The p38 MAPK Pathway Mediates the Growth Inhibitory Effects of Interferon-α in BCR-ABL-expressing Cells*

Received for publication, December 26, 2000, and in revised form, May 11, 2001
Published, JBC Papers in Press, May 15, 2001, DOI 10.1074/jbc.M011685200

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The mechanisms by which interferon-α (IFN-α) mediates its anti-leukemic effects in chronic myelogenous leukemia (CML) cells are not known. We determined whether p38 MAPK is activated by IFN-α in BCR-ABL-expressing cells and whether its function is required for the generation of growth inhibitory responses. IFN-α treatment induced phosphorylation/activation of p38 in the IFN-α-sensitive KT-1 cell line, but not in IFN-α-resistant K562 cells. Consistent with this, IFN-α treatment of KT-1 (but not K562) cells induced activation of the small GTPase Rac1, which functions as an upstream regulator of p38. In addition, IFN-α-dependent phosphorylation/activation of p38 was induced by treatment of primary granulocytes isolated from the peripheral blood of patients with CML. To define the functional role of the Rac1/p38 MAPK pathway in IFN-α signaling, the effects of pharmacological inhibition of p38 on the induction of IFN-α responses were determined. Treatment of KT-1 cells with the p38-specific inhibitors SB203580 and SB202190 reversed the growth inhibitory effects of IFN-α. On the other hand, the MEK kinase inhibitor PD098059 had no effects, further demonstrating the specificity of these findings. To directly determine the significance of IFN-α-dependent activation of p38 in the induction of the anti-leukemic effects of IFN-α, we evaluated the effects of p38 inhibition on leukemic colony formation in bone marrow samples of patients with CML. IFN-α inhibited leukemic granulocyte/macrophage colony formation in a dose-dependent manner, whereas concomitant treatment with p38 inhibitors reversed such an inhibition. Thus, the Rac1/p38 MAPK pathway is activated by IFN-α in BCR-ABL-expressing cells and appears to play a key role in the generation of the growth inhibitory effects of IFN-α in CML cells.

Interferons are potent regulators of malignant hematopoiesis and exhibit growth inhibitory effects in leukemia cells in vitro and in vivo (1–6). Extensive studies have established the efficacy of interferon-α (IFN-α) in the treatment of leukemias, and this cytokine is currently the treatment of choice for patients with chronic myelogenous leukemia (CML) that are not eligible for bone marrow transplantation (7, 8). It is of particular interest that, among several other hematologic malignancies, CML exhibits very high sensitivity to the growth inhibitory effects of IFN-α in vivo (7, 8). CML is a clonal myeloproliferative disorder of hematopoietic stem cells, and the hallmark of the disease is the expression of the BCR-ABL oncprotein in the malignant cells. BCR-ABL is the product of the bcr-abl oncogene, which is generated by the reciprocal translocation between chromosomes 9 and 22, resulting in the fusion of bcr to c-abl and the formation of the abnormal bcr/abl proto-oncogene (9–11). The abnormal bcr/abl proto-oncogene encodes the constitutively active BCR-ABL tyrosine kinase, which plays an essential role in the pathogenesis of the disease (12) via phosphorylation of protein substrates and activation of multiple downstream mitogenic pathways (13).

All type I IFNs (IFN-α, -β, and -ω) bind to a common receptor, the type I IFN receptor, and activate the receptor-associated TYK2 and JAK1 kinases (2–6). Activation of the these tyrosine kinases results in phosphorylation of STAT proteins, which form complexes that translocate to the nucleus and bind to specific elements present in the promoters of IFN-sensitive genes to regulate transcriptional activation (2–6). The IFN-α-induced tyrosine phosphorylated forms of STAT1 and STAT2 associate with interferon regulatory factor-9 to form the IFN-stimulated gene factor-3 complex, which binds to IFN-stimulated response elements in the promoters of IFN-sensitive genes (2–6). On the other hand, STAT1/STAT1 homodimers, STAT5/STAT5 homodimers, and CrkL/STAT5 heterodimers bind to IFN-γ-activated recognition sites in the promoters of IFN-regulated genes to regulate transcription (2–6, 14). Thus, signaling specificity via the IFN-α-activated JAK/STAT pathway appears to be determined by the formation of distinct complexes among STAT proteins that activate different regulatory elements in the promoters of target genes.

