Bcl-xL Functions Downstream of Caspase-8 to Inhibit Fas- and Tumor Necrosis Factor Receptor 1-induced Apoptosis of MCF7 Breast Carcinoma Cells*

(Received for publication, August 7, 1997, and in revised form, October 14, 1997)

Anu Srinivasan, Feng Li, Angela Wong, Lalitha Kodandapani, Robert Smidt, Jr., Joseph F. Krebs, Lawrence C. Fritz, Joe C. Wu, and Kevin J. Tomaselli‡

IDUN Pharmaceuticals, Inc., La Jolla, California 92037

Stimulation of the Fas or tumor necrosis factor receptor 1 (TNFR1) cell surface receptors leads to the activation of the death effector protease, caspase-8, and subsequent apoptosis. In some cells, Bcl-xL overexpression can inhibit anti-Fas- and tumor necrosis factor (TNF)-α-induced apoptosis. To address the effect of Bcl-xL on caspase-8 processing, Fas- and TNFR1-mediated apoptosis were studied in the MCF7 breast carcinoma cell line stably transfected with human Fas cDNA (MCF7/F) or doubly transfected with Fas and human Bcl-xL cDNAs (MCF7/FB). Bcl-xL strongly inhibited apoptosis induced by either anti-Fas or TNF-α. In addition, Bcl-xL prevented the change in cytochrome c immunolocalization induced by anti-Fas or TNF-α treatment. Using antibodies that recognize the p20 and p10 subunits of active caspase-8, proteolytic processing of caspase-8 was detected in MCF7/F cells following anti-Fas or TNF-α, but not during UV-induced apoptosis. In MCF7/FB cells, caspase-8 was processed normally while processing of the downstream caspase-7 was markedly attenuated. Moreover, apoptosis induced by direct microinjection of recombinant, active caspase-8 was completely inhibited by Bcl-xL. These data demonstrate that Bcl-xL can exert an anti-apoptotic function in cells in which caspase-8 is activated. Thus, at least in some cells, caspase-8 signaling in response to Fas or TNFR1 stimulation is regulated by a Bcl-xL-inhibitable step.

Apoptosis is a genetically controlled form of cell death that is conserved from worms to humans (1). A diverse set of stimuli can trigger the apoptotic process in virtually all nucleated cells (1, 2). In some mammalian cells, apoptosis can be triggered by members of the Fas/TNF1 receptor family (3, 4). When activated by receptor aggregation, Fas and TNFR1 induce the activation of a set of cysteine proteases called caspases (5, 6). When activated by diverse stimuli, caspases function in the effector phase of apoptosis by cleaving intracellular protein substrates at specific Asp-X peptide bonds (7, 8). The importance of caspases in mediating Fas- and TNFR1-induced apoptosis has been demonstrated by the ability of both viral and small molecule caspase inhibitors to prevent apoptosis mediated by Fas and TNFR1 signaling (5, 6, 9–13).

The mechanisms by which Fas and TNFR1 stimulation lead to caspase activation are currently being elucidated. In the case of Fas, receptor aggregation either by Fas ligand or by antibody cross-linking induces the formation of a death-inducing signaling complex (DISC) of proteins comprising Fas itself, an adapter protein, FADD, and the inactive, zymogen form of caspase-8 (FLICE, MACH, and MCH5) (14–16). Shortly after formation of the DISC, procaspase-8 is proteolytically cleaved and the active protease, containing p20 and p10 subunits, is released from the DISC (17). A similar DISC, involving TNFR1, TRADD, FADD, and procaspase-8, is thought to mediate TNF-induced apoptosis (15, 18). The ability of certain antiapoptotic viral proteins to specifically inhibit both Fas- and TNFR1-induced apoptosis by targeting the FADD/caspase-8 interaction is consistent with this model (19–21). Once activated, caspase-8 is thought to activate other downstream caspases by proteolytic cleavage of their zymogen forms (16, 22), thus amplifying the caspase signal.

In many cells, overexpression of either Bcl-2 or Bcl-xL inhibits apoptosis induced by a variety of stimuli. Although it is not true in all Fas- and TNFR1-expressing cells (23, 24), Bcl-2 and/or Bcl-xL inhibit Fas- and TNFR1-induced apoptosis in a variety of cell types (13, 25–28). Biochemical studies have demonstrated that Bcl-2 functions upstream of caspase-3 processing and activation in inhibiting Fas-induced Jurkat cell apoptosis (13). Bcl-xL also has been shown to inhibit Fas-induced apoptosis (28). Bcl-2 and Bcl-xL localize in cells to the cytoplasmic membranes of mitochondria, endoplasmic reticulum, and nuclei (29–32). Given the intracellular localization of Bcl-2 family members and the plasma membrane-localized, DISC-associated processing of procaspase-8, we were interested in determining whether Bcl-xL or Bcl-2 inhibit caspase-8 processing during Fas and TNFR1 stimulation. We chose to study MCF7 breast carcinoma cells transfected to express human Fas or doubly transfected to express human Fas and human Bcl-xL. Since MCF7 cells express TNFR1 endogenously, this afforded the opportunity to study both Fas and TNFR1 mediated apoptosis in the same cell. In this study, we demonstrate that, although MCF7 cells overexpressing Bcl-xL are protected from anti-Fas- or TNF-induced apoptosis, caspase-8 is processed with normal kinetics. In addition, Bcl-xL blocks apoptosis induced by microinjection of active caspase-8. Thus, Bcl-xL can block apoptosis in cells in which caspase-8 has been activated.

