Differential Involvement of Initiator Caspases in Apoptotic Volume Decrease and Potassium Efflux during Fas- and UV-induced Cell Death*

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Caspase activation and apoptotic volume decrease are fundamental features of programmed cell death; however, the relationship between these components is not well understood. Here we provide biochemical and genetic evidence for the differential involvement of initiator caspases in the apoptotic volume decrease during both intrinsic and extrinsic activation of apoptosis. Apoptosis induction in Jurkat T lymphocytes by Fas receptor engagement (intrinsic) or ultraviolet (UV)-C radiation (extrinsic) triggered the loss of cell volume, which was restricted to cells with diminished intracellular K⁺ ions. These characteristics kinetically coincided with the proteolytic processing and activation of both initiator and effector caspases. Although the polycaspase inhibitor benzylxycarbonyl-Val-Ala-Asp fluoromethyl ketone completely inhibited the Fas-mediated apoptotic volume decrease and K⁺ efflux, it was much less effective in preventing these processes during UV-induced cell death under conditions whereby caspase activities and DNA degradation were blocked. To define the roles of specific initiator caspases, we utilized Jurkat cells genetically deficient in caspase-8 or stably transfected with a dominant-negative mutant of caspase-9. The results show that the activation of caspase-8, but not caspase-9, is necessary for Fas-induced apoptosis. Conversely, caspase-9, but not caspase-8, is important for UV-mediated shrunken morphology and apoptosis progression. Together, these findings indicate that cell shrinkage and K⁺ efflux during apoptosis are tightly coupled, but are differentially regulated by either caspase-8 or caspase-9 depending on specific pathways of cell death.

Apoptosis is a programmed form of cell death whose execution depends on the complex interplay of intracellular signaling molecules. This cell death is regulated by mechanisms that are both signal- and cell type-specific. During apoptosis, the cell undergoes a series of conserved biochemical and morphological changes, including the loss of cell volume, a defining morphological feature that distinguishes apoptosis from necrosis (1–3). This process, also termed apoptotic volume decrease (4), is an early regulatory event that is essential for successful apoptosis progression (3–5). The signaling components modulating this event remains largely undefined, although previous studies have shown that cell shrinkage is associated with a dramatic loss of intracellular K⁺ and Na⁺ ions (2, 6, 7). Moreover, the efflux of K⁺ ions and the loss of cell volume correlate with several features of apoptosis, including mitochondrial membrane depolarization (8), activation of DEVDases and apoptotic nucleases (7), DNA degradation (5, 7), and ribosomal RNA degradation (9).

In addition to cell shrinkage, an incipient feature of many apoptotic paradigms is the activation of caspases. These proteases initiate and amplify a signaling proteolytic cascade that ultimately cleaves a variety of cellular substrates and facilitates cellular disassembly (10–12). Since caspase activation and the loss of cell volume are critical for execution of apoptosis, we wished to determine whether activation of initiator caspases is necessary for cell shrinkage during apoptosis. We explored the effects of Fas ligation and ultraviolet-C (UV-C) radiation, two distinct stimuli known to activate the extrinsic and intrinsic pathways of apoptosis, respectively. Activation of the Fas receptor by its cognate ligand or agonistic antibody ligation triggers clustering and/or trimerization of the receptor, interaction with adaptor molecules such as Fas-associated death domain adaptor protein, and the recruitment and activation of the initiator pro-caspase-8 (13–15). This death inducing signaling complex in turn activates a sequential caspase cascade, in which caspase-3 functions as a key effector caspase. In contrast, apoptosis induced by UV radiation entails a more complex system that in part involves signaling through DNA damage and cell surface receptors (16–18). One convergent point in these pathways is the mitochondrial release of cytochrome c that facilitates the formation of the apoptosome, which includes Apaf-1 and pro-caspase-9 (19–22). Subsequent processing of pro-caspase-9 to its active form initiates a downstream signaling cascade through multiple effector caspases, including caspase-3, -6, and -7 (23–25).

Here we examined the relationship between cell shrinkage and caspase activation in Jurkat T cells during Fas- and UV-mediated apoptosis. We show that the apoptotic volume decrease induced by both signals is tightly coupled to K⁺ efflux, but the relative contribution of the initiator caspase-8 and -9 to the loss of cell volume and K⁺ ions varies depending on the specific apoptotic pathway that is activated.

