Regulation of the Fas Apoptotic Cell Death Pathway by Abl*

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Relatively little is known about oncogene involvement in the regulation of Fas-mediated apoptosis. Inhibition of Fas-induced cell death by the bcl-2 oncogene has been demonstrated to be only partial. In light of a growing body of evidence for the Abl kinase as a negative regulator of cell death, we sought to determine whether Abl expression could protect against Fas-mediated cell death. To address this question, we utilized two separate strategies. In the first, we expressed human Fas in K562, a chronic myelogenous leukemia cell line, which constitutively expresses bcr-abl and examined the effects of Fas ligation in these cells. Fas-positive K562 transfectants (K562.Fas) were found to be protected against Fas-mediated cell death. However, down-regulation of Bcr-Abl protein levels in K562.Fas cells using antisense oligonucleotides targeted to bcr-abl mRNA rendered these cells highly susceptible to Fas-induced death. In the second approach we utilized a Fas-positive HL-60 cell line, which we transfected with a temperature-regulator of cell death, we sought to determine whether Abl expression could protect against Fas-mediated cell death. To address this question, we utilized two separate strategies. In the first, we expressed human Fas in K562, a chronic myelogenous leukemia cell line, which constitutively expresses bcr-abl and examined the effects of Fas ligation in these cells. Fas-positive K562 transfectants (K562.Fas) were found to be protected against Fas-mediated cell death. However, down-regulation of Bcr-Abl protein levels in K562.Fas cells using antisense oligonucleotides targeted to bcr-abl mRNA rendered these cells highly susceptible to Fas-induced death. In the second approach we utilized a Fas-positive HL-60 cell line, which we transfected with a temperature-sensitive mutant of v-Abl. HL-60.v-Abl mutants were found to be protected from Fas-induced apoptosis at the permissive but not the restrictive temperature for the Abl kinase. Taken together, these observations identify the Abl kinase as a negative regulator of Fas-mediated cell death. Since Abl was also found to block apoptosis mediated by ceramide, a recently proposed downstream effector of the apoptotic pathway initiated by Fas, we propose that Abl exerts its protective effects downstream of the early Fas-initiated signaling events.

Fas (CD95), a 48-kDa cell surface protein belonging to the tumor necrosis factor receptor family (Itoh et al., 1991; Oehm et al., 1992) is expressed in various tissues including thymus, heart, lung, and liver (Watanabe-Fukunaga et al., 1992). Ligation of Fas with anti-Fas antibody (Trauth et al., 1989; McGahon et al., 1995) or its specific ligand (Suda et al., 1993) induces a death signal in many cell types of different hematopoietic origin, including T and B cells (Trauth et al., 1989; Owen-Schaub et al., 1992) and a variety of hematopoietic and nonhematopoietic cell lines (McGahon et al., 1995). Mutations in the fas gene or its specific ligand are responsible for lymphoproliferative disorders in the Ipr and gld mutant mouse, respectively (Watanabe-Fukunaga et al., 1992; Takahashi et al., 1994). A role for Fas in Ca2+-independent T cell-mediated cytotoxicity has also recently been found (Rouvier et al., 1993). Fas-mediated cytotoxic T lymphocyte killing has also been found as an alternative lytic pathway in a perforin-deficient cytotoxic T lymphocyte hybridoma (Walsh et al., 1994) and perforin null mice (Kagi et al., 1994). Activation-induced cell death in T cell hybridomas has recently been demonstrated to proceed via a cell autonomous Fas/Fas ligand interaction (Brunner et al., 1995).

