Role of Phosphatidylinositol 3-Kinase and Specific Protein Kinase B Isoforms in the Suppression of Apoptosis Mediated by the Abelson Protein-tyrosine Kinase*

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Leukemogenic oncogenes, such as the Abelson protein-tyrosine kinases (PTK), disrupt the normal regulation of survival, proliferation, and differentiation in hemopoietic progenitor cells. In the absence of cytokines, hemopoietic progenitor cells die by apoptosis. Abl PTKs mediate suppression of this apoptotic response leading to aberrant survival. To investigate the mechanism of Abl PTK action, we have used an interleukin-3-dependent murine mast cell line that expresses a temperature-sensitive form of the v-ABL PTK, which is active at the permissive temperature of 32 °C and inactive at 39 °C. At the permissive temperature, these cells are resistant to apoptosis induced both by the withdrawal of the hemopoietic growth factor (interleukin-3) and the addition of cytotoxic drugs. We demonstrate that v-Abl associates with and stimulates activation of phosphatidylinositol 3-kinase (PI3K) and, crucially, that this activation results in enhanced cellular levels of the mass of the second messenger phosphatidylinositol-3,4,5-trisphosphate. Activation of PI3K leads to enhanced activity of PKB and increased levels of the anti-apoptotic protein Bcl-xL. Transfection of cells with a dominant negative PKB reduces both the Abl-stimulated PKB activity and the survival effect conferred by activation of this oncogene. Thus, PI3K and PKB are required for the anti-apoptotic effects of Abl PTK.

Hemopoiesis occurs in the microenvironment of the adult bone marrow where progenitor cells are in close contact with bone marrow stromal cells and the associated extracellular matrix. A complex set of interactions between cytokines, integrins, and cell surface receptors on the hemopoietic progenitor cells governs the survival, proliferation, and development of primitive cells in normal hemopoiesis. In the absence of such cytokines, non-leukemogenic progenitor cells die by apoptosis. The normal regulation of survival, proliferation, and differentiation can be disrupted by leukemogenic proteins, e.g. the Bcr/Abi protein expressed in chronic myeloid leukemia. The Abelson protein-tyrosine kinase (PTK) mediates suppression of apoptosis induced by growth factor withdrawal (1). Furthermore, Abi PTK also protects against drug-induced apoptosis, a fact that may explain the resistance of CML progenitor cells to cytotoxic drugs (2, 3). Other oncogenes such as bcl-2 also suppress apoptosis, and it is becoming clear that the bcl-2 family members are regulated transcriptionally and post-translationally by upstream signaling proteins (for review see Ref. 4).

One signal transduction pathway that is proposed to initiate cell survival is the PI3K/PKB pathway. Growth factors, such as nerve growth factor and insulin-like growth factor-1, can stimulate PI3K activity, and this activity is associated with survival of the target cells (5, 6). In hemopoietic cells other cytokines such as IL-3 and stem cell factor also stimulate PI3K and cell survival (7). The lipid product of PI3K, PIP3, functions as a second messenger that stimulates the phosphorylation and activation of PKB by PDK1, via interactions with the pleckstrin homology domain of these two kinases (8). PKB or Akt kinase is the cellular homologue of the viral oncoprotein v-Akt that causes leukemia in mice and is related to protein kinase C (PKC) within the catalytic domain. However, PKB differs from the PKC family members by the presence of a pleckstrin homology domain at its N terminus. It has recently been shown that phosphorylations at Thr-308 and Ser-473 on PKB are required for full activation of PKB activity (8–10). PKB is implicated in protecting cells from apoptosis induced by a number of agents and treatments, including UV irradiation (11, 12), withdrawal of a survival factor, e.g. withdrawal of insulin-like growth factor-1 from neuronal cells (13), and detachment of cells from the extracellular matrix (14).

