Activation of the Abelson Tyrosine Kinase Activity Is Associated with Suppression of Apoptosis in Hemopoietic Cells

Caroline A. Evans, P. Jane Owen-Lynch, Anthony D. Whetton, and Caroline Dive

CRC Molecular and Cellular Pharmacology Group, Manchester University School of Biological Sciences, Oxford Road, Manchester M13 9PT [C. A. E., C. D.], and Department of Biochemistry, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, M60 1QD [P. J. O., A. D. W.], United Kingdom

Abstract

A chromosomal translocation uniquely associated with chronic myeloid leukemia leads to the formation of a chimeric gene, bcr-abl, on the Philadelphia chromosome. The BRC-ABL protein displays an uncontrolled tyrosine kinase activity similar to that seen with the transforming oncogene of the Abelson murine leukemia (ABL) virus (v-ABL). An interleukin 3 dependent cell line, IC.DP, has been transfected with a gene encoding a temperature sensitive v-ABL. In the absence of interleukin 3 at the restrictive temperature for ABL tyrosine kinase activity IC.DP cells died via apoptosis. At the permissive temperature ABL tyrosine kinase activity promoted IC.DP cell survival but not proliferation. ABL therefore can specifically suppress apoptosis.

Introduction

The mechanism of cell death involved in precise regulation of processes such as morphogenesis and the immune response has been shown to occur via a process termed apoptosis (programmed cell death, reviewed in Ref. 1). In the hemopoietic system, programmed cell death plays a key role in the maintenance of a homeostatic supply of mature blood cells from the bone marrow (2-4). Withdrawal of specific growth factors such as IL-3, granulocyte-macrophage colony stimulating factor, and granulocyte colony stimulating factor from growth factor dependent hemopoietic cell lines induces cell death via apoptosis (5). Provision of growth factor promotes not only survival but also the subsequent ability to proliferate and differentiate. In many cases this absolute requirement for hemopoietic growth factor is maintained in primary human leukemias and there is little proof that an autocrine mechanism is involved in the proliferation of leukemic cells (6, 7). There is, however, some evidence to suggest that cells from patients with chronic myeloid leukemia exhibit an enhanced survival potential, in vitro, in the absence of hemopoietic growth factors (8). CML is associated with a chromosomal translocation which results in the formation of a fusion gene (bcr-abl) which encodes a membrane associated, activated ABL tyrosine kinase. Reconstitution of the murine hemopoietic system with stem cells infected with the activated ABL tyrosine protein kinase has shown that there is a positive correlation between this oncogene and leukemogenesis (9-11). We therefore decided to investigate a possible role for activated Abelson tyrosine kinase on the suppression of apoptosis. To achieve this we used the IL-3 dependent mast cell line IC2.9, which has been transfected with a gene encoding a temperature sensitive v-ABL, to generate the subclone, IC.DP (12).

Materials and Methods

Effect of Temperature Switch on p160v-abl Autophosphorylation. Unless stated otherwise reagents were obtained from Sigma (Poole, United Kingdom). Cells (3 x 10⁶/ml) were labeled with 100 μCi/ml 32P¡ in phosphate free Dulbecco's modified Eagle's medium supplemented with 3% X63-Ag-653 cell conditioned media containing IL-3 (13) and 5% dialyzed horse serum, and left for 18 h at 39°C. Cells (1 ml) were then transferred, for the times indicated, to 32°C, with control cells, i.e., time zero hours kept at 39°C for a further 6 h. Cell lysates were then prepared by the addition of ice cold lysis buffer (50 mM Tris-acetate buffer (pH 8.0), 1 mM EDTA, 1 mM ethylene bis(oxyethylentri-trilo)tetraacetic acid, 12 mM NaCl, 1 mM Na₂VO₄, 50 mM NaF, 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonfluoride, 10 μg/ml pepstatin A, 10 μg/ml leupeptin, 10 μg/ml benzamidine, 10 μg/ml antipain, and 1% (w/v) Nonidet P 40) followed by incubation on ice for 30 min. Samples were cleared by centrifugation and then incubated overnight with rabbit polyclonal anti-c-abl (Oncogene Science, New York, NY), 1 μg/ml, and 30 μl/ml of protein A-agarose (Calbiochem, Nottingham, United Kingdom) at 4°C. The immunoprecipitate was collected by washing (4 times) with lysis buffer before separating by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Effect on v-ABL Activation on Cell Viability following Removal of IL-3. Cell lines were maintained in Fischer's medium, supplemented with 3% murine IL-3 conditioned medium and 10% horse serum, and left for 18 h at 39°C to ensure inactivation of v-ABL in IC.DP cells. Cells were washed 3 times to remove the murine IL-3 before being resuspended, in unsupplemented Fischer's medium, at 1 x 10⁶/ml and maintained at either 39 or 32°C. Viable cell counts were obtained every 24 h by subtracting the trypan blue positive cells from the total cell number.