Recent studies have established that, in addition to the STAT pathway, type I IFNs activate MAPKs, including ERK

* This work was supported by National Institutes of Health Grants CA7816 and CA73581 and a merit review grant from the Department of Veterans Affairs (to L. C. P.) and by Canadian Institutes of Health Research Grant MT15094 (to E. N. F.). The costs of publication of this article were defrayed in part by the payment of page charges. 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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1 The abbreviations used are: IFN, interferon; CML, chronic myelogenous leukemia; STAT, signal transducer and activator of transcription; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; MEK, MAPK/ERK kinase; PAK, p21-activated kinase; GST, glutathione S-transferase; PBD, PAK1-binding domain; PAGE, polyacrylamide gel electrophoresis; MAPKAPK, MAPK-activated protein kinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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against p38 to control for protein loading.

IFN-sensitive, BCR-ABL-expressing cell line model systems. In blotting. A polyclonal antibody against p38 was obtained from Santa Inc. Antibodies against the phosphorylated forms of p38 and ERK2 were obtained from New England Biolabs Inc. and used for immuno-

bleotted with an antibody against the phosphorylated/activated form of p38.

were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of p38.

was stripped and reprobed with an antibody against p38 to control for protein loading.

FIG. 1. Activation of the p38 MAPK pathway in the IFN-α-sensitive KT-1 cell line, but not in IFN-α-resistant K562 cells. A, KT-1 cells were incubated for 30 min in the presence or absence of IFN-α as indicated. Equal amounts of total cell lysates (100 μg/lane) were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of p38. B, the blot shown in A was stripped and reprobed with an antibody against p38 to control for protein loading. C, KT-1 or K562 cells were incubated in the presence or absence of IFN-α for the indicated times (min). Equal amounts of total cell lysates (100 μg/lane) were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of p38. D, the blot shown in C was stripped and reprobed with an antibody against p38 to control for protein loading.

FIG. 2. IFN-α-dependent activation of ERK2 in KT-1 and K562 cells. A, KT-1 cells were incubated for 30 min in the presence or absence of IFN-α as indicated. Equal amounts of total cell lysates (100 μg/lane) were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of the ERK kinase. B, the blot shown in A was stripped and reprobed with an antibody against ERK2. C, K562 cells were incubated for 30 min in the presence or absence of IFN-α as indicated. Equal amounts of total cell lysates (100 μg/lane) were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of the ERK kinase. D, the blot shown in C was stripped and reprobed with an antibody against ERK2.

FIG. 3. Tyrosine phosphorylation of the TYK2 kinase in response to IFN-α treatment of KT-1 and K562 cells. A, KT-1 cells were treated with IFN-α as indicated. Cell lysates were immunoprecipitated with an antibody against TYK2 or control nonimmune rabbit immunoglobulin (RigG), and immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an anti-phosphotyrosine (anti-pTyr) antibody (4G10). B, K562 cells were treated with IFN-α as indicated. Cell lysates were immunoprecipitated with an antibody against TYK2 or control nonimmune rabbit IgG, and immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with anti-phosphotyrosine antibody (4G10).

The mechanisms of action of IFN-α in CML are not understood. Very little is known of the signaling events induced by IFN-α in BCR-ABL-expressing cells, primarily due to a lack of IFN-sensitive, BCR-ABL-expressing cell line model systems. In this study, we used the recently established IFN-α-sensitive KT-1 cell line (20) to determine whether p38 and its upstream effector, Rac1, are engaged in IFN signaling in BCR-ABL-expressing cells. We also examined whether the p38 pathway is engaged in IFN-α signaling in primary cells obtained from the peripheral blood of patients with CML. Our data indicate that IFN-α activates the Rac1/p38 MAPK pathway in CML cells. Most importantly, pharmacological blockade of p38 reverses the inhibitory effects of IFN-α in KT-1 cells and primary leukemic bone marrow-derived hematopoietic progenitors, providing the first direct evidence that the p38 MAPK pathway is essential for the generation of the growth inhibitory and antileukemic effects of IFN-α.