To understand the mechanism of the survival advantage conferred by Abi PTKs, we have used the IC.DP cell line model. This murine mast cell line expresses a temperature-sensitive form of v-ABL PTK that is active as a PTK at 32 °C and inactive at 39 °C (15). IC.DP cells are resistant to apoptosis induced both by the withdrawal of the hemopoietic growth factor, interleukin-3 (IL-3), and the addition of cytotoxic drugs (3, 16, 17). Another important feature of this cell line is that apoptotic suppression mediated by v-Abl in IC.DP cells occurs in the absence of cell proliferation. This allows us specifically to examine signal transduction pathways utilized by these PTKs in the suppression of apoptosis. Our previous studies have shown that this is a critical facet of this model. For example, when expressed in other cell lines, Abi PTKs stimulate cellular transformation, i.e. they stimulate both survival and proliferation. In such models the ERK1/2 and the JAK-2/signal transducers and activators of transcription pathways are activated by ABL PTK and linked to the above downstream effects (18–20). However, in IC.DP cells where only a survival response is

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‡ The abbreviations used are: PTK, protein-tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; IL-3, interleukin-3; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PKC, protein kinase C; mIL-3, murine interleukin-3; PAGE, polyacrylamide gel electrophoresis; LY, LY 294002.
observed, these pathways are not activated by Abl PTK but are activated by the growth factor IL-3 (17).

Although it has been shown that Abl PTKs can activate PI3K in other cells (12, 21–23), the mechanism(s) of activation, the effect of this on cellular mass levels ofPIP₃, and the effects of Abl PTK on PKB have remained relatively unexplored. Similarly the role of PI3K and PKB in Abl PTK-mediated cell survival in the absence of proliferation is not clear.

We demonstrate that in the IC.DP cell line, activation of the Abl PTK stimulates PI3K resulting in an increase in the mass of the second messenger PIP₃, which is associated with the activation of specific isoforms of PKB. We also provide evidence that this activation of PI3K/PKB is necessary for v-Abl-mediated anti-apoptotic effects.

EXPERIMENTAL PROCEDURES

Recombinant mouse IL-3 and polyclonal anti-Abl antibodies (Ab-3) were purchased from Calbiochem. Antibodies to phosphotyrosine, PI3K, She, and Cbl were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY).

Cell Culture—IC.29 and IC.DP cells were maintained in Fischer’s medium supplemented with pre-selected batches of horse serum (20% v/v) and murine interleukin-3 (mIL-3). The source of mIL-3 was conditioned medium from a myeloma cell line which expresses the mouse IL-3 gene (24), and this was used at 5% v/v.

Cell Viability Assays—Cells, maintained at 39 °C for 18 h prior to experiments, were washed in Fischer’s medium to remove serum and growth factors and plated in cell culture plates at 2 × 10⁵ cells/ml. Cells were plated in the absence of growth factor (IL-3) and/or inhibitor additions as shown. Duplicate plates were set up, one of which was incubated at the restrictive (39 °C) and one at the permissive (32 °C) temperature. Aliquots of cells were removed from each well, at 24-h intervals, over 3 days, and cell viability was determined by trypan blue exclusion. Results were expressed as percent viable cells in the culture (i.e. viable cell count/viable + dead cell count) × 100%.

In Vitro Measurement of PI3K Activity and Immunoblotting—Cells were maintained at 39 or 32 °C overnight before washing cells three times and resuspending in Fischer’s medium without supplements. Cells were maintained at the relevant temperature for a further 4 h before switching to the alternative temperature or stimulating with recombinant IL-3 for the times shown. Cells were lysed with lysis buffer A (50 mM Tris/HCl (pH 7.5), 1 mM EDTA, 1% (v/v) Triton X-100, 1 mM sodium orthovanadate, 50 mM NaF, 5 mM sodium pyrophosphate, 0.1% (v/v) β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine).

Cell lysates were immunoprecipitated with antibodies raised against p85, Cbl, Abl, or phosphotyrosine as described previously (17). Immunoprecipitated proteins were assayed for PI3K activity as described (25) using [³²P]ATP and phosphatidylinositol and phosphatidylserine containing vesicles as the substrate. An aliquot of the immunoprecipitate was incubated with SDS-PAGE sample buffer, boiled for 5 min, and loaded onto a 7.5% SDS-PAGE gel. Immunoblotting was carried out as described previously (17).

Measurement of PKB Activity—In order to assay the activity of PKB, antibodies to specific PKB isoforms were pre-coupled with protein G beads and incubated with cell lysates (prepared as described above) for an hour at 4 °C with agitation on a shaking platform. The protein G beads were spun down in a microcentrifuge and washed twice with buffer A (as above) and twice with buffer B (50 mM Tris/HCl (pH 7.5), 0.1 mM EDTA, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 5 μg/ml leupeptin, 1% (v/v) Triton X-100). The PKB activities in these immunoprecipitates were assayed as described (9).

