The Suppression of Drug-induced Apoptosis by Activation of v-ABL Protein Tyrosine Kinase

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ABSTRACT

Cells with a temperature-sensitive mutant of the v-abl oncoprotein (IC.DP) were treated with the anticancer drugs melphalan or hydroxyurea. At the restrictive temperature for v-ABL protein tyrosine kinase activity, drug-treated IC.DP cells died by apoptosis. In contrast, apoptotic cell death induced by either drug was suppressed when v-ABL was active. However, melphalan-induced accumulation of cells in the S and G2-M phases of the cell cycle was unaffected by v-ABL activation. Moreover, the continuous presence of v-ABL activity was necessary to suppress apoptosis. This suggested that melphalan had interacted with DNA and that v-ABL activity prevented the coupling of drug-induced damage to the apoptotic pathway. IC.DP cells exhibited similar levels and subcellular localization of the BCL-2 protein irrespective of v-ABL activation status, thus v-ABL-mediated cell survival appeared to be independent of BCL-2.

INTRODUCTION

Inherent and acquired drug resistance are major clinical problems in current cancer chemotherapy. Many mechanisms of drug resistance have already been described (reviewed in Ref. 1); these include amplification of the drug target (2, 3), increased metabolism of applied drugs (4), decreased drug uptake (5), increased drug efflux (6) and enhanced repair of drug-induced damage (7). However, in many cases the mechanism of inherent drug resistance is unexplained (8). We have previously suggested that the response of a tumor cell to a drug may not depend exclusively on the immediate biochemical lesions imposed by the drug, but also on the ability (or inability) of a cell to respond to these lesions (9). One response of drug-sensitive cells to drug treatment is death by apoptosis, a physiological and active cell death pathway (10–12). The suppression of apoptosis could therefore be a novel mechanism of drug resistance which operates downstream of the drug-target interaction and its immediate biochemical consequences. This would obstruct the removal of drug-damaged cells. If such cells exhibited genomic instability, their survival may contribute to progression of the disease. For example, apoptosis induced by treatment of cells with antimitobilite drugs was suppressed by transfection of cells with the oncogene, bcl-2, despite there being similar levels of drug-induced damage in both parent and transfected cells (13). Many similar studies also report drug or radiation resistance due to bcl-2 suppression of apoptosis (14–16). This mechanism of drug resistance may have an important role in the lack of effect observed in drug treatment of relapsed follicular lymphoma, a disease associated with overexpression of bcl-2 (17–18).

CML is also associated with drug resistance, and although anti-cancer agents such as hydroxyurea and busulphan appear to contain the disease during its chronic phase, progression to a fatal blast crisis is inevitable (19). Peripheral blood cells from patients in the chronic phase of CML were exposed to busulphan and found to be more resistant than peripheral blood cells from healthy individuals; at least 1% of the chronic phase cells survived busulphan treatment (20). If these resistant cells were subsequently able to proliferate in vivo they could be responsible for the progression of CML from the chronic phase to the fatal blast crisis of the disease.

Suppression of apoptosis by hemopoietic growth factors is an important regulatory mechanism in hemopoiesis (21, 22). Drug resistance observed in cells from patients in the chronic phase of CML may be associated with an increased survival stimulus afforded to the leukemic cells via the suppression of apoptosis. Indeed, resistance to cytotoxic agents in CML cells via suppression of apoptosis has been reported recently (23). The cytogenetic hallmark of CML is the formation of the Philadelphia chromosome bearing the chimeric bcr-abl gene, which encodes a constitutively activated ABL PTK (reviewed in Ref. 24). A chronic myelogenous leukemia-like syndrome is induced in mice whose hemopoietic stem cells express bcr-abl or v-abl, suggesting a positive correlation between this oncogene and leukemogenesis (25). To evaluate the suppression of drug-induced apoptosis by the ABL PTK we have used the IL-3-dependent mouse mast cell line (IC.2.9) transfected with a temperature-sensitive v-abl PTK to form the IC.DP subclone (26). We previously demonstrated using IC.DP cells that activation of the v-abl-encoded PTK suppressed apoptosis induced by the withdrawal of the hemopoietic growth factor IL-3 (26).

MATERIALS AND METHODS

Unless stated otherwise, reagents were obtained from Sigma Chemical Co. (Poole, UK).

