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Induction of protein-tyrosine-phosphatase activity by interleukin 6 in M1 myeloblastic cells and analysis of possible counteractions by the *BCR–ABL* oncogene

DINA ZAFRIRI*, MIRIAM ARGAMAN†, ELI CANAANI†, AND ADI KIMCHI*‡

Departments of *Molecular Genetics and Virology and †Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

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ABSTRACT **Interleukin 6 (IL-6) induces in M1 myeloblastic cells growth arrest and terminal differentiation toward monocytes. It is reported here that IL-6 reduced by 5- to 20-fold the tyrosine phosphorylation of cellular proteins in these cells. The same -fold reduction was also observed in M1 cells that were transfected with the BCR–ABL deregulated protein kinase. In these stable clones, the levels of tyrosine phosphorylation of cellular proteins were 30- to 100-fold higher than in the parental cells. IL-6 did not reduce the expression levels or the inherent tyrosine kinase activity of BCR–ABL p210. By measuring the protein-tyrosine-phosphatase (PTPase; protein-tyrosine-phosphate phosphohydrolase, EC 3.1.3.48) activity in crude cell lysates, we found that protein dephosphorylation resulted, at least partially, from induction of PTPase activity by IL-6. The induction of PTPase in the *BCR–ABL*-transfected clones was not sufficient to confer the minimal protein phosphorylation levels characteristic of IL-6-treated cells. Yet, the transfected M1 clones showed normal growth and differentiation responses to IL-6. None of the gene responses to IL-6 including suppression in the levels of c-myc, c-myb, and cyclin A mRNA; junB and c-jun mRNA induction; and dephosphorylation of retinoblastoma protein were rescued by the *BCR–ABL* oncogene. The functional relevance of PTPase induction by IL-6 is discussed.**

The t(9;22) chromosomal aberration is considered to be the initiating event in human chronic myelogenous leukemia (CML) (1). This aberration results in fusion of the *ABL* and *BCR* genes and synthesis of a chimeric protein, p210, with deregulated tyrosine kinase activity (2–4). Several *in vivo* experimental systems proved the direct causal effects of *BCR–ABL* in leukemogenesis (5–7). The first mechanistic explanation for the *BCR–ABL* effects came from the observation that introduction of *BCR–ABL* into interleukin 3-dependent hematopoietic cell lines converted them into autonomously growing cells (8–11). Yet, the increased number of myeloid cells in the chronic phase of CML disease could result not only from the reduced dependence of progenitor cells on growth factors but also from a partial loss of negative growth responses to differentiation factors and/or growth inhibitory cytokines. This may happen if *BCR–ABL* antagonizes some of the pathways that are triggered by negative cytokines, leading to a subtle increase in the number of cell divisions during the differentiation process.

Our current understanding of the antiproliferative mode of action of growth inhibitory cytokines [e.g., interferons, transforming growth factor type β (TGF-β), interleukin 6 (IL-6)] implies that they actively antagonize growth factor-induced postreceptor events (reviewed in ref. 12). While the studies have exclusively focused so far on expression and posttranslational modifications of a few common nuclear protein targets [c-*myc*, retinoblastoma (Rb), cyclin A] (12), very little was studied with respect to the issue of protein tyrosine phosphorylation. Mutual antagonism at this level would imply that growth inhibitory cytokines may induce tyrosine dephosphorylation of proteins including those subjected to phosphorylation by growth factors. The rapidly growing information on cytosolic and transmembranal protein-tyrosine-phosphatases (PTPases) (13–15) focused recent interest in this direction. The structural functional analysis of the various members of these two classes of PTPases illustrated the existence of interesting motifs that could play a major role in regulating their enzymatic activity by external signals (13, 14). However, the possibility that growth inhibitory and/or differentiation-inducing cytokines may activate some of the PTPases was poorly explored.