Determination of the Mass of PIP₃ in Cells—Cells were maintained at 39 °C throughout washing cells three times and resuspending in Fischer’s medium without supplements. Cells were maintained at 39 °C for further 4 h and then switched to 32 °C for 2 h in the presence and absence of LY294002. Cells were harvested and lipids were extracted and used in a PIP₃ assay using the detailed procedure described previously (26).

Generation of Transfected Cell Lines—A full-length human PKBα construct was isolated from a skeletal muscle cDNA library (CLONTECH, Palo Alto, CA). A hemagglutinin-antigen epitope tag was incorporated into the N terminus of the PKB gene by polymerase chain reaction, and the resulting construct was subcloned into the EcoRI-

FIG. 1. Activation of PI3K by v-Abl PTK. A, IC.DP cells were preincubated at 39 °C (A) or 32 °C (B) for 18 h, washed to remove horse serum and mIL-3, starved for 4 h, and transferred to 32 °C for 0–18 h (A) or 39 °C for 0–2 h (B). Cells were lysed, and cell lysates were immunoprecipitated using phosphotyrosine-specific antibodies pre-coupled to protein G beads. The PI3K activity associated with the immunoprecipitates was measured as described. Results shown are mean ± S.E. of three experiments.

Kapo site of the pCMV5 vector. Kinase-dead PKB was created by mutating Asp-292 to Ala using Quickchange mutagenesis system (Stratagene). Cell lines were transfected as described previously (27, 28). Briefly, IC.DP cells were transiently transfected with the myeloproliferative sarcoma virus-based vector, pMS alone (control), or vector carrying the dominant negative kinase-dead PKB.

RESULTS

Activation of PI3K in Response to v-Abl Activation in IC.DP Cells—In order to study survival pathways, we used a simple, readily manipulated cell system, the IC.DP murine pre-mast cell line that depends upon v-Abl PTK for survival or IL-3 for survival and proliferation. Our previous data have shown that activation of v-Abl PTK in IC.DP cells resulted in autophosphorylation of v-Abl, which was detectable 2 h after temperature switch and was maximal by 4 h (29). The increase in tyrosine phosphorylation of intracellular proteins, achieved by switching cells to the permissive temperature for v-Abl PTK activity, was also maximal by 4 h and remained elevated indefinitely until the cells were returned to the restrictive temperature (29).

We have now investigated the effect of v-Abl PTK on the PI3K activity associated with tyrosine-phosphorylated proteins. As shown in Fig. 1A, there is detectable increase in the PI3K activity associated with this protein fraction after cells have been incubated at permissive temperature for 1 h, and this activity peaked at 2 h and remained elevated. When cells
were returned to restrictive temperatures, the PI3K activity dropped down to basal level within an hour (Fig. 1B). Thus v-Abl PTK appears to activate PI3K.

\[ \text{v-Abl PTK Activation in IC.DP Cells Leads to an Increase in the Cellular Mass of the Second Messenger PIP}_3 \]

Definitive proof of activation of this PI3K in cells is the presence of elevated mass levels of the product of this enzyme, PIP3. As shown in Table I, there is an approximately 2-fold increase in the mass of PIP3 mass after 2 h at the permissive temperature for Abl PTK activity. Thus v-Abl PTK activates PI3K leading to elevated levels of the second messenger PIP3.

\[ \text{Roles of PI3K Pathway in v-Abl-mediated Cell Survival} \]

In order to determine whether this elevation of PI3K activity has a role in the anti-apoptotic effect of the Abl PTK, experiments were performed using the PI3K inhibitor LY294002 (30). Measurement of PIP3 mass levels after treatment of cells with LY (20 μM) shows that this is sufficient to inhibit completely the rise in PIP3 levels usually observed after temperature switch (Table I).