EXPERIMENTAL PROCEDURES

Cells and Reagents—The KT-1 and K562 cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. Human recombinant IFN-α2 was provided by Hoffmann-La Roche. Human recombinant consensus IFN-α was provided by Amgen Inc. Antibodies against the phosphorylated forms of p38 and ERK2 were obtained from New England Biolabs Inc. and used for immunoblotting. A polyclonal antibody against p38 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies against STAT1, Rac1, and ERK2 were obtained from Transduction Laboratories (Lexington, KY). An antibody that specifically recognizes the tyrosine-phosphorylated form of STAT1 at tyrosine 701 and an antibody that recognizes anti-phosphotyrosine (4G10) were obtained from Upstate Biologies, Inc. and used for immunoblotting. The p38 MAPK inhibitors SB203580 and SB202190 and the MEK kinase inhibitor PD98059 were purchased from Calbiochem.

Cell Lysis and Immunoblotting—Cells were stimulated with 1 × 10^4 IU/ml IFN-α for the indicated times and lysed as previously described (21, 22). Immunoprecipitations and immunoblotting using an enhanced chemiluminescence (ECL) method were performed as previously described (21, 22).

Rac1 Activation Assays—The activation of Rac1 by IFN-α was determined using a recently described methodology (23). Briefly, the pGEX-4T3 construct encoding the GTPase-binding domain of human PAK1...
performed as previously described (17).

K562 cells were treated with IFN-α as indicated. Cell lysates were immunoprecipitated with an antibody against STAT2 or control nonimmune rabbit IgG as indicated, and immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT1 on tyrosine 701. The blot shown in A was stripped and reprobed with an anti-STAT1 monoclonal antibody to control for loading. C, K562 cells were pretreated with SB202190 for 30 min and subsequently treated with IFN-α for 10 min at 37 °C as indicated. Cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT1 on tyrosine 701. D, the blot shown in C was stripped and reprobed with an anti-STAT1 monoclonal antibody to control for loading.

FIG. 4. IFN-α-dependent tyrosine phosphorylation of STAT2 and STAT1 in KT-1 and K562 cells. A, KT-1 cells were treated with IFN-α as indicated. Cell lysates were immunoprecipitated with an antibody against STAT2 or control nonimmune rabbit IgG as indicated, and immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT2 on tyrosine 655. B, K562 cells were treated with IFN-α as indicated. Cell lysates were immunoprecipitated with an antibody against STAT1 or control nonimmune rabbit IgG as indicated, and immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT1 on tyrosine 701. C, KT-1 cells were treated with IFN-α as indicated. Cell lysates were immunoprecipitated with an antibody against STAT1 or control nonimmune rabbit IgG as indicated, and immunoprecipitated proteins were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT1 on tyrosine 701. D, the blot shown in C was stripped and reprobed with an anti-STAT1 monoclonal antibody to control for loading.

FIG. 5. Tyrosine phosphorylation/activation of STAT1 occurs independently of p38 activation in KT-1 cells. A, KT-1 cells were pretreated with SB203580 for 30 min and subsequently treated with IFN-α for 10 min at 37 °C as indicated. Cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT1 on tyrosine 701. B, the blot shown in A was stripped and reprobed with an anti-STAT1 monoclonal antibody to control for loading. C, KT-1 cells were pretreated with SB202190 for 30 min and subsequently treated with IFN-α for 10 min at 37 °C as indicated. Cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the tyrosine-phosphorylated form of STAT1 on tyrosine 701. D, the blot shown in C was stripped and reprobed with an anti-STAT1 monoclonal antibody to control for loading.