Cells. IC.2.9 and IC.DP cells were cultured in Fischer’s medium supplemented with 5% X63-Ag-653 cell-conditioned media containing IL-3 (27), 10% horse serum, 2 mM L-glutamine, 100 units/ml penicillin, and 100 units/ml streptomycin. Cultures were maintained at 37°C with 5% CO2.

Drug Treatment. Log phase IC.DP and IC.2.9 cells were seeded at 2 × 106/ml and incubated at 39°C for 18 h to ensure inactivation of the v-ABL PTK in IC.DP cells (28). Cells were either switched to 32°C to activate v-ABL or left at 39°C for at least 2 h prior to treatment with L-PAM or HU. The cells were then allowed to reattach to 2 × 106/ml, the drugs were added, and the cultures were maintained at 32 or 39°C. After exposure to HU for approximately one cell cycle (24 h), cells were resuspended in fresh medium at the original seeding density. Cells were counted every 24 h using an improved Neubauer hemocytometer. Cell death was assessed by measuring the membrane permeability of cells to trypan blue (0.2% w/v). Morphological features of apoptosis were identified using acridine orange staining (5 μg/ml: Molecular Probes, Inc., Eugene, OR) and fluorescence microscopy (29).

Assessment of DNA Integrity. Cells (106) were resuspended in 20 μl of lysis buffer [10 mM EDTA-50 mM Tris-HCl, pH 8-0.5% (w/v) sodium lauryl sarcosinate] containing proteinase K (0.5 mg/ml) and incubated at 50°C for 1 h. Tris-EDTA (10 μl: 10 mM EDTA, pH 8.0-10 mM Tris-HCl) containing 0.5 mg/ml RNase A was added and the cells were incubated for another hour at 50°C. EDTA (10 μl: 10 mM, pH 8.0) containing 1% (w/v) low melting point agarose was added and the samples were loaded onto a 2% (w/v) agarose gel containing ethidium bromide (0.5 mg/ml, Molecular Probes, Inc., Eugene, OR); the gel was flooded with tris-phosphate-EDTA running buffer. DNA was
subjected to conventional agarose gel electrophoresis at 40 V for 3 h and then visualized under UV illumination (29).

Flow Cytometric Analysis of the Effects of v-ABL Activation and L-PAM on Cell Cycle Distribution. Cells (5 \times 10^4) were fixed in ethanol (70% v/v in PBS) and stained with PI (32 \mu M) for 1 min. The resulting DNA-propidium red fluorescence (630 \pm 22 nm) of 10,000 cells was analyzed using a Becton Dickinson FacsVantage instrument with Enterprise laser excitation at 488 nm and 150 mW (Becton Dickinson, Palo Alto, CA). Cellular debris was excluded from the analysis. Results were analyzed using the Becton Dickinson Cell Fit software and the sum of broadened rectangles program.

Evaluation of the Requirement for Continual Activation of v-ABL to Suppress L-PAM-induced Cell Death. In some experiments cells were re-suspended in fresh medium at a concentration of 2 \times 10^5/ml, 18 h after treatment with 10 \mu M L-PAM and maintained at either 32 or 39°C. Cells were analyzed with respect to total cell number and cell viability every 24 h as described above.

Evaluation of BCL-2 Protein Expression by Western Blotting. Cells were grown at 32 or 39°C as described above and lysed by freeze-thawing in Tris (25 mM), EDTA (2.5 mM) and phenylmethylsulfonyl fluoride (10 \mu l/mil). Samples were diluted in sodium dodecyl sulfate (4% w/v), glycerol (20% v/v), bromophenol blue (0.004% v/v), Tris-HCl (8% v/v, 1 M, pH 6.8) and mercaptoethanol (10% v/v) and boiled for 2 min. After electrophoresis and Western blotting the resultant nitrocellulose blot was incubated with a rabbit polyclonal antiserum to purified BCL-2 from a prokaryotic expression vector (30) (a gift from Dr. G. Nunez, University of Michigan) using a 1:200 dilution in incubation buffer [Tris (2.5 mM), sodium chloride (14.4 mM), Tween 20 (0.5% v/v) and nonfat dried milk (2% w/v)] and incubated for 2 h. This was followed by addition of donkey anti-rabbit antibody (Amersham International, Amersham, UK) diluted 1:10,000 in incubation buffer, incubated for an additional hour, and then washes in incubation buffer for 1 h. The Western blot-antibody protein interaction was developed using the Enhanced Chemiluminescence detection kit from Amersham and detected on blue-sensitive X-ray film (Genetic Research Instrumentation Ltd.).