In agreement with our previously published data, there is rapid cell death after growth factor (IL-3) removal from the culture medium of the parental IC2.9 cell lines at 39 or 32 °C (Fig. 2, A and B) and from v-Abl-transfected IC.DP at restrictive temperature (39 °C) (Fig. 2C). We have previously shown that these cells are dying via apoptosis (1). The PI3K inhibitor LY has no significant effect on the kinetics or extent of apoptosis induced by IL-3 withdrawal under these three conditions. In contrast, activation of v-Abl at the permissive temperature (32 °C) results in maintained cell survival, even in the absence of growth factor, over a 3-day period. This survival is inhibited by over 75% when the PI3K inhibitor is included in the cultures. Thus activation of PI3K appears to play a key role in the survival effects of the abl oncogene.

\[ \text{v-Abl PTK-mediated Activation of PKB} \]

Both the α and γ isoforms of PKC were expressed in the cells, and their expression was independent of temperature. The activity of these two isoforms was markedly increased when cells were incubated at the permissive temperature for Abl PTK activity. PKBα and -γ activation peaked at approximately 4 h and then remained elevated indefinitely (Fig. 3A). Switching off v-Abl PTK activity by maintaining cells at the restrictive temperature (39 °C) resulted in rapid decrease of PKB activity (Fig. 3B). Preincubating cells in the presence of the PI3K inhibitor LY abolished v-Abl-mediated activation of both PKB isoforms (Fig. 3A).

\[ \text{Role of PI3K Pathway in the Up-regulation of Bcl-XL by v-Abl} \]

By having shown that v-Abl can activate PI3K and subsequently

**TABLE I**

| Change in PIP3 mass in IC.DP cells in response to v-Abl activation |
|---------------------------------------------------------------|
| For measurement of PIP3 mass, cells were maintained at 39 °C overnight before washing cells three times and resuspending in Fisher’s medium without supplements. Cells were maintained at 39 °C for further 4 h and then switched to 32 °C for 2 h in the presence and absence of LY. Cells were harvested, and lipids were extracted, and PIP3 mass assays were performed. |
| Experiment 1 | 39 °C | 0.65 ± 0.12 |
| | 32 °C | 1.38 ± 0.18 |
| | 32 °C + LY | Not detectable |
| Experiment 2 | 39 °C | 0.72 ± 0.18 |
| | 32 °C | 1.25 ± 0.12 |
| | 32 °C + LY | Not detectable |
| Experiment 3 | 39 °C | 1.5 ± 0.15 |
| | 32 °C | 2.6 ± 0.5 |
| | 32 °C + LY | Not detectable |

**Fig. 2. The effect of the PI3K inhibitor LY on v-Abl PTK-mediated cell survival.** IC2.9 (A and B) or IC.DP (C and D) cells were preincubated overnight at 39 °C, washed free of IL-3, and plated with or without LY at 32 or 39 °C. Cell viability was assessed every 24 h by trypan blue exclusion over a period of 3 days. Results shown are mean ± S.E. of three experiments.
PKB, we reasoned that this pathway may play a role in the up-regulation of Bcl-XL by v-Abl. As expected, switching on v-Abl PTK activity by culturing cells at the permissive temperature (32 °C) for 6 h resulted in an approximately 2-fold increase in Bcl-XL expression compared with cells cultured at the restrictive temperature (39 °C). This increase in Bcl-XL expression was completely blocked by including LY in the cultures such that the levels of Bcl-XL expression were equivalent to those in cells maintained at the restrictive temperature (Fig. 5). Thus PI3K and PKB may play a role in the up-regulation of Bcl-XL expression, leading to enhanced cell survival.

Role of PKB Activation in v-Abl-mediated Cell Survival—Retroviral mediated transfer of a dominant negative form of PKB was carried out in order to determine whether enhanced PKB activity was required for Abl-induced cell survival. Transfection of IC.DP cells resulted in a reduction in Abl-stimulated PKB activity at 32 °C to 55 ± 5% (n = 2, mean ± range) of the activity in control cells transfected with vector only. This reduction in activity was mirrored by a decline in Abl PTK-stimulated survival in the absence of IL-3 (Fig. 6). Expression of the dominant negative PKB reduced the viability of IC.DP cells at 32°C to 57 ± 11% (n = 3, mean ± S.E.) that observed in the control cells. Thus, Abl-induced activation of PKB is required for the anti-apoptotic effects of this oncogene.