The issue of protein tyrosine dephosphorylation has been studied here by using the M1 myeloblastic cells that, in response to IL-6, undergo terminal growth arrest concomitant with morphological and functional differentiation toward monocytes (16, 17). This system has been recently studied in detail with respect to the downstream nuclear proteins (e.g., c-myc, Rb, cyclin A, c-jun, junB) whose expression or function is modified by IL-6 (17–19, 21–23). This information made the M1 system attractive for studying PTPase induction by IL-6 and analyzing whether abnormal elevated levels of tyrosine phosphorylation due to *BCR–ABL* transfection may interfere with the above-mentioned growth-related gene responses. We report here that IL-6 reduces by 5- to 20-fold the phosphotyrosine content of cellular proteins due to elevation of PTPase activity. The increased PTPase activity could be measured in crude lysates by using a few phosphorylated substrates. Transfection of M1 cells with *BCR–ABL* oncogene led to a 30- to 100-fold increase in the basal levels of protein tyrosine phosphorylation. Yet, none of the examined growth-related gene responses to IL-6 were abrogated by the high tyrosine kinase activity. The functional relevance of PTPase induction by IL-6 is further discussed.

MATERIALS AND METHODS

Cell Lines and Cytokines. S-6, the clone of M1 used in this study, was described elsewhere (17). Human recombinant IL-6 purified to 2×10^7 units/mg was provided by Interpharm (Rehovot, Israel). TGF-β_1 prepared from human platelets was purchased from R&D Systems (Minneapolis).

Expression Vectors and Transfection. A full-length cDNA coding for p210 protein was constructed by substituting the 990-base-pair (bp) *Xho* I/*Kpn* I fragment from a construct coding for p190 (24) with a 2390-bp *Xho* I/*Kpn* I fragment from the CML 11D clone isolated from a K562 cDNA library. The resulting p210 cDNA was cut with *Eco*RI and *Sph* I, blunt ended, and cloned into the *Sma* I site in the pLSV

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Abbreviations: IL-6, interleukin 6; CML, chronic myelogenic leukemia; PTPase, protein-tyrosine-phosphatase; pRb, retinoblastoma protein; TGF-β, transforming growth factor type β.

‡To whom reprint requests should be addressed.

expression vector. The pSV2neo plasmid (25) was cotransfected together with pLSV-p210 at a 1:10 molar ratio. The plasmids were linearized and introduced into 2×10^7 exponentially growing cells by electroporation (26). The cells were then seeded at 10^5 viable cells per well, in 24-well plates, and the antibiotic G418 (GIBCO) was added 48–72 h later to a final concentration of 0.6 mg/ml. G418-resistant clones appeared after 3–5 weeks and were continuously maintained in G418-containing medium.

Northern Blot Hybridization and Immunoblot Analysis. Total cellular RNA was isolated by the LiCl/urea procedure and 20-μg samples were electrophoresed on a formamide/formaldehyde agarose gel, transferred to nitrocellulose, and hybridized as described (27). A 560-bp DNA fragment of the 3′ coding region of human *ABL* was used as a probe for detecting the *BCR–ABL* mRNA transcript. The other DNA probes used were described elsewhere including mouse c-*myc* (28), c-*myb* (29), *junB* (30), c-*jun* (31), *junD* (31), and glyceraldehyde-3-phosphate dehydrogenase (32). All probes were labeled by the oligonucleotide labeling kit using protocols provided by the supplier (Pharmacia).

For protein analysis, cells were lysed in RIPA extraction buffer, fractionated on SDS/7.5% polyacrylamide gels, and electroblotted onto nitrocellulose paper as described (18). The detection of c-myc and pRb proteins with specific monoclonal antibodies was performed as described (18). To detect phosphotyrosine-containing proteins, the immunoblots were incubated for 2 h with anti-phosphotyrosine monoclonal antibodies (Zymed, San Francisco) diluted 1:2000 in blocking buffer (10 mM Tris·HCl, pH 7.2/5% bovine serum albumin/1% ovalbumin/0.9% NaCl/0.1% Tween/0.01% Azid). The second step consisted of a 2-h incubation with goat anti-mouse IgG heavy and light chains diluted 1:10,000 coupled to peroxidase (Jackson ImmunoResearch) and subsequent reaction with reagents of the ECL Western blotting detection system (ECL, Amersham). To detect ABL and BCR–ABL proteins on immunoblots, we used monoclonal antibodies against human ABL (PharMingen, San Diego) and the same second antibodies described above.