Assessment of BCL-2 Cellular Localization by Immunostaining and Confocal Microscopy. Cells (5 \times 10^4) were fixed onto gelatin-coated slides with acetone. The cells were washed in Triton X-100 (0.1% v/v) and incubated for 30 min at room temperature in normal goat serum (10% v/v) in PBS. Slides were washed in PBS and incubated with the primary rabbit-antimouse antibody (31) (a gift from Dr. G. L. Evan, Imperial Cancer Research Fund, London, UK) [1:1000 dilution in PBS plus bovine serum albumin (0.5% v/v)] for 1 h. This was washed off and the secondary goat anti-rabbit biotinylated antibody (1:200 dilution in PBS, Vector) was added for 1 h, then the slides were washed again. The avidin-D fluorescein isothiocyanate was added (1:200 dilution, Vector) in the dark for 1 h and then the slides were washed. Nuclei were stained using PI (0.05 \mu g/ml for 2 min) and the slides were mounted with Citifluor, (Citifluor Ltd.) and viewed using a Bio-Rad Medical Research Council confocal microscope.

RESULTS

We have previously shown that IC.DP cells express a temperature-sensitive v-ABL PTK; at 39°C v-ABL PTK is inactive, whereas a temperature switch to 32°C results in maximal activation of v-ABL PTK within 4 h (28). At the permissive temperature for v-ABL PTK activity (32°C), IL-3 deprived IC.DP cells exhibit enhanced survival associated with the suppression of apoptotic cell death (26). To investigate the effect of v-ABL PTK activity on the response of cells to cytotoxic agents used in chemotherapy, logarithmically growing cells were treated with the DNA-alkylating agent L-PAM or the antimetabolite HU.

Effect of v-ABL Activation on Response of Cells to L-PAM. Fig. 1 shows the kinetics of cell death (determined by the inability of cells to exclude trypan blue) after addition of L-PAM (1–10 \mu M). IC.2.9 and IC.DP cells at 39°C (restrictive temperature for v-ABL) demonstrated a similar concentration-dependent increase in cell death: the number of dead cells 3 days after the addition of 10 \mu M L-PAM was >90% of the total cell number. At 32°C the onset of cell death in IC.2.9 cells was delayed by approximately 48 h; 5 days after addition of 10 \mu M L-PAM >90% of the cells were dead. In comparison, IC.DP cells at 32°C (the permissive temperature for v-ABL) exhibited only 16% cell death at 5 days. This difference between the levels of cell death 5 days after addition of 10 \mu M L-PAM for IC.2.9 and IC.DP cells at 32°C is very highly significant (P < 0.001, Student's t test). It should be noted that although there was minimal cell death in IC.DP cells treated with 10 \mu M L-PAM at the permissive temperature for v-ABL, there was also no observable proliferation as the total cell number remained constant during the experiment (data not shown).

![Fig. 1. Effect of v-ABL PTK activation on cell death kinetics of IC.DP cells at the permissive (32°C) and restrictive (39°C) temperatures for v-ABL activity and IC.2.9 cells at each temperature after treatment with L-PAM. Cell death was measured by the inability of cells to exclude trypan blue and is expressed as a percentage of the total cell count. Columns, mean of 3 experiments; bars, SEM.](image-url)
Suppression of L-PAM-induced Apoptosis by Activation of v-ABL. Many anticancer agents, including those which interact directly with DNA, have been shown to induce apoptotic cell death in hematopoietic cells (10, 12, 15, 32). We therefore investigated the mode of cell death induced by L-PAM. IC2.9 cells at both temperatures and IC.DP cells at 39°C (the restrictive temperature for v-ABL) treated with 10 μM L-PAM exhibited the typical chromatin condensation and nuclear fragmentation patterns of apoptosis (revealed by DNA-binding fluorophore acridine orange, Fig. 2). In contrast, IC.DP cells with active v-ABL had an intact and diffuse nuclear morphology similar to viable control cells.

The suppression of L-PAM-induced apoptosis by v-ABL activation was confirmed qualitatively using conventional agarose gel electrophoresis of extracted DNA. This technique is used to separate the 180-base pair integer oligonucleosomal fragments of DNA formed by an endonuclease(s) which is believed to be activated in most cell types as a late event in apoptosis, and which results in the appearance of a DNA ladder (33). Drug-treated IC2.9 cells at both temperatures and IC.DP cells at 39°C (restrictive temperature for v-ABL) contained 180-base pair integer fragments, whereas control cells contained only high molecular weight DNA (Fig. 3). Both control and drug-treated IC.DP cells at 32°C (with activated v-ABL) contained only high molecular weight DNA.