MATERIALS AND METHODS

Cell Culture and Apoptotic Lysate Preparation—MCF7 cells stably transfected with human Fas cDNA (MCF7/F) or both human Fas and human Bcl-xL cDNAs (MCF7/FB) (kind gifts of Dr. V. Dixit, Genentech, Inc., San Francisco, CA) were grown in RPMI 1640 medium, supple-
mented with 10% fetal bovine serum, 200 units/ml penicillin, 200 μg/ml streptomycin, 200 μg/ml neomycin, and 150 μg/ml hygromycin. Cells at 60–75% confluence were co-treated with either 50 ng/ml anti-Fas (MBL, PanVera Labs, Madison, WI) plus 1 μg/ml cycloheximide or 40 ng/ml TNF (R&D Systems, Minneapolis, MN) plus 1 μg/ml cycloheximide. For TNF treatment, cells were incubated at 37 °C. At various times, cells were harvested by scraping with a rubber policeman and centrifuging at 700 × g. Cells (~20 million/plate) were lysed in 300 μl of lysis buffer (10 mM Hepes, pH 7.4, 42 mM KCl, 5 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA, 0.1 mM EGTA, 0.1 mg/ml pepstatin A, 1 μg/ml leupeptin, 5 μg/ml aprotinin, and 0.5% CHAPS). Following a 30-min incubation on ice, cells were centrifuged at 14,000 × g, and the clear supernatants were used for Western analysis. Protein concentrations of the lysates were measured using the Bio-Rad DC protein determination kit (Bio-Rad).

Preparation of Recombinant Caspase-8—Caspase-8 cDNA with most of the prodomain sequence (corresponding to amino acids 1–212) deleted was cloned into pET21B (Novagen, Madison, WI), transformed into Escherichia coli BL21 (DE3), and expressed as a COOH-terminal 6-His fusion protein. Bacterial cultures grown in LB/ampicillin at 37 °C were induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 4 h at 25 °C, and cell pellets were collected by centrifugation for 10 min at 2000 g. Post-nuclear lysates were made by resuspending the cell pellets in 25 mM Tris, 20 mM NaCl, 0.1% Triton X-100, 0.1 mg/ml lysozyme, and centrifuging at 4 °C (30,000 × g for 40 min). His-tagged caspase-8 was purified from bacterial lysate by nickel chromatography using a Hi-trap column (Pharmacia Biotech Inc.) and eluted with an imidazole gradient buffer (60 mM to 1 M). The protein eluted from the column was found to be processed to p20 and p10 subunits by overnight treatment with 100 mM glycine, pH 2.5 (33). Specificity of the affinity purified caspase-8 antibody was confirmed by Western blotting against a panel of recombinant human caspases (caspase-2, -3, -6, -7, -8, -9, and -10). The caspase-8 antibody strongly recognized the p20 and p10 subunits of caspase-8 and cross-reacted only (caspase-2, -3, -6, -7, -8, -9, and -10). The caspase-8 antibody strongly recognized the p20 and p10 subunits of caspase-8 and cross-reacted only

Caspase Antibodies—To generate antibodies to caspase-8, rabbits were immunized with recombinant caspase-8 prepared as described above. Affinity purification columns were generated by binding denatured caspase-8 to cross-linked 6% beaded agarose through sulfohydroxy groups (Sulfolink Kit, Pierce). Columns were incubated with the immune serum overnight and followed by washing with 10 mM Tris-HCl, pH 7.4, and a high salt buffer (500 mM NaCl in 10 mM Tris-HCl, pH 7.4). Caspase-8 antibodies were eluted using 100 mM glycine, pH 2.5 (33). Specificity of the affinity purified caspase-8 antibody was confirmed by Western blotting against a panel of recombinant human caspases (caspase-2, -3, -6, -7, -8, -9, and -10). The caspase-8 antibody strongly recognized the p20 and p10 subunits of caspase-8 and cross-reacted only with p17, but not the p10, subunit of caspase-3. To generate the monoclonal antibody 1E8, mice were immunized with the peptide CRGTELDCGIDET (corresponding to the COOH terminus of the p20 subunit of human CPP32) conjugated to keyhole limpet hemocyanin through N-hydroxysuccinimide ester linkages. B cells from spleens of mice immunized with 2g/20 myeloma cells. Hybridomas were screened by enzyme-linked immunosorbert assay and Western analysis. Single cell cloning was done by limiting dilution, and IgG was purified from monoclonal supernatants of large scale cultures using a protein G-Sepharose column (33). The 1E8 monoclonal was tested against the panel of recombinant caspases (see above) and found to recognize the p20 and p10 subunits of caspase-3 and -7. The monoclonal antibody to procaspase-3 was purchased from Transduction Laboratories (Lexington, KY).