***In Vitro* Autokinase Assay.** M1 cells (10^7 cells per sample) were incubated for 10 min at 4°C in 1 ml of phospholysis buffer (1% Triton X-100/0.5% deoxycholate/0.1% SDS/0.1 M NaH_2PO_4, pH 7.5/0.1 M NaCl/5 mM EDTA/1% aprotinin/1 mM phenylmethylsulfonyl fluoride). The lysates were centrifuged for 10 min at 11,600 × *g* at 4°C. Immunoprecipitation was performed at 4°C by incubating the lysates for 2 h with 5 μl of anti-BCR polyclonal antibodies (no. 1125 in ref. 33). This was followed by a 30-min incubation with protein A coupled to Sepharose beads (3 mg in 50 μl of phosphate-buffered saline). The washed immune complexes were suspended in 50 μl of reaction mixture containing HNTG buffer (20 mM Hepes, pH 7.5/150 mM NaCl/0.1% Triton X-100/10% glycerol), 20 mM $MnCl_2$, 0.1 μM ATP, and 5 μCi of [γ-^{32}P]ATP (>5000 Ci/mmol; 1 Ci = 37 GBq; Amersham). The *in vitro* phosphorylation reaction was performed for the indicated time periods at 4°C. Sepharose beads were washed with HNTG buffer, suspended in SDS sample buffer, and boiled for 5 min; the proteins were separated on a SDS/7.5% polyacrylamide gel. The gel was dried and autoradiographed. The bands were excised from the gels and the amount of radioactivity was determined.

***In Vitro* Phosphatase Assay in Crude Extracts.** Exponentially growing cells (10^7) were incubated for 10 min at 4°C in 100 μl of lysis buffer (50 mM Tris·HCl, pH 7.4/150 mM NaCl/1% Triton X-100/5 mM EDTA/1 mM phenylmethylsulfonyl fluoride/50 μg of aprotinin per ml/1 mg of bovine serum albumin per ml) with or without vanadate. The lysates were centrifuged for 10 min at 11,600 × *g* at 4°C and supernatant samples were assayed for phosphatase activity at 30°C for the indicated time periods with the following two substrates: (*i*) BCR–ABL: equal cpm of washed immune complexes of phosphorylated p210, obtained by the *in vitro* autokinase assay. After incubation, the Sepharose beads were processed on gels as detailed above. (*ii*) Synthetic peptide: tyrosine phosphorylated synthetic peptide (YINAS) (1 μM ^{32}P-labeled YINAS; 1.5×10^4 cpm) prepared and provided to us by C. D. Diltz and E. H. Fischer (University of Washington, Seattle). The reaction that consists of 20 μl of cell extract and 20 μl of substrate was terminated by the addition of 0.4 ml of acidic charcoal mixture [0.9 M HCl/2 mM Na_2HPO_4/0.1 M sodium pyrophosphate, 10% (vol/vol) Norite A]. The radioactivity in the supernatants was measured.

RESULTS

Isolation of *BCR–ABL* Transfected M1 Clones. We have introduced by electroporation into the S-6 subclone of M1 cells an expression vector coding for BCR–ABL p210 (pLSV-p210) together with a plasmid conferring resistance to G418 neomycin (pSV2neo). Stable neo-resistant clones were analyzed for integration and expression of the *BCR–ABL* gene. Northern blot analysis indicated that two isolated clones, 4-2 and 4-3, expressed high levels of the *BCR–ABL* large mRNA transcript (Fig. 1*a*). No such signal was detected either in a control neo clone (2-1) or in the nontransfected parental cells (S-6). Due to the low abundance of the endogenous ABL mRNA transcripts (of 5.3 and 6.5 kilobases) it was impossible to detect them in total RNA samples (Fig. 1*a*). Functional expression of the *BCR–ABL* gene product, the p210 protein, was confirmed by *in vitro* autokinase assay in immune complexes generated by anti-BCR antibodies. Fig. 1*b* shows that 4-2 and 4-3 M1 clones express functional p210 that undergoes autophosphorylation to an extent comparable with the activity expressed in the K562 cell line (derived from a CML patient). It should be noted that the two *BCR–ABL*-transfected clones displayed normal growth kinetics and cell cycle distribution, characteristic of parental M1 cells.