There is a growing body of evidence that identifies the Abl kinase as a negative regulator of apoptosis. Expression of v-abl can confer growth factor independence on several cell types, including mast cells (Pierce et al., 1986) and lymphoid lines (Mathey et al., 1986). Bcr-Abl expression can also render some myeloid lines IL-3-independent (Daley and Baltimore, 1988; Hariharan et al., 1988). More recent studies have shown that expression of the Abl kinase can confer resistance to apoptosis in some cell types. For example, activation of v-abl is associated with the suppression of apoptosis in hematopoietic cells (Evans et al., 1993) and constitutive expression of the p210 Bcr-Abl protein inhibits apoptosis in CML progenitor cells (Evans et al., 1994). In addition, down-regulation of Bcr-Abl protein levels by antisense oligonucleotides targeted to bcr-abl mRNA have been shown to render K562 cells (an apoptosis-resistant CML cell line that expresses a deregulated form of the abl oncogene: bcr-abl) susceptible to apoptosis (McGahon et al., 1994). Moreover, transfection of a temperature-sensitive mutant of v-abl (Kipreos et al., 1987) into the HL-60 human promyelocytic leukemia cell line also renders cells of this line resistant to agents that are normally very effective inducers of apoptosis in these cells, providing further evidence that deregulated Abl kinase activity can confer resistance to apoptotic cell death.

To examine the potential effect of Abl on Fas-initiated cell killing, we adopted a dual approach. First, we transfected K562, a chronic myelogenous leukemia cell line that expresses bcr-abl with a cDNA encoding human fas. Transfected K562 clones were found to be resistant to Fas-mediated cell death. However, down-regulation of Bcr-Abl expression using antisense oligonucleotides corresponding to bcr-abl mRNA rendered these cells susceptible to Fas-induced death. In the second approach we transfected HL-60 (a Fas-positive cell line) with a temperature-sensitive mutant of v-Abl (v-AblR1), HL-60.v-AblR1 transfecteds were found to be protected from Fas-induced apoptosis at the permissive temperature for the Abl kinase. Finally, we demonstrate that Abl can block apoptosis induced by ceramide, a recently proposed downstream effector of the apoptosis pathway initiated by Fas. Our observations

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1 The abbreviations used are: IL, interleukin; CML, chronic myelogenous leukemia; AS, antisense; NS, nonsense; PI, propidium iodide; FITC, fluorescein isothiocyanate; Ab, antibody.
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demonstrate that Abl can act as a negative regulator of Fas-induced cell death and that it exerts its regulatory effects downstream of Fas-initiated ceramide production.

MATERIALS AND METHODS

Cell Culture and Reagents—All cell lines described were grown in RPMI 1640 supplemented with 5% fetal calf serum and 2 mM l-glutamine. Cells were grown at 37 °C in a humidified 5% CO2 atmosphere and were routinely subcultured every 2–3 days. Anti-Fas IgM monoclonal antibody (CH-11) was purchased from Kamiya Biomedical Co. (Thousand Oaks, CA). N-Hexanoyl sphingosine (C8-ceramide) was purchased from Biomol (Plymouth Meeting, PA).

Flow Cytometry and Selection of the K562LcRAS Fas (+) Cell Line—The human cDNA clone APO14.2 was subcloned into the EcoRI site of pLXSN 90 (Miller and Rosman, 1989) by standard techniques. pLXSN-APO-1 (pLcRASN) was electroplated (250 V, 960 microfarads) into the ecotropic retrovirus packaging line i2, which was then co cultured with the amphotropic retrovirus packaging line pA317, for 2 weeks as described (Bestwick et al., 1988). K562 cells expressing CD95/Fas were made by coculture with pPA317-LcRASN cells overnight with 8 μg/ml Polybrene. Infected K562 cells were selected in 1 mg/ml G418 (active concentration) (Life Technologies, Inc.) for 7 days.

For detection of Fas-positive clones, cells (5 × 10^5 cells/well in a 96-well plate) were washed in phosphate-buffered saline, fixed in 70% ethanol on ice, washed, and then incubated in phosphate-buffered saline containing 2% calf serum to block Fc receptors and minimize nonspecific staining. Cells were stained using a 1:50 dilution of a mouse anti-human Fas IgG monoclonal antibody (UB2, Kamiya Biomedical Co.) followed by a goat anti-mouse FITC-conjugated F(ab)2 Ab at a 1:50 dilution. Clones were analyzed on a FACSCan flow cytometer.