Caspase Immunoblotting—Cells lysates (50 μg of protein/lane) were resolved by SDS-polyacrylamide gel electrophoresis on 16% gels (Novex, La Jolla, CA) and transferred to Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were blocked in PBS, 0.1% Tween 20 (PBST) + 0.4% casein (1-block, Tropix, Bedford, MA). Blots were incubated in 1 μg/ml primary antibody diluted in PBST/ casein for 1 h. Following three washes in PBST, blots were incubated for 1 h in 1:1,000 dilutions of alkaline phosphatase conjugated goat anti-rabbit IgG or goat anti-mouse IgG (Tropix) in PBST/casein. Blots were then washed twice with PBST, twice in assay buffer (10 mM diethanolamine, pH 9.0, 0.1 mM MgCl₂), and then incubated in 250 μM chemiluminescence substrate CSPD (Tropix) assay buffer and exposed to Biomax film (Eastman Kodak Co.) overnight.

Cytochrome c Immunostaining—MC7/F and MC7/FB cells were plated at 25,000 cells per chamber in 8-well chamber slides (Nunc, Naperville, IL). Cells were treated with 40 ng/ml TNF plus 1 μg/ml cycloheximide for various times. Prior to immunostaining, cells were fixed in 10% formalin in PBS for 15 min, washed with PBS, and stored at 4 °C for up to 24 h. Fixed cells were blocked for 1 h in a buffer containing PBS, 10% normal goat serum, and 0.4% Triton X-100. The cells were then incubated for 1 h in 0.5 μg/ml anti-cytchrome c antibody (clone 6H2.B4, Pharmingen, La Jolla, CA) in a buffer containing PBS, 2% normal goat serum, and 0.4% Triton X-100. Following three washes with PBST, cells were incubated with 1 μg/ml Texas Red conjugated goat anti-mouse IgG (Molecular Probes, Portland, OR). Finally, cells were washed three times with PBST and mounted. Phase contrast and fluorescent images were captured on a Sony CamsEye digital camera using appropriate filters. MC7/F7 cells were scored for apoptosis (phase bright, condensed cells) and for cytochrome c immunolocalization. For each time point, at least 150–250 cells were counted; each time course experiment constituted duplicate or triplicate slides. The data presented are from a single experiment but are representative of results obtained in three experiments.

Cell Microinjection—Cell microinjection was performed using a Nikon Diaphot 300 inverted microscope fitted with an Eppendorf pressure injector (model 5246) and micromanipulator (model 5171). Microinjection needles (about 0.1-μm inner diameter) were made from glass capillaries using a horizontal electrode puller (Sutter Instrument, model P-97) and loaded using Eppendorf microlodacers. MC7/F7 or MC7/F7 FB cells were plated on glass coverslips (Eppendorf) 24 h prior to injection. To identify injected cells, the injectate contained 0.3% solution of dextran conjugated to Texas Red (Molecular Probes) in water. Cells were alone or dye plus recombinant caspase-8 were injected into the cytoplasm of MCF7 cells (pressure, 80 to 100 hPa; time, 0.3 s). Cells were switched into fresh medium immediately after injection. The concentration of caspase-8 in the pipette was 60 ng/ml, equivalent to 3 units/μl of active enzyme. Based on the approximate volume delivered per cell (0.05 pl) (34), the concentration of caspase-8 delivered per cell is estimated to be 3 fg or 1.5 × 10⁻⁷ units of activity. 100–150 cells were injected per condition and cells were examined at various time points by phase contrast or fluorescence microscopy. Apoptotic cells were identified morphologically as round, condensed, phase bright cells. Photomicrographs were prepared from digital images obtained using a Sony CamsEye digital camera.