IL-6 Induces Protein Tyrosine Dephosphorylation Through PTPase Activation. We first analyzed the changes in phosphotyrosine content of cellular proteins after *BCR–ABL* transfection and as a consequence of the IL-6 treatment by treating immunoblots prepared from crude extracts with anti-phosphotyrosine antibodies. Subsequently, the autora-

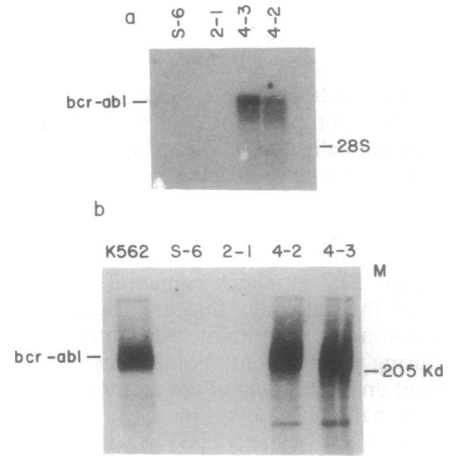

FIG. 1. Analysis of BCR–ABL mRNA expression and *in vitro* autokinase activity of p210 in transfected M1 clones. (*a*) Northern blot analysis. Total RNA was prepared from growing cells and aliquots of 20 μg were fractionated on gels, blotted, and hybridized with human ABL probe. (*b*) *In vitro* autokinase activity of immune complexes precipitated with anti-BCR polyclonal antibodies. S-6, nontransfected M1 parental clone; 2-1, *neo*-transfected M1 clone; 4-2 and 4-3, *BCR–ABL*-transfected clones; K562, cell line derived from a CML patient.

diograms were subjected to densitometric tracing and the relative intensity of each band in the control samples was compared to that of the IL-6-treated samples. Fig. 2 *a* and *b* shows that IL-6 reduced the extent of tyrosine phosphorylation of several but not all cellular proteins; the extent of reduction was between 5- and 20-fold among the different proteins. The reduction was detected in the *BCR–ABL*-transfected (Fig. 2*a*) and the *neo*-transfected clones (Fig. 2*b*), as well as in the parental S-6 cells (data not shown). It is also shown that the activated BCR–ABL kinase led to a 30- to 100-fold increase in phosphotyrosine content of the major IL-6-responsive cellular proteins (Fig. 2*a*, compare lanes 4-2 and 4-3 to lane 2-1). As a consequence, the phosphotyrosine content of proteins after IL-6 treatment of *BCR–ABL*-transfected clones was still 5- to 10-fold higher than the basal levels in the nontransfected cells before their exposure to IL-6 (Fig. 2*a*). Therefore, it seems that *BCR–ABL* transfection eventually prevented IL-6 from reaching the minimal tyrosine phosphorylation levels characteristic of parental S-6 cells. The reduction in phosphotyrosine content of proteins was first detected 12 h after addition of IL-6 and was maximal at 48 h (data not shown). Next, it was found that neither the p210 steady-state levels nor the tyrosine autokinase activity measured in immune complexes was reduced by IL-6. Fig. 2*c* shows that the p210 protein levels detected on immunoblots were not down regulated by IL-6; also the endogenous levels

