Ectopic Expression of Protein-tyrosine Kinase Bcr-Abl Suppresses Tumor Necrosis Factor (TNF)-induced NF-κB Activation and IκBα Phosphorylation

RELATIONSHIP WITH DOWN-REGULATION OF TNF RECEPTORS*

Received for publication, May 14, 2002
Published, JBC Papers in Press, June 11, 2002, DOI 10.1074/jbc.M204748200

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Bcr-Abl, the product of the protooncogene bcr-abl, is a constitutively active protein-tyrosine kinase that is highly expressed in chronic myelogenous leukemia and in acute myeloid leukemia cells. Because Bcr-Abl is known to provide mitogenic signals through suppression of apoptosis, we investigated the effect of this oncogene on signaling by tumor necrosis factor (TNF), a proapoptotic cytokine. We used a bcr-abl-deficient human megakaryocytic leukemia cell line MO7E and an isogenic MBA cell line stably transfected with bcr-abl. Electrophoretic mobility shift assay revealed that TNF activated the nuclear transcription factor NF-κB in MO7E cells but not in MBA cells. The impaired NF-κB activation in Bcr-Abl-expressing cells was not due to absence of the NF-κB proteins p65, p50, or p100 or of IκBα or IκBβ. Okadaic acid-induced NF-κB activation was unaffected by Bcr-Abl expression. TNF induced IκBα phosphorylation and degradation in MO7E cells but not in MBA cells. The suppression of TNF-induced NF-κB activation by Bcr-Abl was not restricted to MBA cells, because ectopic expression of Bcr-Abl in human acute myeloid leukemia HL-60 cells also blocked TNF-induced NF-κB activation. When examined for the TNF receptors by the radioreceptor assay, flow cytometry, or Western blot analysis, we found that Bcr-Abl expression down-regulated the expression of the TNF receptors. The RNase protection assay and Northern blot analysis revealed the transcriptional down-regulation of the TNF receptor by Bcr-Abl protein. Overall, these results indicate that ectopic expression of Bcr-Abl interferes with the TNF signaling pathway through the down-regulation of TNF receptors.

The chimeric oncogene bcr-abl is formed by the reciprocal translocation (Philadelphia translocation) that fuses part of the breakpoint cluster gene (bcr) on chromosome 22 upstream of the Abelson tyrosine kinase (abl) gene on chromosome 9 (1). Depending on the chromosomal fusion point, Bcr-Abl proteins are expressed in three different molecular sizes, 185, 210, and 230 kDa, and are believed to be responsible for acute lymphoblastic leukemia, chronic myelogenous leukemia, and chronic neutrophilic leukemia, respectively (2–4).

Bcr-Abl is a deregulated tyrosine kinase that transforms fibroblasts and immature hematopoietic cells in vitro, and the transformed cells are tumorigenic (5–7). The introduction of a retrovirus vector expressing p210bcr-abl and p185bcr-abl into growth factor (granulocyte-macrophage colony-stimulating factor/IL-3)1-dependent human (MO7E) and mouse (32D) cell lines, respectively, converted them rapidly to growth factor-independent cell lines (8, 9). Bcr-Abl expression has been implicated in the induction of resistance of chronic myelogenous leukemia to apoptosis induced by antileukemic drugs (10, 11). This oncogene has been shown to block apoptosis induced by various stimuli through suppression of mitochondrial release of cytochrome c and by blocking the cytosolic pathway that leads to activation of caspase-3 (12–14). Additionally, Bcr-Abl has been shown to regulate c-jun gene expression, activation of c-Jun N-terminal kinase, and the ras pathway, which may also contribute to suppression of apoptosis, transformation, and tumorigenesis (15–18). It is thus apparent that t(9,22) Philadelphia translocation modulates cellular signaling. In mammalian cells, various signal transduction pathways leading to survival or death are activated depending upon extracellular stimuli.