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1 The abbreviations used are: UV-C, ultraviolet-C; z-VAD-fmk, benzylxycarbonyl-Val-Ala-Asp fluoromethyl ketone; z-IETD-fmk, z-Ile-Glu-Thr-Asp-fmk; Ac-DEVD-pNA, N-acetyl-Asp-Glu-Val-Asp-p-nitroaniline; Ac-IETD-pNA, Ac-Ile-Glu-Thr-Asp-p-nitroaniline; Ac-LEHD-pNA, Ac-Leu-glu-His-Asp-p-nitroaniline; PI, propidium iodide; DN, dominant-negative.
Materials and Methods

Cell Lines—Jurkat T lymphocytes (clone E6.1) were obtained from American Type Culture Collection (Manassas, VA). Jurkat cells (clone I9.2) genetically deficient in caspase-8 and the parental wild-type cells (clone 4A5) were kindly gifts from Drs. P. F. J. and J. Blenis (Harvard Medical School, Cambridge, MA). Jurkat cells stably expressing a dominant-negative (DN) mutant of caspase-9 were generated from the parental clone E6.1 by electroporation (4-mm cuvette, 230 V, 960 microfarads using a Bio-Rad Gene Pulser) with either the empty vector pcDNA3 or pcDNA3-DN Casp9-FLAG (a kind gift of Y. Lazebnik, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY). Clones derived from each cell line were then isolated after single-cell sorting using a FACSVantage SE flow cytometer (Becton Dickinson, San Jose, CA) and selected for neomycin resistance. All cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 75 units/ml streptomycin, and 100 units/ml penicillin. Cells were maintained at 37 °C in a humidified atmosphere containing 7% CO2.

Apoptosis Induction—Cell cultures grown at 5 × 105 cells/ml in complete medium were treated with 20 ng/ml anti-human Fas IgM (clone CH-11, Kamiya Biomedical, Seattle, WA) or irradiated with 60 mJ/cm2 UV-C using a Stratalinker 1800 (Stratagene, La Jolla, CA). In experiments involving the caspase inhibitors, benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (z-VAD-fmk) or z-Ile-Glu-Thr-Asp-fmk (z-IETD-fmk) (Kamiya Biomedical, Seattle, WA), cells were pre-incubated with the inhibitors for 30 min prior to apoptosis induction.

Flow Cytometric Analysis of Cell Size and Viability—Changes in cell volume were examined on a FACSort flow cytometer (Becton Dickinson, San Jose, CA) as described previously (28). The distribution of cell size is represented by forward scatter versus cell number histograms, and gates were set based on control samples to distinguish normal and shrunken cells. The percentage of shrunken cells was determined by statistical analysis of the gated region using CellQuest software. For determination of cell viability, cells were stained with 10 μg/ml propidium iodide (PI, Sigma) for 5 min prior to flow cytometric analysis, and PI-positive cells were considered non-viable.

Immunoblot Analysis of Caspase Processing—Cytosolic extracts were prepared as described above. Fifty μg of the extracts were denatured in Laemmli loading buffer at 99 °C for 5 min, and separated by electrophoresis in a 4–20% SDS-PAGE gel (Novex, San Diego, CA). The proteins were transferred to nitrocellulose membrane for immunoblotting as described previously (27), except that incubation with primary antibodies were performed at room temperature for 1 h. Specific monoclonal antibodies against human caspase-3 (Calbiochem) or -6 (Calbiochem) were used at 1:1000 dilution, and specific protein complexes were detected using ECL reagents (Amer sham Pharmacia Biotech). Immunoblotting of the caspases were performed sequentially on a single membrane with intermediate stripping using 62.5 mM Tris-HCl, pH 6.8, 2% SDS, and 100 mM 8-mercaptoethanol for 20 min at 52 °C.