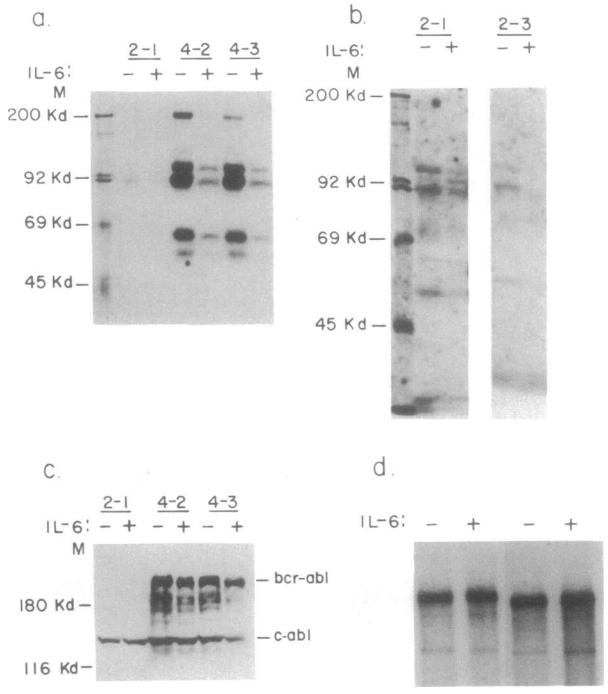

FIG. 2. Inhibitory effects of IL-6 on extent of protein tyrosine phosphorylation in M1 cells. (*a*) Immunoblots after reaction with anti-phosphotyrosine antibodies. M1 clones (2-1, *neo*-transfected clone; 4-2 and 4-3, *BCR–ABL*-transfected clones) were seeded at 1.5 × 10^5 cells per ml and treated with IL-6 (150 units/ml) or were left untreated. After 48 h, cells were extracted and 50-μg protein samples were electrophoresed and immunoblotted. Pattern of stained proteins was identical before and after IL-6 treatment (data not shown). (*b*) Same as in *a* using the 2-1 *neo*-transfected M1 clones and the 2-3 IL-6 growth-resistant M1 clone for analysis by anti-phosphotyrosine antibodies. Autoradiograms were exposed for longer periods of time than in *a*. (*c*) Extracts were prepared as in *a* and immunoblots underwent reaction with anti-c-abl antibodies. (*d*) *In vitro* autokinase activity of p210. Immune complexes precipitated by anti-BCR antibodies were subjected to autophosphorylation after extensive washes and were processed as described. Extracts were prepared from BCR–ABL-transfected clones (4-2 and 4-3) with or without IL-6 treatment for 48 h.

of the ABL protein remained constitutive in the presence of IL-6. Fig. 2*d* illustrates that the *in vitro* autokinase activity of the immunoprecipitated p210 was not reduced at all by IL-6 treatment. A more detailed time course of *in vitro* autokinase activity of BCR–ABL is shown in Fig. 3. It is clear that in the linear range of the reaction, autokinase activity was not reduced by IL-6 but was even slightly stimulated instead. Together, the data rule out the possibility that the reduction in phosphotyrosine content of proteins may result from inhibition by IL-6 of the expression or the inherent tyrosine kinase activity of the BCR–ABL protein. This reduction seems to be triggered by IL-6 itself rather than being the indirect consequence of growth inhibition, since it also took place in a growth-resistant cell variant of M1 S-6 that fails to be arrested by IL-6 (clone 2-3) (Fig. 2*b*). This receptor-positive, growth-resistant clone was described in detail elsewhere (D. Melamed and A.K., unpublished work).

It was interesting to determine whether IL-6-induced tyrosine dephosphorylation results from increased PTPase activity in the cells. Using the *in vitro* autophosphorylated p210 as a substrate, PTPase activity in crude lysates derived from control cells was compared to that of cells treated with IL-6. Fig. 4*a* shows that extracts prepared from IL-6-treated cells at 48 h displayed increased PTPase activity over the basal activity detected in nontreated cells. Addition of sodium vanadate, an inhibitor of PTPase, to the *in vitro* assay abolished the IL-6-induced reduction in p210 phosphorylation (Fig. 4*c*). No increase in PTPase activity was detected after 10 min or 6 h of IL-6 treatment, and the earliest time point at which this activity was detected was 12 h (data not shown). A more quantitative measure of the PTPase activity was obtained by using another substrate, a [^{32}P]tyrosine-labeled synthetic peptide (YINAS), *in vitro*. Fig. 5 shows that PTPase activity was increased by IL-6 by a factor of 2.3 as calculated from the linear phase of the reaction. It was of interest to study whether the increased PTPase activity is also characteristic of other cytokines that induce growth arrest. TGF-β_1 is one of the growth inhibitory cytokines that arrests the M1 cells in the G_0/G_1 phase (18). This cell cycle arrest is reversible and thus differs from the terminal growth arrest induced by IL-6. The pattern of phosphorylated proteins of clone 4-2 treated for 24 h with either TGF-β_1 or IL-6 was compared. There was no reduction, but rather a slight increase in tyrosine phosphorylation levels of the proteins derived from TGF-β_1-treated cells (Fig. 4*b*). Assaying PTPase activity *in vitro* revealed no increase in this enzymatic activity after TGF-β_1 treatment of the cells (Fig. 4*c*).