RESULTS

MC7 breast carcinoma cells stably expressing Fas alone (MC7/F7/F) or Fas plus Bcl-xL (MC7/F7/FB) (35) were treated with anti-Fas/cycloheximide or TNF/cycloheximide or exposed to UV irradiation. As determined by cell rounding and nuclear condensation (Fig. 1A), all three stimuli induced apoptosis in 70–90% of MCF7/F7 cells within 24 h (Fig. 1B). In contrast, about 90% of the cells expressing Bcl-xL were resistant to Fas, TNF, or UV, remaining flat with nuclei that appeared normal by phase contrast or fluorescence microscopy. Apoptotic cells were identified morphologically as round, condensed, phase bright cells. Photomicrographs were prepared from digital images obtained using a Sony CamsEye digital camera.

Downloaded from http://www.jbc.org/ by guest on July 22, 2018
immunolocalization that accompany apoptosis induced by either Fas or TNF-R1 stimulation. In summary, by several criteria (Figs. 1 and 2), heterologous expression of Bcl-xL protects MCF7 cells from Fas- and TNF-R1-induced apoptosis.

Caspase-8 processing following treatment with anti-Fas or TNF was studied by immunoblotting using affinity-purified caspase-8 antibodies that recognize the p20 and p10 subunits, but not the proform, of caspase-8. The specificity of the caspase-8 antibody was determined by immunoblotting of purified recombinant human caspase-2, -3, -6, -7, -8, -9, and -10. The caspase-8 antibody was highly selective for both the p20 and p10 subunits of caspase-8, cross-reacting only with the caspase-3 p20, but not p10, subunit (data not shown). To rule out the possibility that the p20 subunit observed in the MCF7 cells following anti-Fas or TNF treatment (see below) was not caspase-8 p20, caspase-3 expression in MCF7/F cells was determined by immunoblotting with a caspase-3-specific monoclonal antibody. While caspase-3 was readily detected in extracts of Jurkat cells, there was no detectable caspase-3 in extracts of MCF7/F cells (Fig. 3A). Thus, the p20 subunit observed following anti-Fas or TNF treatment corresponds to caspase-8 p20 and not caspase-3 p17.

The p20 and p10 subunits of active caspase-8 were first detectable in lysates of anti-Fas and TNF treated MCF7/F cells at 2 and 4 h post-treatment, respectively (Fig. 3, A and B). Caspase-8 p20 and p10 subunits were detectable out to 8 h following anti-Fas or TNF treatment but declined rapidly thereafter, becoming undetectable by 24 h. Caspase-8 processing previously described in other cell types (17) was similarly transitory but occurred with more rapid kinetics than observed here. Interestingly, caspase-8 processing was not observed in the UV treated MCF7/F cells at any time point following UV irradiation (Fig. 3C). Caspase-8 processing was also not observed following treatment with 1 μM staurosporine (data not shown). Processing of caspase-8 was also studied in MCF7/FB cells following anti-Fas or TNF stimulation. The appearance of caspase-8 p20 and p10 subunits appeared with more rapid kinetics and to the same degree in Bcl-xL-expressing MCF7/FB cells as in the control MCF7/F cells (Fig. 3, A and B). Thus, overexpression of Bcl-xL in MCF7 cells does not block processing of caspase-8, even though it blocks subsequent apoptosis.

Caspase-7 processing was also studied in the MCF7/F and MCF7/FB cells using a monoclonal antibody, 1E8, that recognizes the p20 subunits of both caspase-3 and -7. In MCF7 cells, however, 1E8 recognizes only caspase-7 p20 (Fig. 3, D and E), since caspase-3 is not expressed at detectable levels (Fig. 3G). As described previously for TNF (35), caspase-7 was processed in MCF7 cells in response to anti-Fas, TNF treatment, or UV radiation (Fig. 3, D–F). Caspase-7 p20 was first detected at 4 h and peaked at 16 h following anti-Fas, TNF, or UV (Fig. 3, D–F). In contrast to MCF7/F cells, the appearance of the caspase-7 p20 subunit was substantially attenuated in the MCF7/FB cells following anti-Fas or TNF treatment and completely inhibited following UV irradiation (Fig. 3, D–F). Thus, although caspase-8 is processed normally in MCF7/FB cells, caspase-7 processing is partially inhibited by Bcl-xL.