How Bcr-Abl expression affects signaling to cytokines that either stimulate or inhibit cell growth is poorly understood. It has been shown that Bcr-Abl affects cell growth via autocrine production and action of IL-3 and granulocyte colony-stimulating factor in chronic myeloid leukemia (19). Furthermore, it was recently shown that p210bcr-abl interacts with the IL-3 receptor βc subunit and constitutively induces its tyrosine phosphorylation (20). Here, we investigated the effect of ectopic expression of Bcr-Abl on TNF signaling using the human megakaryocytic leukemia cell line MO7E, which lacks Bcr-Abl, and isogenic MBA, which expresses Bcr-Abl ectopically. Our results indicate that the expression of Bcr-Abl down-regulates TNF-induced NF-κB activation and IκBα phosphorylation through the down-regulation of TNF receptors. We also dem-

* This work was supported by the Clayton Foundation for Research. 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: IL, interleukin; EMSA, electrophoretic mobility shift assay; IκB, inhibitory subunit of NF-κB; IL-6Rα, interleukin-6 receptor α; PARP, poly(ADP) ribose polymerase; TNF, tumor necrosis factor; TNFR, TNF receptor; TGFβRI, transforming growth factor β receptor II; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase.

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onstrate that this effect is not unique to megakaryocytic leukemic cells but also occurs in human T cells and in acute myelogenous leukemia cells. The down-regulation of the TNF receptor by Bcr-Abl occurred at the transcriptional level.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture**—The U-937 cell line was procured from the American Type Culture Collection (Manassas, VA). The human megakaryocytic leukemic cell line M07E, a growth factor-dependent cell line, was obtained from the Genetics Institute (Boston, MA). We used the isogenic M07E cell line transduced with retrovirus vector containing the chimeric bcr-abl gene (MBA cell), which is growth factor independent (8). The stable transfection of human promyelocytic HL-60 cell line with neo or with bcr-abl plasmids has been previously described from our laboratory (14). We also transfected Jurkat cells with doxycycline-inducible Bcr-Abl plasmid. For this, bcr-abl p210 cDNA was cloned into the BamHI site of the plasmid vector pSUPER (21). The resulting plasmid pSSTAR Bcr/Abl was stably transduced into Jurkat cells using LipofectAMINE (Invitrogen). Stable clones were screened using 500 μg/ml of G418 sulfate. After selecting the single clone by limiting-dilution method, the bcr-abl gene was induced using 5 μg/ml doxycycline. All of these cells were regularly grown in RPMI 1640 containing 10% fetal bovine serum and antibiotics-antimycotics, except the medium for M07E cells supplemented with 200 units/ml human granulocyte-macrophage colony-stimulating factor (GM-CSF).

**Materials**—Polyclonal antibodies against IgBα, IgBβ, p50, p52, p65, and PARP raised in rabbits were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal antibodies against the phospho-IgBα (Ser-32) and phospho-p42/44 MAPK were from New England Biolabs, Inc. (Beverly, MA). Biotinylated anti-phosphotyrosine monoclonal antibody, anti-biotin IgG-horseradish peroxidase, and anti-biotin-horseradish peroxidase conjugate and detected by ECL reagent. The proteins were electrotransferred to nitrocellulose membrane, blocked with 5% nonfat milk, and probed with IgG anti-p60 receptor antibodies, respectively. All results were determined in triplicate.

**Electrophoretic Mobility Shift Assay (EMSA)**—Identification of Bcr-Abl Protein and Its Phosphorylated Form—Fifty micrograms whole-cell lysates were resolved on 6% SDS-PAGE gel. After electrophoresis, the proteins were electrotransferred to nitrocellulose membrane, blocked with 2% bovine serum albumin, and probed with anti-Bcr antibody (1:1000) for 1 h. The blot was washed, exposed to horseradish peroxidase-conjugated secondary antibodies for 15 min. The reaction was stopped by boiling beads in SDS sample buffer. Finally, protein was resolved on 10% SDS-PAGE gel. The radioactive bands of the dried gel were visualized and quantitated by phosphorimaging as mentioned earlier.