Analysis of Growth-Related Gene Responses to IL-6 in *BCR–ABL* Transfected Clones. We examined a panel of molecular responses to IL-6, most of which were previously

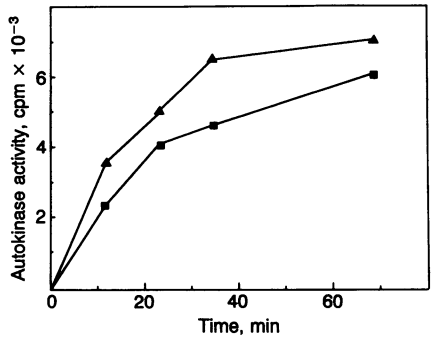

FIG. 3. Autokinase activity of BCR–ABL p210 is not reduced by IL-6. *In vitro* autokinase activity was measured as described in Fig. 2*d* except the reaction was stopped at different time points as indicated. ■, Extracts from untreated 4-2 clone; ▲, extracts from IL-6-treated 4-2 clone (48 h). Values represent cpm counted from excised gel strips.

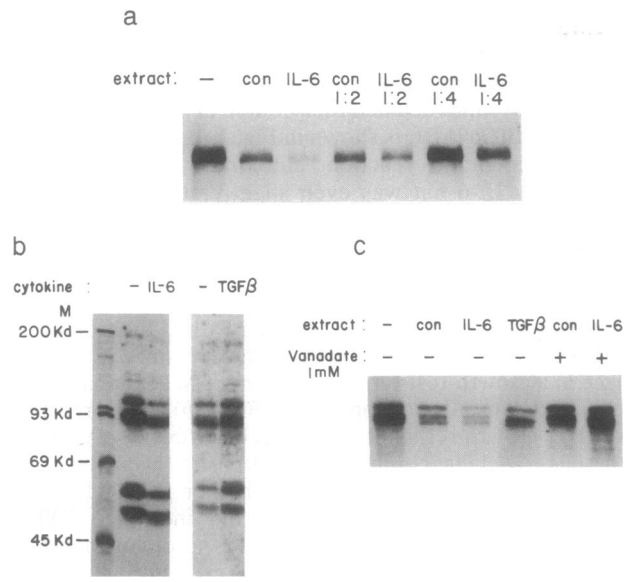

FIG. 4. PTPase activity is increased by IL-6 in M1 cells. (*a*) M1 S-6 cells were grown in the absence (con) or presence of IL-6 (150 units/ml). After 48 h, cell lysates were prepared and tested for phosphatase activity as follows. Samples of 300 μg of protein and 2-fold successive dilutions were added to 75,000 cpm of *in vitro* phosphorylated BCR–ABL immune complexes. After 5 min of incubation at 30°C, the washed immune complexes were fractionated on gels. In the first lane (lane −), lysis buffer was used instead of cell extract. (*b*) Phosphotyrosine content of proteins after TGF-β treatment of M1 cells. The *BCR–ABL*-transfected 4-2 clone was treated for 24 h with either TGF-β_1 (0.7 ng/ml) or IL-6 (150 units/ml). Forty-microgram protein samples were analyzed on immunoblots with anti-phosphotyrosine antibodies. (*c*) Phosphotyrosine phosphatase activities in cell lysates prepared from M1 S-6 cells cultured in the absence (con) or presence of TGF-β_1 (0.7 ng/ml) or IL-6 (150 units/ml). Lysate samples of 300 μg of protein were tested in each case. Phosphatase assay was performed as in *a* except the last two lanes represent reactions that were supplemented with sodium vanadate (1 mM).

shown by genetic and pharmacological approaches to lie along the growth suppressive pathways of IL-6 (17, 18, 21, 22). The Northern blot and the immunoblot in Fig. 6 clearly illustrate that c-myc mRNA and protein levels continued to be strongly suppressed by IL-6 in the 4-2 and 4-3 clones. Also IL-6-mediated pRb dephosphorylation was not interrupted in the *BCR–ABL*-transfected clones. After 48 h of IL-6 treatment, >90% of pRb molecules were retained within the rapidly migrating underphosphorylated form of pRb in the control and *BCR–ABL*-transfected clones (Fig. 6*a*). In untreated cultures, extracts from the *BCR–ABL*-transfected clones consistently displayed a small fraction of rapidly migrating pRb never observed in the control neo clone (Fig. 6*a*) or in the parental M1 cell (18), the significance of which was not investigated further. The Northern blot in Fig. 6*b* also shows that the IL-6-induced reductions in c-myb and cyclin A mRNA were not abrogated as well in the *BCR–ABL*-transfected clones. Also the pattern of *jun* genes responses to IL-6 was not changed—i.e., strong induction of junB and c-jun mRNA and no effects on junD mRNA. The expression of BCR–ABL mRNA remained constitutive in IL-6, as expected from the immunoblot in Fig. 2*c*. Measurements of cell number and cell cycle distribution indicated that *BCR–ABL* expression did not prevent IL-6-induced growth arrest or the differentiation process (data not shown).