Caspase-7 processing in response to UV irradiation was completely suppressed in the MCF7/FB cells, whereas in response to anti-Fas or TNF, caspase-7 processing was not completely suppressed (Fig. 3). This suggested that the processed caspase-8 observed in anti-Fas or TNF treated MCF7/FB cells might be enzymatically active and could contribute to the initial processing of some caspase-7 directly, as observed in vitro (16, 22). To determine if the processed caspase-8 in MCF7/FB cells was indeed active, we attempted to measure caspase-8 protease activity in extracts of anti-Fas and TNF-treated cells.
FIG. 2. Bcl-xL prevents changes in cytochrome c immunostaining in response to TNF. A, phase contrast microscopy (panels A, C, E, and G) and corresponding cytochrome c immunostaining (panels B, D, F, and H) of untreated cells (MCF7/F, panels A and B, MCF7/FB, panels C and D) or cells treated with 40 ng/ml TNF/1 μg/ml cycloheximide for 10 h (MCF7/F, panels E and F, MCF7/FB, panels G and H). Filled arrows indicate normal cells showing perinuclear, punctate cytochrome c staining; open arrows indicate apoptotic cells that are immunonegative for cytochrome c. B, time course of cellular cytochrome c redistribution in MCF7/F (squares) and MCF7/FB cells (circles) following treatment with 40 ng/ml TNF, 1 μg/ml cycloheximide. Each point represents the percentage of anti-cytochrome c labeled cells in which the cytochrome c immunolocalization changed from the punctate, perinuclear pattern observed in untreated cells to either a diffuse cytosolic pattern or to an immunonegative one. Each point represents the mean and standard deviation of determinations made on duplicate cultures run in parallel.

FIG. 3. Bcl-xL functions downstream of caspase-8 processing and inhibits processing of caspase-7. A–F, immunoblots of apoptotic lysates from MCF7/F (control) and MCF7/FB (Bcl-xL) cells following treatment for various time points (hrs.) with 50 ng/ml anti-Fas, 1 μg/ml cycloheximide (panels A and D), 40 ng/ml TNF, 1 μg/ml cycloheximide (panels B and E), or 100 mJ/cm² UV radiation (panels C and F) using antibodies to caspase-8 (panels A, B, and C) or caspase-7 (panels D, E, and F). Processed p20 and p10 subunits of caspase-8 (arrows in panels A and B) were detected using affinity purified rabbit polyclonal antibodies generated against recombinant, active caspase-8; processed caspase-7 p20 subunits (arrows in panels D–F) were detected using the 1E8 monoclonal antibody. G, immunoblot of cell lysates from untreated MCF7/F (lane 1) or Jurkat (lane 2) cells with a caspase-3-specific monoclonal antibody. Procaspase-3 (arrow) is observed in the Jurkat, but not the MCF7/F cell lysate. Arrowheads indicate migration of protein molecular mass markers (in kilodaltons).
at various time points. The tetrapeptide Ac-DEVD-AMC is a substrate for caspase-8 (16) as well as other caspases. However, no significant DEVD-cleaving activity was observed in MCF7 cell lysates at any time point following anti-Fas or TNF treatment (data not shown). Our inability to measure caspase-8 enzymatic activity in extracts of MCF7/F cells is probably due to the 100-fold lower rate of catalysis of DEVD-AMC substrate by caspase-8 as compared with caspase-3.2 Although we were unable to measure caspase-8 activity directly even in control MCF7/F cells, we hypothesized that if the processed caspase-8 in MCF7/FB cells was active, the MCF7/FB cells should be resistant to apoptosis induced by intracellular delivery of active caspase-8. Therefore, we compared the ability of caspase-8 to induce apoptosis in MCF7/F and MCF7/FB cells following microinjection of active, recombinant caspase-8. Microinjection of a solution containing 3 units/μl active caspase-8 was nearly completely inhibited (Fig. 4B). Caspase-8-injected MCF7/FB cells remained normal in appearance with a smooth, flattened morphology and normal nuclei (Fig. 4A). Thus, Bcl-xL was capable of inhibiting apoptosis induced by delivery of active caspase-8 into the cell cytosol.

**DISCUSSION**

This study demonstrates that caspase-8 is processed with normal kinetics in MCF7 cells rendered resistant to Fas- and TNFR1-induced apoptosis by overexpression of Bcl-xL. Thus, Bcl-xL can function to block apoptosis in cells in which the membrane proximal caspase-8 has been activated. Consistent with this observation, Bcl-xL inhibited MCF7 cell apoptosis induced by microinjection of recombinant, active caspase-8.

In several Fas-expressing cell types studied thus far (17), caspase-8 is recruited to the membrane DISC following Fas ligation and is proteolytically processed from its 54-kDa zymogen form to an active protease containing p20 and p10 subunits (17). That caspase-8 functions as a component of a similar DISC comprising TNFR1, TRADD, and FADD has been suggested by the ability of a dominant negative mutation of procaspase-8 to inhibit TNF-induced apoptosis (15). The present study demonstrating activation of caspase-8 in response to TNF treatment provides the first direct evidence that this
caspase also functions in the TNFR1 signaling pathway. Interestingly, apoptosis induced by UV irradiation or staurosporine did not lead to caspase-8 processing in MCF7 cells, suggesting that these stimuli engage the apoptotic pathway independently of caspase-8.

