. 6C) because of its relatively large size. In summary, blasts derived from patient 960421 expressed a PTK that could be responsible for induction of constitutive STAT5 activity. The nature of this kinase has yet to be determined.

Samples with Constitutive STAT3 or Both STAT3 and STAT5 Activity. Data on in vitro and in-gel kinase assays, as well as Western blotting in samples with constitutive activity of STAT3 alone or both STAT3 and STAT5, were available for six patient samples. Representative data are shown in Fig. 6, A–C (right). Our data demonstrate constitutive PTK activity in one sample with constitutive STAT3 (960586) and STAT5 activity (960586). The in vitro kinase assay shows clear bands in the Mr 190,000 region (arrowhead), between the Mr 47,000 and 89,000 molecular weight markers and below the Mr 47,000 marker (Fig. 6A). The in-gel kinase assay demonstrates a strong band below the Mr 47,000 molecular weight marker (Fig. 6C). The significance of these bands is exemplified by the Western blotting (Fig. 6B). The band at Mr 190,000 represents the chimeric p190BCR/ABL molecule in the blasts of this patient with t(9;22)(q34; q11), and the smaller bands represent degradation products of the Bcr/Abl kinase in these cells (Fig. 6D). Therefore, in this patient's blasts, p190BCR/ABL most probably has a role in the activity of STAT3 and STAT5, as suggested previously (20–23).

DISCUSSION

Our data demonstrate that 78% of the patients' samples expressed STAT isoforms. STAT3 and/or STAT5 are constitutively activated in blasts derived from a small subset of AML patients. STAT activity is cytokine responsive in some patients, whereas in others, the STATs

Constitutive PTK Activity

We hypothesized that presence of a deregulatable PTK might be responsible for constitutive STAT activity. To test for constitutive kinase activity, we used three complementary methods: (a) in vitro phosphorylation of proteins; (b) in-gel kinase method; and (c) Western blotting with antiphosphotyrosine antibody and specific anti-PTK antibodies. This method was used to complement the other two methods.

Fig. 5. The kinetics of STAT activation. Activation of STAT3 (top) and STAT5 (bottom) protein complexes in extracts from cells of a representative patient exposed to G-CSF (10 ng/ml), GM-CSF (10 ng/ml), TPO (40 ng/ml), or no growth factor (control) for 15, 30, and 60 min. The positions of STAT3 and STAT5 are indicated.
are cytokine unresponsive. For the first time, a subset of patients was recognized in whom exposure to cytokines resulted in down-regulation of STAT activation. Finally, we demonstrate the potential contribution of cellular PTKs, members of at least two kinase families, to constitutive STAT activation.

It has been shown that STAT3 and STAT5A and STAT5B exist in different isoforms, a short one (β) and a long one (α). In all cases, the short form (termed β) is the COOH-terminally truncated form of the long one. This COOH-terminal sequence, missing in the β-forms for both STAT3 and STAT5, encodes the transcriptional activation domain (5, 34, 35). It is not clear how these isoforms are generated: either as the products of separate mRNA species or as posttranslational proteolysis of a single α form. Alternative mRNA splicing has been suggested as originally observed for STAT1 (36). It was shown that the exon that appears in STAT1β after exon 22 contains an in-frame translational stop signal after amino acid 701. In the STAT1α, there are two exons after amino acid 700. The upstream exon that is used to produce the STAT1β mRNA is skipped in producing the STAT1α mRNA. Similar data have been generated for STAT3 (32) and STAT5 (33). However, truncations do not necessarily arise only by alternative splicing. Several researchers suggested that the truncated STAT5 isoforms can also be generated by a STAT5-cleaving protease (8, 37). Our preliminary findings that samples containing STAT3β isoform also contain the STAT5β isoform suggest that there is a general phenomenon concerning the appearance of the truncated forms in AML blasts. This is further supported by our preliminary data that samples with the β isoforms maintain the same forms upon exposure to exogenous cytokines. Finally, previous work has shown that cell lines and AML patient samples that express both STAT3 protein isoforms express both mRNA sequences (5). Exposure of the cells to G-CSF, however, appeared to result in the activation of the β isoform only. Although suggested, it seems unlikely that the exogenous cytokine can transduce signal by using only one of the two STAT isoforms. Alternatively, and more likely, a cytokine-induced conversion of α to β occurs posttranslationally. Because the appearance of the β isoforms seems to be a detrimental event in the signal transduction pathway and may be involved in leukemogenesis, events controlling β isoform production can provide tools to modulate its expression.