**Receptor-binding Assay**—Human recombinant TNF was labeled with Na32P using the IODO-GEN procedure as described (25). The specific activity of the labeled TNF was 38 μCi/μg. The binding assays were performed by using the 96-well method as previously described (26).

**Electrophoretic Mobility Shift Assay**—The expression of p60 and p80 TNF receptor was calculated by subtracting TNF-specific binding in the absence of antibody from that in the presence of either anti-p80 or anti-p60 receptor antibodies, respectively. All results were determined in triplicate and expressed as the mean ± S.E.

**Flow Cytometric Analysis of TNFR Expression**—Analysis of TNFR expression, M07E and MCA cell lines were harvested, centrifuged, and resuspended in Dulbecco’s phosphate-buffered saline containing 10% fetal bovine serum and 0.1% sodium azide. The cells were incubated with polyclonal, affinity-purified rabbit anti-p60 and p80 antibodies (27). Following a 1-h incubation at 4°C, the cells were washed and incubated for an additional 1 h with biotin-conjugated anti-rabbit IgG monoclonal antibody (Jackson ImmunoResearch, West Grove, PA). The cells were washed and incubated for 1 h at 4°C with phycoerythrin-conjugated streptavidin (Molecular Probes, Inc., Eugene, OR). Thereafter, the cells were analyzed using a FACScan Vantage flow cytometer and CellQuest acquisition and analysis programs (Becton Dickinson, San Jose, CA).

**Receptor-binding Affinity Assay**—To prepare the cell extracts, MBA, M07E, U937, and KMB-5 cells (2 × 106) were incubated for 30 min on ice in 100 μl of lysis buffer (20 mM HEPES, pH 7.4, 2 mM EDTA, 250 mM NaCl, 0.1% Nonidet P-40, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml benzamidine, and 1 mM dithiothreitol). The lysates were then centrifuged for 4 min at 14,000 rpm at 4°C, the supernatants were collected, and protein was measured. The cell extracts (100 μg) was resolved on a 10% SDS-polyacrylamide gel, electrotransferred onto nitrocellulose membrane, and blocked with blocking solution containing 5% nonfat milk in phosphate-buffered saline containing 0.5% Tween 20. The membrane was blotted first with anti-p80 TNF receptor antibody (1:1000 dilution in blocking solution) for 1 h, washed three times (10 min each) with phosphate-buffered saline/Tween, and then blotted with horseradish peroxidase-conjugated IgG as the secondary antibody for 1 h, washed three times (10 min each), and then detected by chemiluminescence (ECL; Amersham Biosciences).

**Receptor-binding Affinity Assay**—The expression of p60 and p80 TNF receptor mRNA was determined by RNase protection assay using human cytokine receptor multiprobe template sets (hCR-4) as per the manufacturer’s instruction. Briefly, each cell type (5 × 106) was washed two times with phosphate-buffered saline to remove medium protein. Total RNA was isolated using Trizol Reagent (Invitrogen). Ten-microgram RNA samples were hybridized with 32P-labeled antisense mRNA probes against IL-1RI, IL-1RII, p60TNFR, p80TNFR, IL-6Rα, gp130, TGFRRI, TGFRRII, L32, and glyceraldehyde-3-phosphate dehydrogenase and digested with RNase and T1 nucleases. The quantitation was performed by using the Imagequant software (Molecular Dynamics, Inc., Sunnyvale, CA) using ImageQuant software.
Northern Blot Analysis—Cell cultures seeded at 1 x 10^6 cells/ml were incubated in 75-cm^2 flasks. Total RNA was extracted from cells using Trizol reagent. For electrophoresis, 30 μg of RNA was fractionated on 1.2% agarose gels containing 2.2% formaldehyde at 50–100 V for ~3 h. Thereafter, the gels were rinsed with diethyl pyrocarbonate, and the RNA was transferred to Hybond nylon membranes (Amersham Biosciences). After alkaline transfer (overnight), the filter was stained with methylene blue to visualize 28 S RNA. Prehybridization was carried out at 65 °C for 1 h in a buffer containing 7% SDS, 50 mM sodium phosphate, 1 mM EDTA, pH 7.2 (Church buffer or hybridization buffer). Filters were then hybridized for 16–20 h with p60 or p65 cDNA probes (approximate specific activity 2 x 10^6 cpm/μg DNA) in a hybridization buffer containing denatured salmon sperm DNA (200 μg/ml). After hybridization, membranes were washed several times at 65 °C with 40 mM sodium phosphate containing 1% SDS. The filters were exposed to Eastman Kodak Co. Xar-5 film with intensifying screens at ~70 °C for 1–3 days. Equal loading of lanes was demonstrated by examination of gels after methylene blue staining of the 28 S RNA.