Effect of L-PAM on Cell Cycle Distribution in the Presence and Absence of v-ABL PTK Activation. The effects of L-PAM on cell cycle distribution in the presence or absence of v-ABL PTK activity were examined using PI staining and flow cytometric analysis. The results obtained are shown in Table 1. The activation of v-ABL had no major effect on the cell cycle distribution of untreated control IC.DP cells over a 72-h period. Treatment of IC2.9 and IC.DP cells at 39°C (restrictive temperature for v-ABL) with L-PAM (5 and 10 μM) induced accumulation of cells in S and G2-M phases by 24 h, with increased G2-M block by 72 h (Table 1). At 32°C (permissive temperature for v-ABL), the effects of L-PAM on the cell cycle were similar but more pronounced in both IC2.9 and IC.DP cells. This lack of effect of v-ABL on L-PAM-induced cell cycle perturbations is further illustrated in Fig. 4, which compares the effects at 24 h of 5 μM L-PAM on cell cycle distributions of IC.DP and IC2.9 cells at both temperatures.

Continuous Activation of v-ABL PTK is Required for the Suppression of L-PAM-induced Apoptosis. Cells at either the permissive or the restrictive temperature for v-ABL activity were exposed to 10 μM L-PAM for 18 h, washed, reseeded in drug-free medium, and maintained at either temperature for 5 days (Fig. 5). IC2.9 cells treated at 32 or 39°C and reseeded at either temperature showed a decrease in viable cell number; after 5 days less than 40% of the cells were alive in either case (Fig. 5, A and B). IC.DP cells treated and reseeded at the permissive temperature for v-ABL (32°C) did not die during the 5 days of the experiment but did not show an overall increase in cell number (Fig. 5C). When these cells were reseeded at the restrictive temperature for v-ABL (39°C) there was >80% cell death at 5 days. More than 90% of IC.DP cells treated and reseeded at the restrictive temperature for v-ABL (39°C) underwent cell death after 5 days (Fig. 5D). The same cells reseeded instead at the permissive temperature for v-ABL (32°C) also showed a decrease in viable cell number but with slower kinetics than those reseeded at 39°C.

Effect of v-ABL Activation on Response of Cells to HU. To examine whether activation of v-ABL could suppress apoptosis induced by a second cytotoxic agent (with a different mode of action to L-PAM), logarithmically growing cells were treated with the antime-tabolite HU. As it is an S-phase-specific agent, HU (10–20 mM) was left in contact with the cells for 24 h (sufficient time to ensure the majority of cells went through one cell cycle). The cells were then resuspended in drug-free medium and their ability to survive and proliferate was assessed. Fig. 6 shows the kinetics of cell death after addition of HU (10–20 mM). At 39°C (restrictive temperature for v-ABL), cell death in both IC2.9 and IC.DP cells treated with 10 mM HU was maximal 24 h after drug addition (46% and 64%, respectively). After withdrawal of HU, remaining viable cells repopulated the culture (data not shown) and the levels of cell death subsequently decreased. The untreated cells exhibited a decrease in viability toward the end of the experiment as they approached plateau phase.
SUPPRESSION OF DRUG-INDUCED APOPTOSIS BY V-ABL

Table 1 Flow cytometric analysis of cell cycle distribution of IC.DP cells at the permissive (32°C) and restrictive (39°C) temperatures for v-ABL PTK activity and IC2.9 cells at each temperature 24 and 72 h after treatment with 5 or 10 µM L-PAM. Cellular debris was excluded based on light scatter parameters and red fluorescence (630 ± 22 nm, PI-DNA) of 10,000 cells was analyzed for each cell sample. Cell cycle histograms were analyzed using the Cellfit software and the sum of broadened rectangles program (Becton Dickinson). The results shown are the average of three repeat experiments.

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<th>G0-G1 (%)</th>
<th>S (%)</th>
<th>G2-M (%)</th>
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24 h after melphalan addition 72 h after melphalan addition

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<th>S (%)</th>
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*a*, Cellfit unable to analyze data due to indistinct G2-M peaks.