Induction of Apoptosis by Fas Ligation—For induction of apoptosis, cells (1 × 10^6) were incubated with anti-Fas IgM monoclonal antibody (clone CH-11, Kamiya Biomedical Co.) at the concentrations indicated in the legend to each figure. After an overnight incubation at 37 °C, Leukostat-stained (Fisher Scientific, Orangeburg, NY) cytocentrifuged preparations were examined for the morphological characteristics of apoptosis using previously defined criteria (Wyllie et al., 1980; McGahan et al., 1995). Typical identifiable apoptotic features included membrane blebbing and chromatin condensation. Apoptosis was quantitated by scoring triplicate fields, with a minimum of 100 cells/field.

Cell death was also quantitated by flow cytometry. The criteria used for assessment of cell death in this case were based on two parameters: changes in light scattering properties of dead cells due to cell shrinkage and increased granularity (Yamada and Ohyama, 1980; Wyllie and Morris, 1982; McGahan et al., 1995), and their permeability to the DNA binding dye propidium iodide (PI). Cells were incubated with 5 μg/ml PI at room temperature and analyzed immediately.

Antisense Treatment of K562.Fas Clones—K562 cells (2.5 × 10^5/ml) were incubated with either 10 μM AS-bcr-abl or 10 μM NS-bcr-abl for a 48-h time period, these conditions having been previously established as being optimal for Bcr-Abl protein depletion (McGahan et al., 1994). Phosphorothioate-derivatized antisense (AS) oligodeoxynucleotides (Framingham, MA) corresponding to the first 18 bases of the transla tion start site of Bcr-Abl were used. A nonsense (NS) sequence of the same base composition of the AS oligodeoxynucleotide was used as a positive control.

Oligodeoxynucleotides were used as follows: AS-bcr-abl: 5'-GCCACCGGGTCACCAT-3'; NS-bcr-abl: 5'-GCCGCTCTGGCC-AAAGC-3'.

Inmunoblot Analysis—For immunoblotting, 30 μg of total cellular lysates were resolved by SDS-polyacrylamide gel electrophoresis and transferred onto an Immobilon filter, as described previously (Martin et al., 1995). The filter was preblocked in PBS-Tween (Tris-buffered saline, 0.1% Tween 20) for 1 h at room temperature. Bcr-Abl protein levels were detected by probing with an anti-Bcr-Abl antibody BE9 (McWhirter and Wang, 1993) at a 1:1000 dilution for 1 h at room temperature. Following wash in PBS-Tween, detection of the bound primary antibody was performed using an enhanced chemiluminescent blotting detection system (Amersham Corp.). The filter was probed with a secondary antibody conjugated to horseradish peroxidase at 1:2000 dilution for 1 h, washed for 1 h, and probed with ECL reagents before exposure to Hyperfilm (Amersham).

Agarose Gel Electrophoresis of Nuclearosomal DNA Fragmentation—A total of 10^6 apoptotic cells or untreated control cells were washed, resuspended in 20 μl of lysis buffer (100 mM Tris, pH 8.0, 20 mM EDTA, 0.8% sodium lauryl sarcosinate), followed by addition of 10 μl of RNase A (10 mg/ml) (Amresco, Cleveland, OH) and incubated at 37 °C for 30 min. 10 μl of proteinase K (20 mg/ml) (Amresco) was then added, and the cells were incubated for 2 h at 50 °C. The resulting DNA was subjected to agarose gel electrophoresis in 1.5% agarose in TAE as described previously (Shi et al., 1992).

RESULTS

Expression of Human Fas in K562 Cells—To explore the effects of the Abl kinase on Fas-mediated cell killing, we chose a CML cell line, K562, which constitutively expresses the Bcr-Abl chimeric protein but is negative for Fas expression (Fig. 1A), and retrovirally infected these cells with a cDNA encoding human Fas. Infected cells were stained by limiting dilution, and clones were screened for Fas expression by flow cytometry. The J urkat cell line (a T cell leukemia line that constitutively expresses Fas), served as a positive control for Fas staining. For the experiments described in this report, we selected a single Fas-positive clone, K562.Fas (Fig. 1A), and a K562 clone that was negative for Fas expression, K562, which acted as a negative control for our experiments (Fig. 1A). All data presented in this paper were obtained using these two clones, while similar results were obtained using an additional clone of each (data not shown).