Results

The Loss of Cell Volume Is Tightly Coupled to K+ Efflux and Correlates with Caspase Activation—We have examined the kinetics of cell shrinkage during apoptosis induced by anti-Fas antibody or UV-C radiation by flow cytometry, which can distinguish cells of different sizes based on light scatter. A decrease in the amount of forward-scattered light correlates with cell shrinkage (26). Fig. 1A shows the volume distribution histo-
grams of control cells and those induced to undergo apoptosis with either anti-Fas antibody or UV radiation. After 10 h, the treated cells displayed a marked and progressive decrease in forward scatter. Gates were drawn based on control cells as shown in Fig. 1A to designate cells with a normal size and those that were shrunken. Additionally, both the normal and shrunken cells were individually sorted following apoptotic induction for morphological analysis. Fig. 1B shows that cells derived from the “normal” gate displayed a well rounded morphology that was distinct from the smaller cells collected from the “shrunken” gate. We used the flow cytometric plots to determine the percentage of shrunken cells in the first 10 h following apoptosis activation, during which over 95% of cells were PI-negative. Treatment of Jurkat cells with either anti-Fas antibody or UV radiation induced both a time-dependent (Fig. 1C) loss of cell volume. In both cases, the apoptotic volume decrease was stochastic and occurred as early as 1–2 h after treatment.

We next examined the relationship between the apoptotic volume decrease and intracellular K\(^+\) in both anti-Fas- and UV-treated Jurkat cells. Fig. 2 shows the normal forward scatter and intracellular PFBFI (K\(^+\)) fluorescence in control cells. In contrast, treatment with either anti-Fas or UV induced cell shrinkage and a concomitant decrease in PFBFI (K\(^+\)) fluorescence, indicating that the shrunken population of cells had reduced intracellular K\(^+\). Thus, a direct relationship between cell size and intracellular K\(^+\) is observed with both apoptotic stimuli.

To determine whether the loss of cell volume induced by anti-Fas antibody or UV correlated with the activation of caspases, we measured the kinetics of caspase activation based on the in vitro release of p-nitroaniline (pNA) from the peptide substrates Ac-IETD-pNA, Ac-LEHD-pNA, or Ac-DEVDE-pNA. The former is most selective for caspase-8, -10, -6, and -1, whereas Ac-LEHD-pNA has a preference for caspase-9, -4, and -5, and Ac-DEVDE-pNA is cleaved by several caspases including caspase-3, -8, -7, -10, -6, and -1 (28). The results in Fig. 3 show that both Fas receptor ligation and UV radiation caused significant increases in IETDase, LEHDase, and DEVDase activities that, similar to the apoptotic volume decrease, commenced at 1–2 h and reached apparent maximal values during 6–8 h after treatment. Although the anti-Fas- and UV-induced activation of IETDases and LEHDases were comparable with maximal increases of ~4-fold, a more robust activation of at least 15-fold was observed when Ac-DEVDE-pNA was used as a substrate, suggesting an amplification effect of certain DEVDases in the caspase cascade.

Given that activation of caspases is associated with the proteolytic processing of these enzymes (10–12), we examined the cleavage of caspase-8, -9, and -3. Results from Western blot analyses show that apoptosis induction by either anti-Fas antibody or UV radiation triggered a time-dependent loss of procaspase-8 and the formation of two cleavage fragments (Fig. 3). Similarly, proteolytic processing was also observed with procaspase-9. The cleavage of these caspases was first detectable at 2 h and corresponded with the increased caspase activity and apoptotic volume decrease. In contrast, processing of procaspase-3 appeared significantly delayed until 4 h following both anti-Fas and UV treatments. Collectively, these data show that treatment of Jurkat cells with these apoptotic stimuli induced a time-dependent loss of cell volume, which was restricted to cells with decreased K\(^+\) levels and temporally correlated with the initial phase of caspase activation.

**Apoptotic Volume Decrease and K\(^+\) Efflux Are Differentially Inhibited by z-VAD-fmk and z-IETD-fmk During Fas- and UV-induced Apoptosis**—To determine if caspases are essential for the loss of intracellular K\(^+\) ions and cell volume, we initially examined the effects of z-VAD-fmk, an inhibitor of many but not all caspases (28–32). The efficacy of z-VAD-fmk was determined by its ability to inhibit caspase activities in Fas- and UV-treated cell extracts. At the lowest concentration tested (10 \(\mu\)M), z-VAD-fmk completely inhibited the Fas- and UV-induced IETDase, LEHDase, and DEVDase activities (Fig. 4A), as well as the processing of caspase-8, -9, and -3 (data not shown). We also used DNA degradation as a known caspase-dependent event of apoptosis (33) to evaluate the functional effectiveness of z-VAD-fmk in intact cells. Fig. 4B shows the complete protection by z-VAD-fmk at 10 or 50 \(\mu\)M against both Fas- and UV-induced DNA degradation. Thus, z-VAD-fmk can effectively block both caspase activation and DNA degradation during either Fas- or UV-induced apoptosis.