At 32°C the amount of cell death increased in the HU-treated IC2.9 cells up to a maximum of 57% after 5 days. At this time point only 11% cell death was observed in IC.DP cells with the active v-ABL PTK treated with HU. The difference between the levels of cell death 5 days after addition of 10 mM HU in IC2.9 and IC.DP cells at 32°C is significant (P < 0.05, Student’s t test). At this lower temperature IC2.9 and IC.DP cell did not repopulate the cultures after withdrawal of HU although the viable cell number of IC.DP cells was maintained throughout the experiment (data not shown).

Suppression of HU-Induced Apoptosis by Activation of v-ABL.

Acridine orange staining and fluorescence microscopy of IC2.9 cells at both temperatures and IC.DP cells at the restrictive temperature for v-ABL after HU treatment revealed condensed and fragmented chromatin in a proportion of cells typical of apoptosis at all time points (data not shown). In contrast, HU-treated IC.DP cells at the permissive temperature for v-ABL contained intact nuclei with diffuse chromatin staining characteristic of viable cells. This result was confirmed using conventional agarose gel electrophoresis of extracted DNA. This showed nonrandom DNA fragmentation (DNA laddering) after HU exposure in the absence of v-ABL activation in IC.DP cells and in IC2.9 cells at either temperature (Fig. 7). IC.DP cells at 32°C (with the activated v-ABL PTK) contained only high molecular weight DNA typical of viable cells.

Characterization of BCL-2 in the IC2.9 and IC.DP Cell Lines.

Western analysis revealed that both IC.DP and IC2.9 cells routinely cultured at 37°C expressed BCL-2 levels comparable to those of a murine leukemic cell line (L1210) expressing BCL-2 after transfection.4 To investigate whether v-ABL might suppress apoptosis in IC.DP cells via an increased expression of BCL-2, cells were maintained at 32 or 39°C for up to 3 days and BCL-2 levels were examined using Western blotting. The level of BCL-2 was not increased by activation of v-ABL (data not shown). In preliminary experiments v-ABL activation also did not upregulate BCL-2 levels in cells deprived of IL-3 for 48 h or cells exposed to melphalan (10 µM for up to 48 h) (data not shown). BCL-2 is found in multiple cellular locations including the periphery of mitochondria, perinuclear membrane, and throughout the cytoplasm (34). Immunostaining was used to determine whether activation of v-ABL PTK altered the subcellular localization of BCL-2; BCL-2 was found to be localized to the...
cytoplasm in both cell types and was independent of PTK activation status (data not shown).

DISCUSSION

One major problem in cancer chemotherapy is inherent or acquired resistance (35). Many mechanisms of drug resistance have been described but the reasons why some cells are inherently easier to kill than others are still unknown. The expansion of a tumor cell population must depend not only on the rate of proliferation but also on the rate of cell death by apoptosis. Tumor cells may be resistant to chemotherapy not only because of classical mechanisms of drug resistance, such as an alteration in the drug target or an increased drug metabolism, but also because they cannot engage and complete the active apoptotic pathway (9). The resulting increased longevity of a proportion of the cell population may then allow them to undergo further genetic changes which could lead to disease progression.

The purpose of this study was to examine the effect that expression of activated v-ABL PTK had on cells treated with the anticancer drugs L-PAM, a DNA-alkylating agent (36), or HU, an antimetabolite which inhibits ribonucleotide reductase [an enzyme involved in the synthesis of DNA bases (37)] and ultimately induces DNA strand breaks (38). We have shown that the mode of cell death induced by both HU and L-PAM in the absence of v-ABL activity was apoptosis [as demonstrated by the classically condensed and fragmented nuclear morphol-
ogy (29) and confirmed by the cleavage of DNA into 180-base pair
consistently with apoptosis being an active process.

Experiments where cells were both treated and reseeded in
drug-free medium at either the permissive or restrictive
temperatures for v-ABL PTK activity demonstrated that continuous activation of
v-ABL PTK was necessary for the suppression of L-PAM-induced apoptosis (Fig. 5).
These experiments also provide an insight into the possible
mechanisms of v-ABL PTK suppression of L-PAM-induced apoptosis.
IC.DP cells treated at 32°C (with activated v-ABL) and reseeded at 32°C neither died nor proliferated. The activation of
v-ABL did not prevent cell cycle changes induced by L-PAM; cell
cycle analysis showed that L-PAM-treated IC2.9 and IC.DP cells at
either temperature blocked initially in S phase and then in G2-M
phases of the cell cycle. This suggests that L-PAM treatment caused
DNA damage in cells with activated v-ABL, which may not have been
sufficiently repaired to allow DNA replication. IC.DP cells treated
with L-PAM when v-ABL PTK was activated but reseeded with
inactive v-ABL PTK subsequently died. This observation strengthens
the hypothesis that these cells had sustained sufficient L-PAM-in-
duced damage to undergo an apoptotic cell death and that damage had
not been adequately repaired. However, as long as v-ABL was acti-
vated, DNA damage was not "coupled" to the apoptotic pathway.