Resistance of K562 Fas Transfectants to Fas-induced Cell Death—To determine whether K562.Fas cells were susceptible to Fas-mediated cell death, K562 and K562.Fas cells were incubated overnight with various concentrations of anti-Fas IgM Ab and cell viability was then assessed on morphological criteria (% apoptosis) and light scattering properties (% cell death). The flow cytometry criteria chosen for analysis was based on the observed changes in light scattering properties of apoptotic cells as well as their increase in permeability to PI dye during the late stages of apoptosis (Martin et al., 1994).

Using these criteria, we found K562.Fas cells to be resistant to Fas-mediated death (Fig. 1B). In contrast, similar concentrations of anti-Fas were sufficient to completely kill J urkat cells (Fig. 1B). As expected, the Fas-negative K562 cells were also resistant to Fas-induced death under the same incubation conditions (Fig. 1B).

It remained a possibility that the expressed Fas was not capable of activating the apoptotic pathway in transfected cells. Therefore we examined the ability of murine P815 cells infected with the same Fas-expressing retrovirus (P815.Fas) to undergo apoptosis upon Fas ligation. These mastocytoma cells do not express Bcr-Abl protein and express the c-Abl protein at normal levels (data not shown). P815.Fas cells expressed cell surface human Fas at levels that were similar to those of K562.Fas cells (Fig. 1C) and were significantly susceptible to Fas-mediated cell killing under the same incubation conditions used for the K562.Fas transfectants (Fig. 1D).

Antisense Oligonucleotides Directed Against bcr-abl Renders K562.Fas Transfectants Susceptible to Fas-induced Cell Death—We and others (Bedi et al., 1994; McGahan et al., 1994) have previously identified a role for Bcr-Abl in the maintenance of resistance of CML cells to apoptotic cell death, induced by a wide variety of agents. Antisense (AS) oligodeoxynucleotides targeted to the mRNA of bcr-abl (AS-bcr-abl) have been shown to down-regulate Bcr-Abl protein levels in K562 cells (Szylak et al., 1991; Mariat et al., 1993; McGahan et al., 1994) and in turn render these cells susceptible to apoptosis induced by various agents (McGahan et al., 1994). Using a similar approach we sought to determine if Bcr-Abl was responsible for the resistance of K562.Fas transfectants to Fas-induced cell death. As shown in Fig. 2A, Bcr-Abl protein levels were found to be decreased in K562.Fas cells after preincubation of these cells with 10 μM AS-bcr-abl for a period of 48 h, as previously reported (McGahan et al., 1994). In contrast, Bcr-Abl protein levels in K562.Fas clones treated with 10 μM of a nonsense
control (NS-bcr-abl) for the same time period, remained comparable with Bcr-Abl levels in the untreated controls. Actin protein levels remained constant following treatment of K562.Fas cells with either the AS or NS oligonucleotides, confirming that the oligonucleotides used were specific for the bcr-abl mRNA (Fig. 2A). Similarly, we observed AS-bcr-abl-mediated down-regulation of Bcr-Abl protein levels in the parental K562 cells, as previously reported (McGahon et al., 1994, and data not shown).

The effect of lowering Bcr-Abl protein levels in K562 and K562.Fas cells on susceptibility to Fas-mediated cell death was then examined. Untreated, AS-bcr-abl-treated, and NS-bcr-abl-treated K562 and K562.Fas cells were incubated overnight in varying concentrations of anti-Fas, and cell death was assessed as before. K562.Fas cells treated with AS-bcr-abl oligonucleotides underwent extensive cell death upon Fas ligation (Figs. 2B and 3B). Cell death under these conditions was confirmed to be apoptotic by examination of stained-cytospun preparations of the treated cells (Figs. 2B and 3A). In contrast, NS-bcr-abl-treated or untreated cells remained resistant to Fas-mediated apoptosis. AS or NS-bcr-abl treatment alone had no effect on cell viability (data not shown). Furthermore, AS or NS-bcr-abl treatment had no effect on the cell viability of K562 cells upon exposure to anti-Fas (Fig. 2B).

To rule out the possibility that AS or NS-bcr-abl treatment of the Fas transfectants was modulating the levels of Fas expression in these cells, thereby affecting the susceptibility of these clones to Fas-mediated death, cells were examined for Fas expression after treatment with the various oligonucleotides. These experiments confirmed that Fas expression in the K562 and K562.Fas cells remained constant following AS or NS-bcr-abl pretreatment (data not shown).