RESULTS

In this report, we investigated the effect of ectopic expression of Bcr-Abl protein on TNF-mediated cellular responses in the human megakaryoblastic cell line M07E, which was originally derived from an acute megakaryocytic leukemia patient. To confirm that the effects of Bcr-Abl on these cellular responses are not unique to one cell line, we also used the human acute myelogenous leukemia HL-60 cell line.

Differential Expression of p210bcr-abl Protein and Kinase Activity in M07E and MBA Cells—We first examined the expression of Bcr-Abl protein by Western blot using Bcr-specific antibodies. As shown in Fig. 1A, M07E cells did not express Bcr-Abl protein, whereas MBA cells expressed a large amount of p210bcr-abl protein. We next examined whether this protein exhibited protein-tyrosine kinase activity. The chimeric oncogene product was tyrosine-phosphorylated as shown by phosphotyrosine Western blot analysis (Fig. 1A, lower panel).

Bcr-Abl Down-regulates TNF-induced NF-κB Activation—The activation of NF-κB is one of the earliest cellular responses to TNF in most cells (29). We investigated whether expression of Bcr-Abl modulates TNF-mediated NF-κB activation. M07E and MBA cells were treated with 0.1 and 1 nM TNF for 30 min, and nuclear extracts were prepared and analyzed by DNA-binding assay using EMSA. The results in Fig. 1B show that TNF activated NF-κB in M07E cells almost to the maximum at 0.1 nM, but in MBA cells TNF even at 1 nM had no effect. These results indicate that Bcr-Abl down-regulates TNF-induced NF-κB activation (Fig. 1B).

To ensure that the activated NF-κB in M07E cells was composed of transcriptionally active heterodimers of p50 and p65 subunits, the TNF-treated nuclear extracts were incubated with anti-p65 or anti-p50 antibodies before EMSA. The EMSA showed that the NF-κB-DNA complex was either abrogated or supershifted when nuclear extract was treated with p50/p65 antibodies (Fig. 1C). The DNA binding was not prevented by treatment of nuclear extracts with irrelevant cyclin D1 antibodies or preimmune sera, indicating specificity of the heterodimer. The specificity of the TNF-induced NF-κB-DNA complex was further confirmed by demonstrating that the binding was disrupted in the presence of a 100-fold excess of unlabeled κB-oligonucleotide but not by mutant oligonucleotide (Fig. 1C).

Bcr-Abl Does Not Affect the Expression of Various NF-κB Proteins—It is possible that Bcr-Abl down-regulated the expression of NF-κB proteins, making MBA cells unable to respond to TNF-induced NF-κB activation. To determine this, we prepared cytoplasmic extracts from both cell types and examined the expression of p65 (c-Rel), p50, p100, IκBα, and IκBβ by Western blot analysis using specific antibodies. Fig. 2A shows that all the NF-κB proteins are expressed to a similar level in both cell types, thus suggesting that Bcr-Abl had no effect on the expression of various NF-κB proteins.

Bcr-Abl Does Not Affect the NF-κB Activation Induced by Okadaic Acid—Since Bcr-Abl did not affect the expression of various NF-κB proteins, we examined whether it affected the NF-κB activation induced by other agents. For this, cells were treated with 0.5 μM okadaic acid for 4 h, and we prepared the nuclear extracts and examined the NF-κB activation by EMSA. Activation by TNF was used as a control. As shown in Fig. 2B, okadaic acid activated NF-κB in both M07E and MBA cell lines, indicating that ectopic expression of Bcr-Abl has no effect on NF-κB activation by other agents.