Stable expression of Bcl-xL in MCF7 cells greatly attenuated apoptotic changes observed following anti-Fas, TNF or UV treatment. A similar inhibition by Bcl-xL of anti-Fas- and TNF-induced apoptosis has also been observed in MCF7 cells transiently transfected with Bcl-xL and in cells microinjected with purified recombinant Bcl-xL. Our observations place Bcl-xL downstream of activated caspase-8 processing in the Fas- and TNF-induced apoptotic cascade in MCF7 cells. Consistent with this conclusion, Bcl-xL was capable of inhibiting apoptosis induced by microinjection of active caspase-8. Biochemical studies have shown that processed, active caspase-8 is released from the DISC following receptor stimulation (17). Although we did not study Fas-induced DISC formation directly, we would predict that it occurs normally in the Bcl-xL-overexpressing cells. Although the subcellular localization and targets of activated caspase-8 are unknown, caspase-8 microinjected into the cytosol may have access to the targets of endogenous caspase-8. If true, then the caspase-8-injected cells are a good model of the anti-Fas- and TNF-treated cells. In both paradigms, Bcl-xL overexpression curtailed the apoptotic signaling cascade initiated by active caspase-8. A previous study demonstrated that Bcl-xL could inhibit apoptosis in Jurkat cells in which caspase-like activity was not completely suppressed (28). Our data suggest that caspase-8 could have been activated in that Bcl-xL paradigm as well.

How does Bcl-xL prevent apoptosis after the activation of the receptor-associated caspase-8 protease? The target substrates of caspase-8 in cells are unknown. Based on in vitro data, caspase-3, -6, and -7 could be downstream targets of caspase-8 (16, 22), thus propagating the caspase signal. Alternatively, caspase-8 could cleave noncaspase targets, thereby altering their functions (40). Whatever proteins function as caspase-8 targets, overexpression of Bcl-xL must attenuate either cleavage or the consequences of those cleavages. Bcl-xL, like Bcl-2, is thought to exert its antia apoptotic function in association with the intracellular membranes of the mitochondria, endoplasmic reticulum, and nuclei (29–32). We could consider several possible models for Bcl-xL function in the system described herein. Bcl-xL could function to inhibit cytosolic caspase-8 protease activity directly. This would require co-localization of Bcl-xL and caspase-8 proteins, perhaps involving translocation of activated caspase-8 from the plasma membrane to internal membranes. To date, however, neither Bcl-xL nor Bcl-2 have been shown to have caspase inhibitory activity. Alternatively, the key targets of caspase-8 could be physically associated with and regulated by Bcl-xL. Bcl-xL has been shown to interact with several proteins, including Bax (41) and the Caenorhabditis elegans cell death effector, CED4 (42, 43). Evidence for an indirect interaction between Bcl-xL and certain caspases via a mammalian CED4-like molecule has been presented (44). However, whether any Bcl-xL-interacting proteins are targets of caspase-8 is unknown. Bcl-xL might also regulate the availability of a cofactor necessary either for caspase-8 to cleave its substrates or for the consequences of those cleavages to ensue. Recent evidence has suggested that Bcl-2 and Bcl-xL regulate the release of cytochrome c from pre-apoptotic cells (37, 39). In the presence of additional cofactors present in cytosol, cytochrome c is capable of activating caspase-3 (45). In the present study, Bcl-xL had a profound effect on changes in the intracellular immunolocalization of cytochrome c induced by anti-Fas and TNF. If a key function of caspase-8 is to stimulate cytochrome c release from mitochondria and subsequent propagation of the caspase cascade, then Bcl-xL is in the right intracellular compartment to regulate this event. Bcl-xL could accomplish this directly by binding to and sequestering cytochrome c (38) or indirectly as a consequence of its membrane channel-forming ability (46). In either case, Bcl-xL would function upstream of cytochrome c release, as suggested by previous studies (36, 37). The inability of Bcl-xL to inhibit apoptosis induced by direct intracellular microinjection of cytochrome c (47) is consistent with this model.

Regardless of the mechanism of Bcl-xL, our data suggest that caspase-8 signaling in this paradigm is propagated through a Bcl-xL-inhibitable step. This is surprising given the possibility that caspase-8 could activate downstream caspases directly (16, 22). Data in Fig. 3 indicate that, in the Bcl-xL-expressing cells, activation of caspase-8 by anti-Fas or TNF leads to the processing of a small amount of caspase-7, whereas in UV-treated cells, in which caspase-8 is not activated, Bcl-xL completely prevents caspase-7 processing. If caspase-8 directly processes some caspase-7, it appears that this level of processing is below the threshold for inducing marked apoptotic changes in the MCF7/ FB cells. The need for a Bcl-xL-inhibitable amplification of the caspase-8 signal may vary from one cell type to another (23, 24) depending, in part, on the complement of downstream caspases that are present. In this respect, MCF7 cells may be unique in their lack of expression of caspase-3. The model described here provides an opportunity to address this question.