RESULTS

We sought to determine whether the p38 MAPK pathway is activated in response to IFN-α treatment in BCR-ABL-expressing cells of CML origin. We first performed studies with the CML-derived human leukemia cell line KT-1, which expresses BCR-ABL and is sensitive to the growth inhibitory effects of IFN-α (20, 27). Cells were incubated in the presence or absence of IFN-α; and after cell lysis, total lysates were resolved by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of p38. IFN-α induced strong phosphorylation/activation of p38 MAPK in these cells (Fig. 1, A and B). On the other hand, IFN-α treatment of the CML-derived K562 cell line, which is resistant to the growth inhibitory effects of IFN-α (27), failed to induce phosphorylation/
activation of p38 (Fig. 1C). It should be pointed out that the inability to detect signals in anti-phospho-p38 immunoblots using lysates from K562 cells was not due to lack of p38 expression, as the p38 protein was abundantly expressed in these cells (Fig. 1D). Thus, p38 is phosphorylated/activated in an IFN-α-dependent manner in KT-1 (but not K562) cells, indicating that the upstream components of this pathway are functional in the KT-1 cell line, but defective in K562 cells. Interestingly, the ERK2 kinase, another member of the MAPK family that has also previously been shown to be activated in non-BCR-ABL-expressing cells (15, 16), was phosphorylated in an IFN-α-dependent manner in both KT-1 and K562 cells (Fig. 2).

In parallel studies, we sought to determine the activation of other IFN-α-inducible signaling elements in KT-1 and K562 cells. Consistent with our previous report (27), the TYK2 tyrosine kinase was phosphorylated/activated in an IFN-α-dependent manner in KT-1 cells (Fig. 3A). TYK2 was also phosphorylated/activated in K562 cells (Fig. 3B), indicating that defective activation of p38 in these cells does not result from lack of TYK2 activation. In studies to determine whether STAT proteins are activated in KT-1 and K562 cells, we found that the IFN-α-dependent tyrosine phosphorylation of STAT1, which functions as an upstream effector for p38 MAPK and STAT pathways appear to function independently of each other in BCR-ABL-expressing cells, and activation of the p38 kinase does not play a regulatory role in phosphorylation/activation of STAT1.

We subsequently determined whether the small GTPase Rac1, which functions as an upstream effector for p38 MAPK (19, 28–30), is activated by IFN-α in BCR-ABL-expressing cells. KT-1 or K562 cells were treated with IFN-α for the indicated times; and after cell lysis, lysates were bound to a GST fusion protein encoding the GTPase-binding domain of PAK1 (23) to detect GTP-bound Rac1. IFN-α treatment induced strong activation of Rac1 in KT-1 cells (Fig. 6A). In K562 cells, some base-line activation of Rac1 was detectable, but there was no further increase in the amount of GTP-bound Rac1 in lysates from IFN-α-treated cells (Fig. 6B), indicating that IFN-α-inducible activation of Rac1 is defective in these cells. We
also performed studies to determine whether the activation of Rac1 in KT-1 cells is tyrosine kinase-dependent. The IFN-α-inducible activation of Rac1 was blocked by pretreatment of cells with the tyrosine kinase inhibitor genistein, but not the phosphatidylinositol 3'-kinase inhibitor wortmannin (Fig. 6C). Thus, engagement of Rac1 in IFN-α signaling in BCR-ABL-expressing cells requires upstream activation of a tyrosine kinase(s), but not the function of the phosphatidylinositol 3'-kinase, which is also activated by the type I IFN receptor (16).

Taken together, our data established that the Rac1/p38 MAPK pathway is activated independently of the STAT pathway in the IFN-α-sensitive, BCR-ABL-expressing KT-1 cell line. To obtain information on the functional relevance of p38 activation in the induction of IFN-α responses in CML cells, we performed experiments in which the effects of pharmacological inhibition of p38 on the generation of the antiproliferative and antiviral activities of IFN-α were examined. KT-1 cells were preincubated in the presence or absence of the p38 MAPK inhibitor SB203580 or SB202190 and subsequently treated with IFN-α in the continuous presence or absence of the p38 MAPK pharmacological inhibitors. Cell proliferation was subsequently assessed using an MTT assay. As expected (20, 27), treatment of KT-1 cells with IFN-α suppressed the growth of KT-1 cells (Fig. 7, A and B). However, concomitant treatment of cells with SB203580 (Fig. 7A) or SB202190 (Fig. 7B) reversed the growth inhibitory effects of IFN-α. On the other hand, treatment of cells with PD98059, which selectively inhibits activation of ERK kinases (but not p38), did not abrogate the IFN-α-inducible growth inhibition (Fig. 7C). On the contrary, it slightly enhanced the generation of an IFN-α antiproliferative response in these cells.