Constitutive activity of STAT3 and STAT5 were detected in samples from 28% and 22% of AML patients. These data differ from those of Gouilleux-Gruart et al. (38), who found that samples from five of five AML patients had constitutive STAT3 activity. They, however, analyzed only a small number of patients. Our data demon-
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Suggesting STAT3 activation following a prolonged culture period might provide another explanation for its activity in all samples tested by Gouilleux-Gruart et al. (38). Our data concerning STATs are compatible with those of Gouilleux-Gruart et al. (38), who showed constitutive STATs activity in 40% of AML patients' samples. Although these data, as well as those of others (39, 40), suggest a role for constitutive STAT activity in leukemogenesis, the evidence is not complete. Luo et al. (41) and Hanratty et al. (42) generated JAK mutants that hyperphosphorylated and hyperactivated STAT proteins. These mutants developed, among other abnormalities, leukemia-like defects in the Drosophila melanogaster. However, introduction of loss-of-function STAT mutants suppressed premature differentiation processes but did not prevent overproliferation of the hematocytes (41).

Our work is the first to demonstrate down-regulation of STAT proteins in fresh leukemic cells in response to exogenous cytokines. The findings could be attributed to lack of resident STATs or to cell death. However, all samples contained similar levels of immunodectectable resident STATs. Moreover, an intracellular signaling reaction could be triggered by exposure to some of the cytokines. These data support the concept that sufficient resident STAT proteins exist in the cells and defy the concept of cell death, as does the short incubation period (15 min). Although the mechanism of STAT inhibition is unclear, a likely possibility is activation of JAK inhibitors (11-18), which, upon exposure to exogenous cytokines, would block prolonged signal transduction by STATs toward differentiation. Lack of differentiation may then result in leukemogenesis.

Finally, several concepts can be advanced concerning the constitutive activation of STATs by PTKs. On the basis of studies of leukemic cells, we have shown that p190BCR/ABL is primarily located in the cytoplasm (31), which would place it in proximity to STAT proteins. Therefore, the fact that substantial activity of resident STAT proteins was not detected in samples with active PTKs whereas the addition of exogenous cytokines was very effective in inducing STAT activity may suggest that PTKs and STATs may reside in different subcellular compartments in some cases. Another possibility is that these kinases may involve other signaling processes that are different from those that are recognizable by the activation of STATs. That is particularly interesting in view of the recent suggestion that SCF involves a modulation of STAT5 through serine phosphorylation (43, 44). Furthermore, the DNA-binding activity of STAT proteins has been ascribed to the phosphorylation of the tyrosine residue at the COOH-terminal portion of the molecule that serves as binding substrate for a second STAT molecule (1, 2). Upon dimerization, the STAT complexes display DNA-binding activity and undergo nuclear translocation. We have previously traced the effect of STAT activation by p190BCR/ABL and p210BCR/ABL to gene induction in hematoma cells by usage of prominent cytokine response elements (45). We expect that STAT proteins may exert similar effects in hematopoietic cells; however, target genes relevant in these cells remain to be defined. Although the results were somewhat preliminary, they helped recognize representative PTK patterns associated with AML.

Taken together, the current knowledge suggests that STATs have the potential to be involved in leukemogenesis via more than one mechanism: (a) constitutive expression of aberrant STATs may be involved in blockage differentiation, (b) exogenous cytokines may activate STAT-inhibitory pathways, and (c) STAT activation may be induced by PTKs in some AML blasts without exposure to exogenous cytokines. Because AML encompasses significant biological heterogeneity, correlation of patterns of STAT activation with karyotype, immunophenotype, and other biological variables may reveal subtypes of AML with novel mechanisms of leukemogenesis.

REFERENCES


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