Mechanisms for the v-Abl PTK-mediated Activation of PI3K—As shown in Fig. 1 v-Abl activation leads to the increase of PI3K activity associated with anti-phosphotyrosine immunoprecipitates. This finding lead us to investigate the molecular mechanisms involved in the activation of PI3K by Abl PTK. The adapter protein SHC is known to be associated with PI3K in some v-Abl- and Bcr-Abl-transformed cells (12). In IC.DP cells, IL-3 stimulation resulted in phosphorylation of SHC, and we have previously demonstrated that Abl PTK also stimulates phosphorylation of SHC on tyrosine residues (17). By using antibodies raised against SHC, we can demonstrate that SHC protein co-immunoprecipitates with the p85 subunit of PI3K in IC.DP cells at 39 and 32 °C. However, this association is constitutive (i.e. independent of temperature), and the associated PI3K is inactive at both the restrictive and permissive temperatures (Fig. 7D). Thus in IC.DP cells, enhanced activity of PI3K in response to v-Abl PTK is not mediated directly through enhanced interaction with this phosphorylated adapter

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FIG. 3. v-Abl-mediated activation of PKB isoforms. ts-v-Abl-transfected IC.DP cells were preincubated at 39 (A) or 32 °C (B), washed to remove horse serum and IL-3, starved for 4 h and transferred to 32 °C for 0–18 h (A) or 39 °C for 0–2 h (B). Cells were lysed, and cell lysates were immunoprecipitated using PKB isoform-specific antibodies pre-coupled to protein G beads. PKB activities were assayed as described under “Experimental Procedures.” Results are mean ± S.D. of triplicates from a representative of three experiments.

FIG. 4. Expression of PKB isoforms in IC.DP cells and the effect of LY on PKB activity. ts-v-Abl-transfected IC.DP cells were preincubated at 39 °C, washed to remove horse serum and IL-3, starved for 4 h, and transferred to 32 °C for 2 h in the presence and absence 20 μM LY. Cells were lysed, and cell lysates were immunoprecipitated using PKB isoform-specific antibodies pre-coupled to protein G beads. PKB activities were assayed as described under “Materials and Methods” (A). Cell lysates from cells in the absence of LY were also loaded onto a 10% SDS-PAGE gel and blotted onto polyvinylidene difluoride membrane. The immunoblot was probed with PKB isoform-specific antibodies (B).

2 D. Xenaki and P. J. Owen-Lynch, unpublished observations.
Bcl-X\textsubscript{L}. The figure shown is from a representative experiment of three.

![Image](85x449 to 261x601)

**FIG. 5.** The effect of LY and v-Abl on Bcl-X\textsubscript{L} expression. IC.DP cells were preincubated at 39 °C, washed to remove horse serum and IL-3, starved for 4 h, and transferred to 32 °C for 2 h in the presence (C) and absence (B) of 20 \( \mu \)M LY. Control cells were maintained at 39 °C over the same period (A). Cells were lysed, cell lysates were loaded onto a 10% SDS-PAGE gel and blotted onto polyvinylidene difluoride membrane. The immunoblot was probed with antibody to Bcl-X\textsubscript{L}. The figure shown is from a representative experiment of three.

**FIG. 6.** Effect of dominant negative PKB expression on Abl-induced cell survival. IC.DP cells were transfected with retrovirus containing the dominant negative PKB construct as described. 24 h post-infection, cells were washed free of IL-3 and plated in the absence of added growth factor at 32 °C. Cell viability was assessed every 24 h by trypan blue exclusion over a period of 2 days. Results shown are from a representative of three experiments.

**Fig. 7**

By using immunoprecipitation and Western blotting approaches, we found that, in common with the results obtained with Shc, Cbl and p85 were constitutively associated, i.e. the amount of p85 associated with Cbl did not increase following IL-3 stimulation or Abl activation (Fig. 7C). To provide further evidence of the association between p85 and Cbl, anti-Cbl immunoprecipitates were assayed for PI3K activity. As shown in Fig. 7D, v-Abl activation resulted in a 1.5-fold increase in the activity of the fraction of PI3K associated with Cbl.

![Image](212x613 to 554x729)

Similarly, the PI3K activity associated with anti-Abl immunoprecipitates was examined. V-Abl activation resulted in an approximately 2-fold increase in PI3K activity associated with the Abl protein. However, again, activation of the Abl PTK did not affect the levels of p85 associated with v-Abl, i.e. a fraction of the cellular p85 is constitutively associated with Abl in a temperature-independent manner, and increased activity of Abl PTK leads to enhanced activity of this fraction.

**Discussion**

v-Abl protein-tyrosine kinase potently suppresses apoptosis, induced by cytokine withdrawal, in various hemopoietic cell lines, including pre-mast cells (1, 16, 17). The molecular mechanisms involved in the suppression of apoptosis by the v-Abl protein-tyrosine kinase are not yet clear. By using the IC.DP cell line model, where Abl stimulates survival but not proliferation upon growth factor removal from the cells, we have investigated the role of PI3K in the anti-apoptotic effects of this oncogene.