Measurement of Apoptosis. AO (Molecular Probes, Inc., Eugene, OR, 10 μg/ml in phosphate buffered saline) staining to identify apoptotic cells was performed as described previously (14). AO stained samples were analyzed by confocal microscopy using an MRC 600 confocal microscope (Bio-Rad, Richmond, CA). DNA was extracted from 2 x 10⁶ cells and subjected to gel electrophoresis as described by Smith et al. (4).

Results

IC.DP cells constitutively express p160v-abl tyrosine kinase. Fig. 1 shows the effect of temperature switch from 39°C (the restrictive temperature) to 32°C (the permissive temperature) on the phosphorylation state of v-ABL. It can be seen that the phosphorylation state increases up to 2 h and remains elevated above basal levels over 6 h. Apart from the autophosphorylation site (which is associated with activation of the tyrosine kinase activity), v-ABL has other sites which are constitutively phosphorylated. This would explain the presence of the faint band at 39°C. Measurements of levels of tyrosine phosphorylated proteins indicate that these increase within 1 h of temperature switch (from restrictive to permissive temperatures) and remain elevated for over 6 h. Taken together these results show that the v-ABL tyrosine kinase is constitutively active at 32°C but not at 39°C.

The number of viable cells decreased when IC.DP and IC2.9 cells were cultured at 39°C in the absence of IL-3 (Fig. 2A). At 32°C loss of viable cells was observed only in the parent IC2.9 cell line. Using...
ABL TYROSINE KINASE ACTIVITY

p160—¿ 116
1 2 4
HOURS

Fig. 1. Effect of temperature switch, from the nonpermissive to permissive temperature, on the autophosphorylation of v-ABL. An autoradiograph of polyclonal anti-ABL antibody immunoprecipitates of 32P-labeled cell lysates separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis is shown. Cells were maintained at 39°C for 18 h before switching to 32°C for the times shown.

As expected, removal of IL-3 from IC2.9 cells at either temperature resulted in fragmentation of DNA to 180-200 base pairs integers (Fig. 4, Lanes 1 and 3) indicative of the activation of an apoptosis-associated endonucleolytic activity (15). The DNA extracted from 1C.DP cells maintained at 39°C (the nonpermissive temperature) was similarly fragmented to give the classical DNA “ladder” typical of apoptotic cells (Fig. 4, Lane 2). Consistent with only a basal number of cells displaying morphological changes associated with apoptotic cell death (4%), no evidence of endonucleolytic fragmentation was seen in DNA extracted from IC.DP cells 72 h after removal of cytokine when v-ABL was activated (Fig. 4, Lane 4).

v-ABL activation suppressed apoptosis in IC.DP cells throughout the time course of the experiment which was carried out in the absence of serum (Fig. 2B). There was no significant change in cell number during the experiment (Fig. 2A) suggesting that activation of v-ABL in the absence of serum did not permit cellular proliferation. Moreover, there was minimal incorporation of radiolabeled thymidine in IC.DP cells at 32°C following removal of IL-3. However, these IC.DP cultures at 32°C were induced to proliferate on readdition of IL-3 (results not shown). The presence of serum had no measurable effect on IL-3 withdrawal induced apoptosis (results not shown).

Discussion

The expansion of tumor cell populations must depend not only on the rate of proliferation but also on the rate of cell death (1). The data presented here show that while activation of v-ABL tyrosine kinase in the absence of IL-3 and serum did not cause an increase in cell number, v-ABL protein tyrosine kinase activity did provide hematopoietic cells with a survival advantage via the suppression of apoptosis. This may in part account for the discordant development observed in the chronic phase of CML which results in granulocytosis. It is possible that CML progenitor cells which are present in the bone marrow or peripheral blood have a survival advantage over normal progenitor cells. Whereas normal progenitor cells would die in some locations, CML progenitors survive and can then proliferate when they encounter sufficient concentrations of cytokine(s) to promote DNA synthesis. A similar role in promoting cell survival via abrogation of apoptosis induced by growth factor deprivation and cytotoxic stress has been demonstrated for the oncogene bcl-2 in B-cell malignancies (16, 17). It may be significant that bcl-2, although absent in most cases during the chronic phase of CML, was present in large quantities in 3 cases of blast crisis analyzed (18). It is possible...
that while abl contributes to the development of CML via suppression of apoptosis, the additional survival advantage provided by bcl-2 augments the effect of abl and results in a blast crisis which is chemotherapy resistant. Furthermore, transfection of myeloid leukemia cells with the wild type p53 tumor suppressor gene induces apoptosis (19) and the accelerated and chronic phases of CML are in some patients associated with mutations in p53 (20). While the biochemical functions of the BCL-2 and p53 proteins are unclear, ABL is known to be a member of the SRC family of non-receptor tyrosine kinases. The signal transduction pathways involved in the initiation of apoptotic cell death are unknown. We envisage that the finding that an activated tyrosine kinase transduces signals which act to suppress apoptosis (in the absence of proliferation signals) may reveal new targets which in turn may expedite rational design of novel anticancer agents specifically targeted to oncogene mediated suppression of apoptosis.

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References

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