Acknowledgments—We gratefully acknowledge Dr. Vishva Dixit for providing the Fas-transfected and Fas/Bcl-xL-transfected MCF7 cells, Dr. Robert Armstrong for helpful discussions, Teresa Ajin for help with cell culture, Salma Salchi for help with antibody purifications, and Lisa Trout for administrative assistance.

REFERENCES

1. Steller, H. (1995) Science 267, 1445–1449
2. Thompson, C. B. (1995) Science 267, 1456–1462
3. Nagata, S., and Golstein, P. (1995) Science 267, 1449–1455
4. Cleveland, J. L., and Ihle, J. N. (1995) Cell 81, 479–492
5. Enari, S., Hug, H., and Nagata, S. (1995) Nature 375, 78–81
6. Los, M., Van de Craen, M., Penning, L. C., Schenk, H., Westendorp, M., Baeseuerle, P. A., Droge, W., Kramer, P. H., Fiers, W., and Schulte-Osthoff, K. (1995) Nature 375, 81–83
7. Nicholson, D. W., Ali, A., Thornberry, N. A., Vaillancourt, J. P., Ding, C. K., Gallant, M., Gareau, Y., Griffin, P. R., Labelle, M., Lazeznik, Y. A., Munday, N. A., Raja, S. M., Smulson, M. E., Yamin, T.-T., Yu, V. L., and Miller, D. K. (1995) Nature 376, 37–43
8. Lazeznik, Y. A., Kaufmann, S. H., Desnoyers, S., Poirier, G. G., and Earnshaw, W. C. (1994) Nature 371, 346–347
9. Tewari, M., and Dixit, V. M. (1995) J. Biol. Chem. 270, 3255–3260
10. Bump, N. J., Hackett, M., Hugunin, M., Seshagiri, S., Brady, K., Chen, P., Ferenz, C., Mankovich, J., Shi, L., Greenberg, A. H., Miller, L. K., and Wong, W. W. (1995) Science 269, 1885–1888
11. Tewari, M., Quinn, L. G., O’Rourke, K., Desnoyers, S., Zeng, Z., Beidler, D. R., Poirier, G. G., Salvesen, G. S., and Dixit, V. M. (1995) Cell 81, 801–809
12. Darmon, A. J., and Bleackley, R. C. (1996) J. Biol. Chem. 271, 21699–21702
13. Armstrong, R. C., Ajin T. N., Xiang, J., Gaur, S., Kreis, J. F., Hoang, R., Bax, I., Korsmeyer, S. J., Karanewsky, D. S., Fritz, L. C., and Tomaselli, K. J. (1996) J. Biol. Chem. 271, 16850–16853
14. Muzio, M., Chinnaiyan, A. M., Kischkel, F. C., O’Rourke, K., Shevchenko, A., Ni, J., Scaffidi, C., Bretz, J. D., Zhang, M., Gentz, R., Mann, K., Krammer, P. H., Peter, M. E., and Dixit, V. M. (1996) Cell 85, 817–827
15. Boldin, M. P., Goncharov, T. M., Golsteyn, Y. V., and Wallach, D. (1996) Cell 85, 803–815
16. Sriramusvala, S. M., Ahmad, M., Fernandez-Alnemri, T., Liwack, G., and Alnemri, E. S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14486–14491
17. Medema, J. P., Scaffidi, C., Kischkel, F. C., Shevchenko, A., Mann, M., Krammer, P. H., and Peter, M. E. (1997) EMBO J. 16, 2794–2804
18. Hsu, H., Shu, H.-B., Pan, M.-G., and Goeddel, D. V. (1996) Cell 84, 299–308
19. Bertin, J., Armstrong, R. C., Ozturk, S., Martin, D. A., Wang, Y., Banko, S., Wang, G. H., Senkevich, T. G., Alnemri, E. S., Moss, B., Lenardo, M. J., Tomaselli, K. J., and Cohen, J. I. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1172–1176
20. Thome, M., Schneider, P., Hofmann, K., Fickenseher, H., Meinel, E., Neipel, F., Mattmann, C., Burns, K., Bodmer, J.-L., Schroter, M., Scaffidi, C., Krammer, P. H., Peter, M. E., and Dixit, V. M. (1997) J. Biol. Chem. 272.
22. Muzio, M., Salvesen, G. S., and Dixit, V. M. (1997) J. Biol. Chem. **272**, 2952–2956
23. Huang, D. C., Cory, S., and Strasser, A. (1997) Oncogene **14**, 405–414
24. Erhardt, P., and Cooper, G. M. (1996) J. Biol. Chem. **271**, 17601–17604
25. Chinnaiyan, A. M., Orth, K., O’Rourke, K., Duan, H., Poirier, G. G., and Dixit, V. M. (1996) J. Biol. Chem. **271**, 4573–4576