Bcr-Abl Suppresses TNF-induced IκBα Phosphorylation—TNF-induced NF-κB activation requires phosphorylation of IκBα at serine residues 32 and 36 (30). We investigated whether Bcr-Abl suppresses TNF-induced NF-κB activation through suppression of IκBα phosphorylation. Cells were treated with TNF in the presence of N-acetyldeucylleucylleucinorluclinal (a proteasome inhibitor), which prevents the degradation of the phosphorylated form of IκBα, and then examined for
Bcr-Abl Protein Down-regulates TNF Signaling

nonphosphorylated and phosphorylated forms of IκBα using specific antibodies. Fig. 2C shows that the phosphorylated form of IκBα appeared in MO7E cells but not in MBA cells, thus suggesting that Bcr-Abl prevents the phosphorylation of IκBα.

**Bcr-Abl Activates JNK and p44/p42 MAPK Activation**—TNF is also a potent activator of JNK and MAPKK (29). We examined whether Bcr-Abl also suppresses the TNF-induced activation of JNK and MAPKK. Cells were treated with 0.1 and 1 nM TNF, and whole-cell extracts were prepared and analyzed for JNK by the immune complex kinase assay and for p44/p42 MAPK by Western blot analysis using specific antibodies. As Fig. 2D shows, JNK was constitutively active in MBA cells and not in MO7E cells, suggesting that Bcr-Abl expression leads to JNK activation. Interestingly, TNF failed to activate JNK in MO7E cells, whereas in MBA cells no further enhancement was found.

The results in Fig. 2E show that MAPKK, which is an upstream kinase to MAPK, is also constitutively active in MBA cells but not in MO7E cells, suggesting that Bcr-Abl expression leads to MAPKK activation. Similarly, TNF was unable to activate MAPKK in MO7E cells, whereas in MBA cells the constitutive expression of MAPKK was not further increased by the ligand.

**Down-regulation of TNF-induced Cellular Responses by Bcr-Abl Is Not Specific to MO7E**—It is possible that the effect of Bcr-Abl on TNF-mediated cellular responses is unique to megakaryoblastic cells. To determine whether Bcr-Abl down-regulates TNF responses in other cell types, we employed human acute myelogenous leukemia HL-60 cells. These cells were transfected with bcr-abl plasmid and then examined for TNF-mediated cellular responses. As shown in Fig. 3A, normal HL-60 cells did not express Bcr-Abl, but transfected cells did. As was the case for MBA cells, expression of Bcr-Abl in HL-60 cells down-regulated TNF-induced NF-κB activation (Fig. 3B) without any significant loss of NF-κB proteins (Fig. 3C). Thus, the effects of Bcr-Abl on TNF signaling were not cell type-specific.
TNF-induced cytotoxicity is known to require activation of caspases that cleave various cellular substrates including PARP (31). To determine whether Bcr-Abl affects TNF-induced PARP cleavage, we treated cells with TNF in the presence and absence of cycloheximide (which suppresses the synthesis of antiapoptotic proteins), prepared cell extracts, and analyzed them by Western blot using anti-PARP antibodies. The results showed that TNF-induced PARP cleavage in HL-60 cells but not in HL-60 cells transfected with Bcr-Abl, thus suggesting that Bcr-Abl also suppresses TNF-induced activation of caspases (Fig. 3D).

We also examined the effect of doxycycline-inducible Bcr-Abl in Jurkat cells on TNF-induced NF-κB activation. The results showed that TNF activated NF-κB in control cells but not in Bcr-Abl-expressing cells (data not shown).