We have now confirmed, in agreement with others (12, 21–23), that activation of PI3K by Abl PTK does occur and, more importantly, by using our novel mass assay for PIP\textsubscript{3}, that this activation does result in an increase in the mass of the product PIP\textsubscript{3} in the cells. This result is important. Previously due to radiolabeling difficulties in these and other hemopoietic cells, it was not possible to demonstrate that in vitro PI3K activity measurements did correlate with an increase in the amount of the PIP\textsubscript{3} second messenger *in vivo*.

Recently there have been a number of reports examining the effects of PI3K inhibition on cell survival. It was noted that PI3K was important for cell survival of MC-9 mast cells when they were grown in stem cell factor or IL-3 (7), and PI3K is important for the survival of PC12 cells, a model system for sympathetic neurones (31). By using the PI3K inhibitor LY, we found that the effect of v-Abl on cell survival in the absence of IL-3 can be inhibited by preincubating cells with LY. Thus PI3K is necessary for the suppression of apoptosis induced by the oncogene v-abl.

Elevated cellular levels of the second messenger PIP\textsubscript{3} lead to activation of PKB, another protein kinase that has been shown to be important as the downstream effector of PI3K leading to cell survival (see Introduction). PKB is activated following v-Abl PTK activation in IC.DP cells, and this PKB activity is dependent on PI3K activity. Of the three isoforms of PKB assessed in this study, only PKB\textalpha and PKB\textbeta are expressed in IC.DP cells. Abl PTK activates both of these isoforms (up to a 6-fold enhancement of activity) in a PI3K-dependent manner.

Several reports examining the signal transduction pathways leading to growth factor-induced survival of hemopoietic cells (35–37) and aberrant survival in cancer cells (38) have suggested that activation of PKB is not a necessary component of survival signaling. Our experiments using expression of a dominant negative PKB to reduce the level of PKB activation in response to the Abl PTK demonstrate that activation of PKB is required for Abl-mediated survival effects.

The mechanism of PKB-mediated survival has been partly elucidated in other cell systems. In growth factor-stimulated cells, activated PKB phosphorylates the pro-apoptotic protein, BAD (39, 40). This protein, in its dephosphorylated form, constitutively associates with the Bcl-2 family member, Bcl-X\textsubscript{L}, and induces apoptosis of some cells. However, once phosphorylated by PKB at Ser-136 BAD dissociates from Bcl-X\textsubscript{L}, and Bcl-X\textsubscript{L} is free to exert its anti-apoptotic effect (39, 40). In oncogenic transformation by Abl PTKs and other oncogenes, increases in the levels of anti-apoptotic Bcl-2 family members are often observed (12, 41). We have now demonstrated up-regula-
activity associated with anti-Abl immunoprecipitates at the tyrosine at the permissive temperature is comparable to the contrast, the extent of activation associated with anti-phosphotyrosine immunoprecipitates. In the PI3K activity as compared with the permissive temperature, but this is a very small proportion of the overall activation of the PI3K activity as compared with the fraction of PI3K that is associated with either of these two adapter protein Shc (17) both become tyrosine-phosphorylated in IC.DP cells after v-Abl activation, and both of these can provide us the dominant negative PKB construct.

Our previous work has examined other signal transduction pathways that may be involved in Abl PTK function. In contrast to the results in other cell lines where Abl PTKs stimulate cellular transformation, i.e. they stimulate both survival and proliferation, the ERK1/2 and the JAK-2/signal transducers and activators of transcription pathways are not activated by ABL PTK in IC.DP cells, where only a survival response is observed (17). However, evidence does point to a role for a specific protein kinase C isoform, PKCαII. This protein is translocated to the nucleus upon activation of Abl PTK, and inhibition of PKC reverses the survival response (16). In this paper we have shown that Abl PTK-induced activation of PI3K activity is associated with enhanced cellular levels of the second messenger PIP3, consequent PKB activity, and enhanced levels of Bcl-XL. Both PI3K and PKB are necessary for the anti-apoptotic effects of Abl PTK and thus may play a role in vivo in the growth and survival advantage conferred on leukemia cells by this oncogene. It remains to be determined whether there are any interrelationships between the PKCαII and PI3K/PKB pathways and how these mediate up-regulation of Bcl-XL expression in response to Abl PTK activation.

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