26. Lee, R. K., Spielman, J., and Podack, E. R. (1996) Int. Immunol. **8**, 991–1000
27. Mandal, M., Maggirwar, S. B., Sharma, N., Kaufmann, S. H., Sun, S.-C., and Kumar, R. (1996) J. Biol. Chem. **271**, 30534–30539
28. Boise, L. H., and Thompson, C. B. (1997) Proc. Natl. Acad. Sci. U. S. A. **94**, 3559–3674
29. Hockenbery, D. M., Nunex, G., Milliman, C., Sereiber, R. D., and Korsmeyer, S. J. (1990) Nature **348**, 334–336
30. Krajewski, S., Tanaka, S., Takayama, S., Schibler, M. J., Fenton, W., and Reed, J. C. (1993) Cancer Res. **53**, 4701–4714
31. Lithgow, T., van Driel, R., Bertram, J. F., and Strasser, A. (1994) Cell Growth Differ. **3**, 411–417
32. Gonzalez-Garcia, M., Perez-Ballester, R., Ding, L., Duan, L., Boise, L. H., Thompson, C. B., and Nunez, G. (1994) Development **120**, 3033–3042
33. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 285–317, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
34. Minaschek, G., Bereiter-Hahn, J., and Berthold, G. (1989) Exp. Cell Res. **183**, 434–442
35. Duan, H., Chinnaiyan, A. M., Hudson, P. L., Wing, J. P., He, W.-W., and Dixit, V. M. (1996) J. Biol. Chem. **271**, 1621–1625
36. Yang, J., Liu, X., Bhalla, K., Kim, C. N., Ibrado, A. M., Cai, J., Peng, T.-I., Jones, D. P., and Wang, X. (1997) Science **275**, 1129–1132
37. Kluck, R. M., Bossey-Wetzel, E., Green, D. R., and Newmeyer, D. D. (1997) Science **275**, 1132–1136
38. Kharbanda, S., Panday, P., Schofield, L., Iareals, S., Roncineske, R., Yoshida, K., Bharti, A., Yuan, Z.-M., Saxena, S., Weichselbaum, R., Nalin, C., and Kufe, D. (1997) Proc. Natl. Acad. Sci. U. S. A. **94**, 6939–6942
39. Kim, C. N., Wang, X., Huang, Y., Ibrado, A. M., Liu, L., Fang, G., and Bhalla, K. (1997) Cancer Res. **57**, 3115–3120
40. Rudel, T., and Bokoch, G. M. (1997) Science **276**, 1571–1574
41. Seldlak, T. W., Olvai, Z. N., Yang, E., Wang, K., Boise, L. H., Thompson, C. B., and Korsmeyer, S. J. (1995) Proc. Natl. Acad. Sci. U. S. A. **92**, 7834–7838
42. Wu, D., Wallen, H. D., and Nunez, G. (1997) Science **275**, 1126–1129
43. Ottolie, S., Wang, Y., Banks, S., Chang, J., Vigna, N. J., Weeks, S., Armstrong, R. C., Fritz, L. C., and Oltersdorff, T. (1997) Cell Death Differentiation **4**, 526–533
44. Chinnaiyan, A. M., O’Rourke, K., Lane, B. R., and Dixit, V. M. (1997) Science **275**, 1122–1126
45. Liu, X., Kim, C. N., Yang, J., Jemmerson, R., and Wang, X. (1996) Cell **86**, 147–157
46. Minn, A. J., Velez, P., Schendel, S. L., Liang, H., Muchmore, S. W., Fesik, S. W., Fill, M., and Thompson, C. B. (1997) Nature **385**, 353–357
47. Duckett, C. S., Li, F., Wang, Y., Tomaselli, K. J., Thompson, C. B., and Armstrong, R. C. (1997) Mol. Cell. Biol. **18**, 608–615
Bcl-xL Functions Downstream of Caspase-8 to Inhibit Fas- and Tumor Necrosis Factor Receptor 1-induced Apoptosis of MCF7 Breast Carcinoma Cells
Anu Srinivasan, Feng Li, Angela Wong, Lalitha Kodandapani, Robert Smidt, Jr., Joseph F. Krebs, Lawrence C. Fritz, Joe C. Wu and Kevin J. Tomaselli

J. Biol. Chem. 1998, 273:4523-4529.
doi: 10.1074/jbc.273.8.4523

Access the most updated version of this article at http://www.jbc.org/content/273/8/4523

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 27 of which can be accessed free at http://www.jbc.org/content/273/8/4523.full.html#ref-list-1