Bcr-Abl Down-regulates TNF Receptors—Our results so far indicated that most of the TNF-induced cellular responses were down-regulated by Bcr-Abl irrespective of cell type. It is possible that Bcr-Abl may have suppressed TNF-induced cellular responses through down-regulation of TNF receptors. Most leukemic cells express two types of TNF receptor (viz. p60 and p80) (32). It is known that most of the TNF signals are mediated through the p60 receptor. We examined the effect of Bcr-Abl expression on the cell surface expression of these two receptors using radioreceptor assays and receptor-specific antibodies. Because both types of TNF receptor are well characterized on U-937 cells, we used these cells as a control. As shown in Fig. 4A, U-937 cells expressed almost 65% p80 and 35% p60 TNF receptors. Similarly, most of the ligand binding in MO7E cells was due to p60 receptor; very little p80 receptor was found. Amazingly, MBA cells were found to lack any specific TNF binding. Thus, these results indicate that Bcr-Abl down-regulates TNF receptors in megakaryoblastic cells. The cell surface of TNF receptors was also examined by flow cytometry. These results also showed that MO7E cells express both the p60 and p80 forms of the TNF receptors, but MBA cells expressed neither of the receptors (Fig. 4B). Whether Bcr-Abl expression down-regulates the TNF receptor protein was examined by Western blot analysis. As shown in Fig. 4C, MO7E cells expressed significant levels of TNF p80 receptor protein, and these levels were comparable with other myeloid cell lines such as U-937 and KMB-5 cells. Two different bands observed suggest a breakdown of the p80 receptor. In contrast, MBA cells did not express TNF p80 receptor protein, suggesting that Bcr-Abl expression down-regulates the TNF receptor protein. No antibody was found sensitive enough to detect the p60 receptor by Western blot analysis.
Bcr-Abl Down-regulates the mRNA for TNF Receptor—Whether Bcr-Abl down-regulates the expression of TNF receptors at the transcriptional level was determined by isolating the mRNA from different cell types and performing the RNase protection assay using specific probe kits. Fig. 5, A and B, shows that MO7E cells expressed the mRNA for p60 and p80 receptor, and the expression for p60 was higher than p80. The expression of Bcr-Abl in MO7E eliminated the expression of mRNA for both p60 and p80 receptors. The effects of Bcr-Abl were not unique to TNF receptors, in as much as the mRNA for IL-6Rα and TGFβRII were also completely down-regulated in Bcr-Abl-expressing MBA cells. These results indicate that Bcr-Abl can down-regulate the mRNA for various cytokine receptors.

The down-regulation of the mRNA by Bcr-Abl expression was further confirmed by Northern blot analysis. The results in Fig. 5C indicate that MO7E expressed the mRNA for both the p60 and p80 form of the TNF receptors, whereas MBA cells did not express either of the receptor mRNAs.

DISCUSSION

In this report, we investigated the effect of Bcr-Abl on TNF-mediated NF-κB activation. Our results show that Bcr-Abl suppresses TNF-induced NF-κB activation, IkBα phosphorylation, and caspase-mediated PARP cleavage, and this suppression correlates with down-regulation of TNF receptor expression both at the mRNA and protein levels. Our results also indicate that these effects are not cell type-specific, since Bcr-Abl was effective both in megakaryoblastic, acute myelogenous leukemia cells and in Jurkat T cells.

Our results indicate that Bcr-Abl by itself had no effect on constitutive NF-κB activation in either of the human leukemic cell lines. These results, however, differ from two earlier reports of Reuther et al. (33) and Hamdane et al. (34), which showed that Bcr-Abl causes constitutive NF-κB activation. Both of these investigators used murine myeloid 32D and DA1 cell lines. Whether the difference in our results from those previously reported is due to the cell line is not clear. We used three different human cell lines and found similar results. Rather than stimulating NF-κB by itself, Bcr-Abl suppressed TNF-induced NF-κB activation in our study. This suppression occurred through the inhibition of IkBα phosphorylation needed for NF-κB activation. This is the first report to our knowledge to indicate that Bcr-Abl can modulate the signaling of any cytokine other than IL-3. IL-3 is known to be produced by Bcr-Abl-expressing leukemic cells and acts as an autocrine growth factor (19). Furthermore, Bcr-Abl can interact with the IL-3 receptor β-chain and induce constitutive tyrosine phosphorylation (20).