In other studies, we determined whether KT-1 cells are sensitive to the antiviral effects of IFN-α and whether p38 plays a role in the induction of an antiviral state by IFN-α. As shown in Fig. 8, KT-1 cells were susceptible to the cytopathic effects of encephalomyocarditis virus infection, whereas treatment with IFN-α inhibited virus replication in a dose-dependent manner. Treatment with SB203580 partially inhibited the induction of IFN-α-regulated antiviral responses, suggesting a role for p38 in the IFN-α induction of antiviral effects.

We subsequently sought to identify putative downstream effectors of p38 that may mediate the induction of IFN-α-dependent growth inhibitory responses in KT-1 cells. We determined whether the MAPKAPK-2 and MAPKAPK-3 kinases, which are activated downstream of p38 in other systems (31, 32), are engaged in IFN-α signaling in BCR-ABL-expressing cells. KT-1 cells were treated with IFN-α in the presence or absence of the inhibitor SB203580. The cells were lysed and immunoprecipitated with specific antibodies against MAPKAPK-2 or MAPKAPK-3. Subsequently, in vitro kinase assays were carried out on the immunoprecipitates using HSP25 as an exogenous substrate. As shown in Fig. 9, treatment of KT-1 cells with IFN-α resulted in strong activation of MAPKAPK-2 and MAPKAPK-3. Such activation was blocked by pretreatment of cells with SB203580, indicating that activation of the kinase domains of MAPKAPK-2 and MAPKAPK-3 is p38-dependent. Thus, pharmacological inhibition of p38 reverses the biological effects of IFN-α in the KT-1 cell line, and such reversal correlates with inhibition of MAPKAPK-2/3 kinases (but not STAT) activation (Fig. 5), raising the possibility that MAPKAPK-2 and MAPKAPK-3 are downstream targets of p38 that mediate IFN-α-dependent growth inhibition in BCR-ABL-expressing cells.

Based on the data from the studies with the KT-1 cell line, the p38/MAPKAPK-2/3 pathway appeared to play an important role in the generation of the antiproliferative effects of IFN-α in BCR-ABL-expressing cells. This prompted us to ex-
tend these studies to determine the biological relevance of this pathway in the generation of the anti-leukemic effects of IFN-α in primary cells from patients with CML. We first determined whether p38 is phosphorylated/activated in an IFN-α-dependent manner in isolated granulocytes obtained from the peripheral blood of four different patients with CML. The granulocytes were treated with IFN-α; and after cell lysis, total lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated/activated form of p38. Treatment of cells with IFN-α induced phosphorylation/activation of p38 MAPK (Fig. 10, A, C, E, and G), whereas there was no change in the levels of p38 protein expression (B, D, F, and H).

We subsequently determined whether the p38 MAPK pathway plays a role in inducing the antiproliferative effects of IFN-α on primary leukemic hematopoietic progenitors. We determined whether two specific inhibitors of p38 (SB203580 and SB202190) reversed the generation of the growth inhibitory effects of IFN-α on leukemic progenitors in clonogenic assays in methylcellulose. Bone marrow samples from four CML patients were obtained and studied. IFN-α inhibited colony formation of myeloid progenitors isolated from the bone marrow samples of all patients with CML (Fig. 11, A–D). Concomitant treatment of cells with SB203580 reversed the growth inhibitory effects of IFN-α on the leukemic progenitors (Fig. 11, A–D). In a similar manner, SB202190 also reversed the inhibitory effects of IFN-α on leukemic progenitor colony formation, whereas treatment with PD098059 had no effects (Fig. 11, A and B). Thus, pharmacological inhibition of the p38 MAPK pathway abrogates the growth inhibitory effects of IFN-α on clonogenic hematopoietic progenitor growth in the bone marrow samples of CML patients, indicating that the function of p38 is essential for the generation of the anti-leukemic effects of IFN-α.