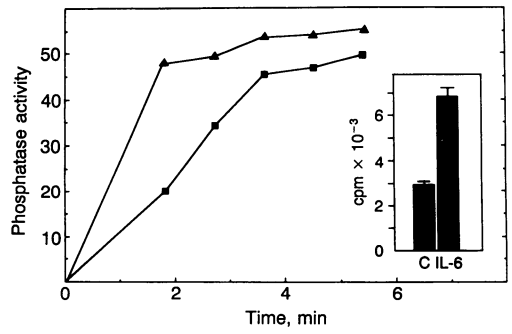

FIG. 5. Quantitation of PTPase activity in crude cell lysates before and after IL-6 treatment. *In vitro* incubation with ^{32}P-labeled YINAS peptide (1.3×10^4 cpm) was done with 20-μl samples of cell lysates (containing 60 μg of protein) at 30°C for 2, 3, 4, 5, or 6 min as described. Values represent percentage radioactivity released from ^{32}P-labeled YINAS into supernatants after charcoal precipitation at these time points. ■, Lysates from untreated cells; ▲, lysates from cells 48 h after IL-6 treatment. (*Inset*) Average value of phosphatase activity in three independent measures using nontreated (C) and IL-6-treated (IL-6) cell extracts in 2-min incubations.

DISCUSSION

While growth stimulatory factors elevate protein tyrosine phosphorylation through activation of several types of protein kinases (34), very little is known about whether growth

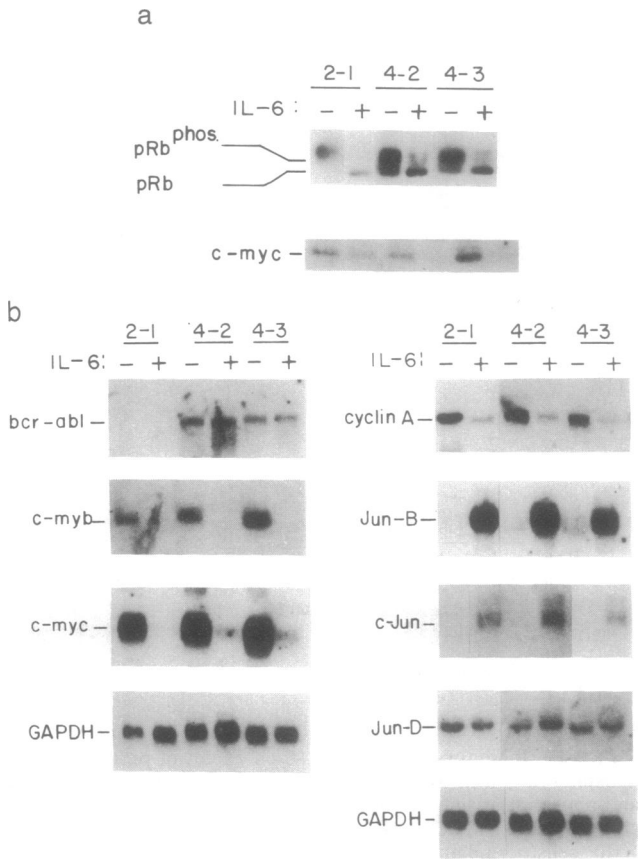

FIG. 6. Transfection with *BCR–ABL* does not interfere with the IL-6-induced gene responses. (*a*) Immunoblot analysis of IL-6-induced responses of pRb and c-myc proteins. Three M1 clones were treated with IL-6 (150 units/ml) for 48 h or were left untreated. Proteins were extracted, and 40-μg samples were fractionated on gels, immunoblotted, and incubated with anti-pRb and anti c-myc antibodies. (*b*) Northern blot analysis. M1 clones were seeded at a density of 1.5×10^5 cells per ml in the absence or presence of IL-6 (150 units/ml) for 48 h. Total RNA samples (20 μg) were fractionated and analyzed. Blots were hybridized sequentially with the above-mentioned DNA probes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used as an internal control.