We found that ectopic expression of Bcr-Abl by itself activated JNK. This result is in agreement with previous reports, one by Raitano et al. (16) on human embryonic kidney cells and one by Burgess et al. (15) on MO7E cells. Whereas Bcr-Abl expression leads to constitutive activation of JNK in MO7E cells, TNF did not. That these cells are insensitive to TNF-induced JNK activation is not due to lack of TNF receptors. Furthermore, these receptors are functional, since they activated NF-κB in MO7E cells. It is possible that TNF receptor-associated factor 2, which is needed for JNK activation but not for NF-κB activation, is either not expressed or not functional due to the expression of TNF receptor-associated factor 2 inhibitors in parental MO7E cells.

Our results also indicate that Bcr-Abl suppressed TNF-induced cytotoxicity. TNF was not highly cytotoxic to MO7E cells. The suppression of TNF-induced cytotoxicity in our studies was consistent with down-regulation of TNF-activated caspase activation. Several reports indicate that Bcr-Abl provides a growth advantage to the cells by blocking apoptosis (35–38), thus promoting transformation and tumorigenesis. However, there is very little known about how Bcr-Abl modulates cytokine signaling. IL-3 is known to be produced by Bcr-Abl-expressing leukemic cells and acts as an autocrine growth factor (19). Additionally, Bcr-Abl may also provide growth advantage to the leukemic cells through suppression of cytokine-mediated apoptosis. Alternatively, it is possible that Bcr-Abl may induce antiapoptotic proteins, such as Bcl-2, as reported previously (12), which could mediate the suppression of TNF-induced apoptosis. Indeed, we did find that Bcr-Abl-expressing MBA cells co-express Bcl-xL, whereas MO7E did not.

Our results indicate that most of the effects of Bcr-Abl on TNF signaling can be explained through the down-regulation of TNF receptors. The down-regulation of death receptors is a novel mechanism through which Bcr-Abl could provide a proliferative advantage to the leukemic cells. Our preliminary studies indicate that it is not the TNF receptor alone, but the mRNA for TGF-βRII and IL-6 receptor, that are also down-regulated by Bcr-Abl. IL-6 has inhibitory effects on human and murine leukemic cell lines in vitro (40). By suppressing transcription of IL-6Rα, Bcr-Abl-expressing cells escape the growth-inhibitory effects of IL-6. The role of TGFβ as a negative autocrine growth factor for tumorigenesis has been reported (41). Most normal cells are growth-inhibited by TGFβ. However, tumor cells lose their responsiveness to TGFβ in several ways (42). One of the possible ways by which tumor cells protect themselves from inhibitory effects of TGFβ is by losing TGFβRIIs (39, 43). We believe that Bcr-Abl-expressing leukemic cells could escape suppression of growth also by down-modulation of TGFβ receptors.

Previously, Bcr-Abl had been shown to interact with the IL-3 receptor β-chain and induce constitutive tyrosine phosphorylation (20). Our studies suggest that down-regulation of receptors involved in antiproliferative effects may be another mechanism through which Bcr-Abl provides a growth advantage. Overall, the studies described here provide a novel mechanism through which Bcr-Abl may interfere with cytokine signaling, especially those involved in suppression of cell growth. Whether TNF receptors and TNF signaling are down-regulated in samples from chronic myelogenous leukemia or AML patients should be investigated in the future.

Acknowledgments—We thank Dr. Jian Ni and Bharati Matta for assistance with Northern and RNA protection assay analysis.

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Ectopic Expression of Protein-tyrosine Kinase Bcr-Abl Suppresses Tumor Necrosis Factor (TNF)-induced NF-κB Activation and IkBα Phosphorylation: RELATIONSHIP WITH DOWN-REGULATION OF TNF RECEPTORS
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J. Biol. Chem. 2002, 277:30622-30628. doi: 10.1074/jbc.M204748200 originally published online June 11, 2002

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