Expression of a Temperature-sensitive v-Abl Mutant in HL-60 Cells—To determine whether resistance to Fas-induced

FIG. 1. A, expression of human Fas in K562 cells. The parental K562 cells and their transfectants were stained with an FITC-conjugated secondary antibody alone, GAM-FITC (control), or with an anti-Fas Ab followed by a GAM-FITC Ab (anti-Fas), and Fas expression was determined on a FACSscan as described under “Materials and Methods.” B, resistance of K562.Fas transformants to Fas-mediated cell death. K562 (●) and K562.Fas (■) cells (10⁵/ml) were incubated for an 18-h period with varying concentrations of anti-Fas Ab and apoptosis was quantitated using morphological criteria as described under “Materials and Methods.” An independent measure of cell viability as assessed by PI uptake was also performed. The ability of the Fas-positive Jurkat (▲) cell line to undergo Fas-mediated killing under the same culture conditions was also assessed by both morphological criteria and PI uptake. All data presented are averages of triplicate experiments. C, expression of human Fas in murine P815 cells. The parental P815 cells (P815) and their transfectants (P815.Fas) were stained with an FITC-conjugated secondary antibody alone, GAM-FITC, or with an anti-Fas Ab, followed by a GAM-FITC Ab, and Fas expression was determined on a FACSscan as described under “Materials and Methods.” D, dose-dependent killing of P815.Fas transfectants with anti-Fas Ab. P815.WT (■) or P815.Fas (□) transfectants (10⁵/ml) were incubated in 96-well plates for an 18-h period with varying concentrations of anti-Fas Ab, and cell viability was assessed by PI uptake as described under “Materials and Methods.”
death was mediated by the kinase activity of the abl oncogene, we transfected HL-60 cells (a Fas-positive line) (Fig. 4A) with a temperature-sensitive mutant of v-Abl, which is active only at the permissive temperature of 32 °C (Kipreos et al., 1987). This provided a convenient system in which we could modulate the kinase activity of v-Abl and explore the effects of this on Fas-mediated cell death in these cells. Thus HL-60.v-Ablts and vector-transfected cells were incubated overnight in the presence of anti-Fas antibody at the permissive (32 °C) and restrictive (39 °C) temperatures for the kinase, and the effects of Fas ligation on cell death at both temperatures were examined.

Vector-only transformants were extremely susceptible to Fas-mediated death at both temperatures (Fig. 4B), as were HL-60.v-Ablts cells, which were maintained at the restrictive temperature for the Abl kinase (Fig. 4B). However, HL-60.v-Ablts cells were protected from Fas-induced apoptosis at the permissive temperature for v-Abl (Fig. 4B).

As expected, the v-Abl kinase also protected against DNA...
exposed to various concentrations of ceramide then Abl should block these effects. To test whether Abl kinase activity in the HL-60 v-Abl transfectants could protect against apoptosis induced by ceramide. Therefore, HL-60.v-Abl\textsuperscript{TS} cells were incubated with varying concentrations of C\textsubscript{6}-ceramide at permissive and non-permissive temperatures for the Abl kinase. As shown in Fig. 5B, the active Abl kinase provided a partial protection against ceramide-induced apoptosis in HL-60.v-Abl\textsuperscript{TS} transfectants at the permissive temperature. In contrast, HL-60.vector transfectants were found to be extremely susceptible to apoptosis induced by ceramide at both permissive and non-permissive temperatures (Fig. 5B).