inhibitory cytokines may trigger antagonistic activities that reduce tyrosine phosphorylation as part of their antiproliferative mode of action. The issue has been approached in this work from two different angles by using the M1 cell system where IL-6 operates as a potent and irreversible growth inhibitor. One approach was aimed at studying directly whether IL-6 triggers dephosphorylation of proteins at tyrosyl residues. In the second, indirect, approach, we tested whether introduction of deregulated protein tyrosine kinase into M1 cells may release the cells from some of the negative growth constraints of IL-6 by abrogating one or more of the gene responses to the cytokine.

The direct approach led to the finding that IL-6 reduced by 5- to 20-fold the phosphotyrosine content of proteins in M1 cells. This reduction turned out to lie along the signaling pathways of the cytokine since it was still preserved in a growth-resistant M1 clone that had lost many but not all the growth-related gene responses to IL-6. The latter data ruled out the possibility that the reduction may simply reflect the change in growth state of the cells. Consistently, another cytokine that caused G_1 block in M1 cells—i.e., TGF-β_1—failed to reduce the phosphotyrosine content of proteins, suggesting some specificity toward the IL-6-induced signaling pathways. The slow and persistent nature of the reduction in phosphorylation classified it as a late-response event similar to the responses of the cell cycle-controlling proteins (18). The finding that the inherent tyrosine kinase activity of p210 remained constitutively high in IL-6 prompted us to look for IL-6-induced PTPase activity. Indeed, *in vitro* assays showed elevated levels of vanadate-sensitive PTPase activity in lysates that were prepared from IL-6-treated cells. The increased levels of PTPase activity could account, at least in part, for the IL-6-induced changes in phosphotyrosine content of proteins. Obviously, it will be interesting to identify in the future which PTPase gene product is activated by IL-6. It will be determined in this respect whether IL-6-responsive activity is generated by a transmembranal or a soluble intracellular PTPase (13, 14) and whether IL-6 alters the protein expression or triggers posttranslational modifications of a preexisting PTPase. Increased levels of PTPase activity have been documented in differentiated HL-60 cells and in peripheral blood monocytes and granulocytes (20, 35). In the latter case, the activity was resistant to vanadate and therefore differed from the IL-6-induced PTPase. The M1 system represents one of the few available *in vitro* model systems in which PTPase activity is regulated by extracellular signals that are generated by growth inhibitory cytokines.

It is apparent from analysis of the transfected clones that expression of p210 changed the balance between phosphorylation and dephosphorylation of proteins after IL-6 treatment. The deregulated protein kinase caused a 30- to 100-fold increase in phosphotyrosine content of proteins. As a consequence, the extent of phosphorylation after IL-6 treatment was still 5- to 10-fold higher than basal levels in parental cells before treatment. Yet the *BCR–ABL* oncogene failed to rescue, even partially, the growth inhibitory responses to IL-6. It did not confer any growth advantage to the IL-6-treated cells as measured by cell counts and cytofluorimetric studies. Moreover, the *BCR–ABL* oncogene did not interfere with any of the growth-related molecular responses to IL-6.

A simple interpretation of the data would be that none of the putative protein substrates of the deregulated p210 tyrosine kinase falls into the IL-6 growth suppressive pathways. Yet, there exists a second intriguing possibility that implies that both BCR–ABL and the IL-6-activated PTPase share common crucial protein substrates. However, due to a redundancy in the IL-6-induced mechanisms that modify one or more of the aforementioned genes, the antagonistic effects of BCR–ABL on substrates subjected to dephosphorylation by the PTPase remain latent in the system. Therefore it would be interesting to study whether *BCR–ABL*-transfected M1 clones are "predisposed" to be liberated from negative growth control and could grow as immature blasts in the continuous presence of IL-6 after an additional genetic hit. If this turns out to be the case, then the *BCR–ABL*-transfected M1 clones could serve as a convenient *in vitro* tool to isolate the collaborating genetic hits that together with the deregulated kinase are responsible for the blast crisis phase in CML disease.

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