**DISCUSSION**

Despite the well documented clinical effects of IFN-α in CML, very little is known regarding the mechanisms of IFN signaling in cells expressing BCR-ABL. In this study, we provide the first evidence that IFN-α activates the p38 MAPK pathway in CML-derived IFN-sensitive cells. Most importantly, our data provide direct evidence that this signaling cascade plays a critical role in the induction of the anti-leukemic activities of IFN-α in BCR-ABL-expressing cells. This is revealed by the finding that pharmacological inhibition of p38 reverses the biological effects of IFN-α in an IFN-α-sensitive CML line and primary leukemic bone marrow-derived myeloid progenitors. The SB203580 and SB202190 inhibitors of the p38 MAPK pathway used in our studies have been previously shown to exhibit specificity for p38 MAPK. They act by binding to the ATP site and by inhibiting the kinase activity of p38 MAPK (33–35). Both SB203580 and SB202190 have similar target specificities; and in addition to inhibiting p38 (also called p38α), they inhibit the p38β/δ isoform (but not the p38γ and p38ε isoforms) of the same family (36–39). Thus, our data demonstrating reversal of the growth inhibitory effects of IFN-α by treatment with these pyridinyl imidazole compounds provide strong evidence for an important role of p38 (p38α) and possibly p38β/δ in the induction of the growth inhibitory effects of IFN-α in CML.

Our data clearly establish that the small GTPase Rac1, which functions as an upstream effector of p38 in various systems, including the IFN system (19, 28–30), is rapidly activated during IFN-α treatment of the IFN-sensitive KT-1 CML cell line, but not the IFN-resistant K562 cell line. This activation of Rac1 in KT-1 cells is inhibited by the tyrosine kinase inhibitor genistein, but not the phosphatidylinositol 3′-kinase inhibitor wortmannin. Thus, the function of a tyrosine kinase(s) is required for Rac1 activation in KT-1 cells, but such activation does not require upstream engagement of the IFN-α-dependent insulin receptor substrate/phosphatidylinositol 3′-kinase pathway (16, 21, 24). It is therefore likely that acti-

**FIG. 10. IFN-α-dependent activation of p38 in isolated granulocytes from the peripheral blood of patients with CML.** Granulocytes isolated from the peripheral blood of four different patients with CML (A, C, E, and G) were treated with IFN-α for the indicated times (min). Total cell lysates were analyzed by SDS-PAGE and immunoblotted with an antibody against the phosphorylated form of p38 MAPK to detect activated p38 MAPK (A, C, E, and G). The blots shown in A, C, E, and G were subsequently stripped, and each of them was reprobed with an antibody against p38 MAPK to control for protein loading (B, D, F, and H, respectively).

| A | B |
|---|---|
| CML1 | CML1 |
| Blot: anti-phospho-p38 | Blot: anti-p38 |
| IFN-α 0 30 | IFN-α 0 30 |

| C | D |
|---|---|
| CML2 | CML2 |
| Blot: anti-phospho-p38 | Blot: anti-p38 |
| IFN-α 0 30 | IFN-α 0 30 |

| E | F |
|---|---|
| CML3 | CML3 |
| Blot: anti-phospho-p38 | Blot: anti-p38 |
| IFN-α 0 30 | IFN-α 0 30 |

| G | H |
|---|---|
| CML4 | CML4 |
| Blot: anti-phospho-p38 | Blot: anti-p38 |
| IFN-α 0 30 | IFN-α 0 30 |
vation of the TYK2 and/or JAK1 kinase, both of which are associated with the type I IFN receptor, regulates downstream activation of Rac1 and p38. Such a regulation should involve an intermediate protein substrate that links JAK kinase activation to Rac1, and the identity of such a Rac1 regulator remains to be established.