**DISCUSSION**

In the present study we examined the effect of the abl oncogene on Fas-mediated apoptosis. K562.Fas transfectants were highly resistant to Fas-induced apoptosis, in contrast to murine P815 cells, which were infected with the identical fas construct. Using antisense oligonucleotides targeted to the mRNA of bcr-abl, we have found that down-regulation of Bcr-Abl protein levels in K-562 Fas cells renders these cells highly susceptible to Fas-mediated apoptosis. This suggests that bcr-abl is responsible for the resistance of these cells to Fas-mediated apoptosis. The concept of bcr-abl as a negative regulator of apoptosis is relatively new. Although this oncogene has been demonstrated to prolong cell survival in various growth factor-dependent myeloid cell lines (Daley and Baltimore, 1988; Harigai et al., 1988), it was not until quite recently that these observations were extended to the study of various apoptosis-inducing stimuli. We have recently shown that bcr-abl is responsible for the resistance of K562 cells to apoptosis induced by a wide variety of chemotherapeutic agents (McGahon et al., 1994). Using a similar antisense strategy Bedi et al. (1994) also demonstrated that constitutive expression of bcr-abl could inhibit the apoptotic death seen in CML myeloid progenitor lines upon IL-3 withdrawal.

The viral homolog of the c-abl gene is a 160-kDa tyrosine kinase, v-Abl (p160) (Abelson and Rabstein, 1970). This v-Abl kinase can also function as a negative regulator of apoptosis. Infection of early myeloid, granulocyte, or primitive lymphoid lines with A-MuLV results in the generation of IL-3-independent lines (Mathey et al., 1986; Pierce et al., 1986; Rovera et al., 1987). Using a temperature-sensitive mutant of v-Abl in which the kinase activity is greatly reduced at 39°C, Kipreos and Wang (Kipreos et al., 1987) demonstrated that the kinase activity of v-Abl is essential for the maintenance of factor-independent proliferation in an IL-3-dependent line. Using the same v-Abl temperature-sensitive mutant, we have demonstrated in this study that HL-60.v-Abl\textsuperscript{TS} transfectants are resistant to Fas-mediated apoptosis at the permissive temperature for the Abl kinase. Moreover, the differential sensitivity to Fas-mediated apoptosis was not due to differential Fas expression at the different temperatures, as was demonstrated by anti-Fas staining. These data provide further evidence that the resistance to Fas-mediated apoptosis is due to Abl kinase activity.

These results are of particular interest given the lack of information concerning oncogene regulation of Fas-mediated apoptosis. To date, Bcl-2 has been the only oncogene implicated in the regulation of Fas-induced apoptosis. Itoh et al. (1993) demonstrated a partial inhibition of Fas-mediated apoptosis in both an IL-3-dependent FDC-PI line and WR9L cells transfected with Bcl-2. We have also observed that the Abl kinase can protect against a wide variety of apoptosis-inducing agents.\textsuperscript{2} Whether these stimuli share a similar signal trans-

\textsuperscript{2} A. J. McGahon, S. J. Martin, R. P. Bissonnette, N. J. Yao, M. Harigae, T. G. Cotter, J. Reed, and D. R. Green, submitted for publication.
duction pathway in the generation of apoptosis is, however, unclear. Since both the Bd-2 and Abl protein products have been demonstrated to protect against Fas-mediated death, one obvious possibility is that Abl may be exerting its anti-apoptotic activity through the induction of Bd-2. However, recent experiments by our group suggest that the anti-apoptotic activity of the v-Abl kinase is independent of Bd-2 activity. These observations may explain the differences in inhibition of Fas-mediated apoptosis induced by Abl and Bd-2, as both repressors are working independently of each other. One other possibility is that Abl and Bd-2 may be acting at different stages of the Fas-mediated signaling pathway that leads to apoptosis.

Elucidation of the signal transduction events triggered by Fas ligation will provide a greater understanding of how Abl may exert its anti-apoptotic effects on Fas-mediated apoptosis. Recent investigations demonstrate that apoptotic signaling through Fas activates an acidic sphingomyelinase (Cifone et al., 1994), which results in sphingomyelin hydrolysis and the generation of ceramide. Ceramide can serve as a second messenger, which in turn can lead to the activation of Ras (Gulbins et al., 1995). Ceramide has been demonstrated to be a potent inducer of apoptosis in many cell types, including U937 cells (J. arvis et al., 1994) and HL-60 cells (Obeid et al., 1993). In the present study we found that both the Bcr-Abl and v-Abl tyrosine kinases inhibit ceramide-induced cell death. Hence, it is likely that Abl is acting downstream of ceramide-signaling events to regulate Fas-induced apoptosis.