In the converse experiment where IC.DP cells were treated with
L-PAM with v-ABL inactive and reseeded with v-ABL PTK active,
some cell death still occurred but to a lesser extent and with slower
kinetics than when cells were maintained throughout with an inactive
v-ABL PTK. It is likely that when those cells with inactive v-ABL at
the time of drug addition reached DNA damage sensor(s) or check-
point(s) (39), they received the appropriate signals to die by apoptosis.
v-ABL activation may have either prevented cells from progressing to
such checkpoints so that DNA damage was not sensed by the cell
and/or suppressed or delayed the coupling of DNA damage to the
initiation of apoptosis.

v-ABL PTK was also protective against the cytotoxic effects of
HU, an agent routinely used in the treatment of CML (40). At 39°C
(inactive v-ABL) HU killed a larger number of IC.DP cells than IC2.9
cells. This was not seen after L-PAM treatment at 39°C and may
reflect differences in the length of S phase in each cell type. HU is
known to have differential effects on cell death dependent on cell
cycle phase; Chinese hamster ovary cells synthesizing DNA at the
time of drug exposure were lethally damaged, cells in G1 survived but
were prevented from DNA synthesis, and G2 cells survived and
progressed to the next G1 (41). The block in cell cycle progression
observed was, however, reversible on removal of drug (41). In our
studies a similar cell cycle effect may be affecting cell death kinetics.
When treated at 39°C more IC.DP cells may have been in S phase
compared with IC2.9 cells; hence the greater initial cytotoxicity.
When cells at 39°C were reseeded in fresh medium some were
able to proliferate and repopulate the culture (Fig. 6). Flow cytometric
analysis showed that these cells were blocked in G1 phase until the
drug was removed (data not shown). At 32°C there was also a block
in G1 phase of the cell cycle but none of the cells repopulated cultures
after drug removal over the subsequent 96 h.

One protooncogene known to function as a suppressor of apoptosis
is bcl-2 (42). It is possible that v-ABL mediates its suppression of
apoptosis via some modulation of BCL-2 function. In the IC.DP
model system ABL PTK activation did not up-regulate BCL-2 levels;
nor did it affect subcellular localization of BCL-2. These results imply
that v-ABL may suppress apoptosis via a BCL-2-independent mech-
nanism. However, several proteins which, bind to BCL-2 and modulate
its survival function have been recently identified, including BAX,
BCLxL, and r-RAS (43–45); the possibility remains that v-ABL may
alter BCL-2 function indirectly by affecting one or more of its protein
partners.

In summary, our results show that activation of the v-ABL PTK
induced drug resistance to both L-PAM and HU via the suppression of
apoptosis. v-ABL PTK presumably interferes somewhere along the
effector pathway downstream of the drug-target interaction. Previous
work demonstrated the suppression by v-ABL PTK of apoptosis
induced by removal of the growth factor IL-3 from IC.DP cells, where
v-ABL activation resulted in a cascade of signaling events, some but
not all of which were common to those elicited by IL-3 (26, 46).
However, IL-3, at a concentration which maintained IC.DP and IC2.9
viability and which stimulated proliferation in the absence of v-ABL
activation, was present throughout the studies with L-PAM and HU.
Moreover, in preliminary experiments where IC.DP cells were ex-
posed to L-PAM in the absence of IL-3, v-ABL PTK still provided a
significant degree of drug resistance. Thus, v-ABL does not merely
mimic IL-3 in its effects but provides an additional "protection" via an
enhanced ability to survive exposure to anticancer agents. The sup-
pression of apoptotic cell death by v-ABL may in part account for the
discordant development observed in chronic phase CML where CML
progenitor cells in the bone marrow or peripheral blood may have a
survival advantage. Where normal cells would die in some circum-
stances, (e.g., because of a lack of growth factors or after exposure to
anticancer chemotherapy), CML progenitor cells could survive and
consequently be susceptible to other genetic changes (47) which
would lead to blast crisis. It will now be of interest to establish
whether BCR-ABL PTK activity modulates cell survival via the
suppression of apoptosis after drug treatment in Philadelphia-positive
progenitor cells.

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