In previous studies, we have established that the function of p38 is required for transcriptional regulation of IFN-sensitive genes that express IFN-stimulated response elements or IFN-γ-activated sites in their promoters (17, 19), including the PML gene (19), which mediates IFN-regulated growth inhibitory responses (41). It is possible that the requirement for p38 MAPK in the induction of the growth inhibitory effects of IFN-α is mediated by up-regulation of expression of this gene and possibly other related genes with tumor suppressor activity. However, activation of the p38 MAPK pathway by IFN-α may have additional effects that mediate growth inhibitory responses such as regulation of signals that modify cell cycle progression in CML cells. Our data indicate that the MAPKAPK-2 and MAPKAPK-3 kinases are downstream effectors for p38, activated by IFN-α in BCR-ABL-expressing cells. It is therefore possible that MAPKAPK-2 and MAPKAPK-3 play important roles in the induction of the anti-leukemic effects of IFN-α in CML cells either via regulation of gene transcription or via engagement of other downstream effectors that regulate cell cycle progression. Regardless of the precise mechanisms involved, p38 appears to play a critical role in the generation of the antiproliferative effects of IFN-α on leukemic progenitors as well as on normal bone marrow erythroid and myeloid progenitors. Thus, the p38 MAPK pathway may participate in the generation of both the anti-leukemic effects of IFN-α as well as the documented hematologic toxicity that this cytokine exhibits when administered to humans (7, 8).

It remains to be determined whether the p38 pathway acts in cooperation with other IFN-activated pathways such as the STAT pathway to regulate induction of growth inhibition by IFN-α in CML cells. Interestingly, it was recently demonstrated that IFN-α treatment induces formation of STAT5-CrkL complexes in KT-1 cells and that such complexes bind to one of the IFN-γ-activated sites of the promyelocyte leukemia gene promoter (27). The promoter of the PML gene also contains IFN-stimulated response elements regulated by the IFN-stimulated gene factor-3 complex, which involves STAT2-STAT1 complexes. The fact that the p38 MAPK pathway is required for transcriptional regulation of the promoter of this gene (19), which is also regulated by different STAT-binding complexes, suggests a coordination of signaling functions between the p38 MAPK and STAT pathways. Such a coordination may be important in the induction of the direct anti-leukemic effects of IFN-α in CML cells.

It has been previously shown that IFN-α down-regulates expression of the bcr-abl oncogene, which causes the malignant transformation (42). Our data suggest the existence of a direct mechanism, distinct from inhibition of BCR-ABL expression, that mediates the antiproliferative effects of IFN-α. It is possible that the p38 MAPK pathway also contributes to the induction of the anti-leukemic effects of other pharmacological agents such as STI571 that exhibit selective growth inhibitory effects in CML cells (40). A potential scenario may be that BCR-ABL exhibits constitutive negative regulatory effects on the p38 MAPK pathway. Activation of the IFN-α-dependent tyrosine kinases or inhibition of BCR-ABL kinase activity by STI571 may overcome such negative regulatory effects of BCR-ABL on p38 MAPK and

FIG. 11. IFN-α inhibits the growth of leukemic myeloid progenitors from the bone marrow samples of CML patients in a p38-dependent manner. Bone marrow mononuclear cells from four different CML patients (A–D) were plated in a methylcellulose culture assay system with the indicated doses of IFN-α (IU/ml) in the presence or absence of the indicated MAPK inhibitors. The data are expressed as percent control of granulocyte/macrophage colony-forming units for untreated cells. PD 098, PD098059; SB 202, SB202190; SB 203, SB203580.
result in inhibition of malignant cell growth. Future studies in that direction are warranted and may provide further insights into the mechanisms of BCR-ABL-mediated leukemogenesis as well as clarification of the mechanisms by which drugs and cytokines block BCR-ABL-mediated cell growth.

Acknowledgment—We thank Dr. Gary Bokoch for providing the pGEX construct for the production of the GST-PBD fusion protein.

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J. Biol. Chem. 2001, 276:28570-28577.
doi: 10.1074/jbc.M011685200 originally published online May 15, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011685200

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