These observations lead us to consider how Abl may be signaling to prevent Fas-mediated apoptosis. One possibility arises from studies which indicate a role for protein kinase C activation in Fas-mediated abrogation of IL-3 dependence (Owen et al., 1993). Activation of the v-Abl tyrosine kinase stimulates phospholipase C-mediated breakdown of phosphatidylcholine to generate diacylglycerol, which may then activate protein kinase C. Protein kinase C activation by phorbol esters abolishes apoptosis in response to some stimuli (Tomei et al., 1988; Rajotte et al., 1992). More recently protein kinase C activation has been demonstrated to block ceramide production and ceramide-induced apoptosis (Hainovitz-Friedman et al., 1994). It is possible that Abl may activate protein kinase C to block ceramide-induced apoptosis, lending support to our hypothesis that Abl functions as a downstream regulator of Fas-initiated apoptosis.

Ras activation has also recently been found to be a critical component of the Fas-mediated signaling pathway (Gulbins et al., 1995). Ras is involved in the signaling events during both v-Abl- and Bcr-Abl-mediated transformation (Stacey et al., 1991; Pendergast et al., 1993). Expression of constitutively activated Ras in some cell types can prevent death after withdrawal of trophic support (Guerrero et al., 1986; Rukenstein et al., 1991). Transformation of murine myeloid cells to IL-3 independence by Bcr-Abl is dependent of the activation of p21 Ras (Mandanças et al., 1993). Thus a second possibility is that Abl may activate Ras via an alternative pathway and thereby prevent Fas-mediated Ras activation, thus preventing apoptosis. Bcr-Abl-mediated Ras activation can be achieved by direct interaction with the SH2 domain of the GRB-2 adaptor protein (Stacey et al., 1991; Pendergast et al., 1993), whereas activation of Ras by v-Abl may be achieved by phosphorylation of the SH2-containing protein SHC (Rozakis-Adcock et al., 1992). Experiments involving GRB-2 binding mutants of Abl further demonstrate that Ras activation is necessary step for transformation (Pendergast et al., 1993). Whether Ras activation by Abl is a necessary step in the prevention of Fas-mediated apoptosis remains unclear. In addition, since Abl is a tyrosine kinase and its signal transduction events involve the phosphorylation of many intracellular substrates, including the recently identified CRKL protein (ten Hoeve et al., 1994; Nichols et al., 1994), examination of the intracellular substrates necessary for Abl signaling may yield useful insight into how it is working as a negative regulator of apoptosis.

Finally, our observations lead us to consider the potential role of abl-mediated resistance to Fas-induced apoptosis, in the control of hematopoiesis. Bone marrow transplantation experiments indicate that the turnover of hematopoietic precursor cells are dependent upon Fas/Fas ligand interactions such that Fas-negative hematopoietic cells have a growth advantage (Ettenger et al., 1994). These studies suggest that resistance to Fas-mediated apoptosis may be an important element in proliferation control. The C-abl kinase has also been implicated in the control of hematopoietic cell growth. Evidence that c-Abl function is necessary for normal hematopoiesis includes the demonstrations that homozygous null mutation of murine c-Abl results in lymphopenia and neonatal mortality (Schwartzberg et al., 1991; Tybulewicz et al., 1991). Thus a potential, yet unexplored mechanism for c-Abl-mediated control of hematopoiesis, is that Abl-mediated resistance to Fas-induced apoptosis may serve as a negative regulator of proliferation in hematopoietic cells. Recent studies (Owen-Schaub et al., 1995) have demonstrated that Fas/APO-1 is a target gene for transcriptional activation by p53. K562 cells transfected with a temperature-sensitive p53 mutant show a dramatic up-regulation of Fas at the permissive temperature only. These cells remain resistant to Fas-induced apoptosis, however, lending support to our current observations.

In summary, we have identified the Abl kinase as a negative regulator of Fas-mediated apoptosis. Inhibition of Fas-mediated apoptosis by Abl appears to be downstream of early signaling events mediated by Fas ligation. Knowledge of how the Abl kinase may signal in the prevention of apoptosis remains unclear. Further elucidation of the signaling events involved in Abl-mediated suppression of apoptosis will yield valuable insight into its regulation of Fas-mediated apoptosis.

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