We next examined the propensity of z-VAD-fmk to block cell shrinkage and K\(^+\) loss during apoptosis. Although z-VAD-fmk alone had no effect on cell volume, it completely abrogated cell shrinkage and K\(^+\) loss induced by Fas receptor ligation (Fig. 5), but was much less effective against UV radiation even at a concentration 5-fold greater than that required to inhibit caspase activities or DNA degradation (see Fig. 4). It is important to note that z-VAD-fmk, especially at high concentrations, has the potential to inhibit non-caspase proteases such as cathepsins (34) and calpains (35). Nonetheless, these findings implicate an essential role of a z-VAD-sensitive component for the apoptotic volume decrease and K\(^+\) efflux in the Fas pathway, but only a partial involvement in the UV response. We further observed that z-IETD-fmk blocked both the apoptotic volume decrease and K\(^+\) efflux induced by anti-Fas antibody.
(Fig. 6), a finding consistent with the prominent role of caspase-8 in the Fas pathway. However, z-IETD-fmk was ineffective against the UV response. These data suggest that z-VAD- and specifically z-IETD-sensitive proteases are critical for the Fas receptor-mediated apoptotic volume decrease and K<sup>+</sup>/H<sub>11001</sub> loss, but play a relatively minor role in the UV response.

Caspase-8 Is Required for Fas-induced Cell Shrinkage and K<sup>+</sup> Efflux but Is Largely Dispensable in the UV Pathway—Although caspase-8 plays an apical role during the initiation phase of Fas receptor-mediated apoptosis (13–15), the exact role of this caspase in the UV apoptotic pathway is not well understood. Several reports have suggested that UV radiation employs the Fas pathway to induce apoptosis (36–39), and caspase-8 also may be involved in the UV response. To examine this idea, we utilized Jurkat cells genetically deficient in caspase-8 (40).

Immunoblot analyses of cytosolic extracts revealed the absence of caspase-8 in the caspase-8-deficient cells but the presence of caspase-9 and -3 at similar levels to parental wild-type counterparts (Fig. 7A). Upon treatment with anti-Fas antibody, wild-type cells displayed processing of caspase-8, -9, and -3 in a time-dependent manner (Fig. 7A). Under the same conditions, no processing of caspase-9 or -3 was detected in the caspase-8-deficient cells, affirming the initiator role of caspase-8 in the Fas-mediated pathway. Conversely, when the caspase-8-deficient cells were exposed to UV radiation, caspase-9 and -3 were processed to a similar extent as wild-type cells. Therefore, unlike the Fas pathway, processing of caspase-9 and -3 can occur independently of caspase-8 following UV radiation. We extended these findings by examining specific caspase activities in the caspase-8-deficient and wild-type cells. Consistent with the results in Fig. 7A, cytosolic extracts from wild-type...
cells treated with anti-Fas antibody exhibited a profound time-dependent increase in IETDase, LEHDase, and DEVDase activities, whereas no such changes were observed in the mutant cells (Fig. 7B). In contrast, UV radiation induced significant activation of LEHDases and DEVDases in both wild-type and caspase-8 mutant cells. Interestingly, a time-dependent increase in IETDase activity was detected in the caspase-8-deficient cells following UV but not anti-Fas treatment. This UV-induced activity presumably arose from non-caspase-8 IETDases such as caspase-10, -6, and -1. For all caspase activities measured, the extent of UV-induced caspase activation in the mutant cells appeared slightly lower relative to wild-type cells, reflecting a partial contribution of caspase-8 to the caspase cascade.

To determine whether caspase-8 is required for the apoptotic volume decrease and K⁺ efflux. Jurkat cells were pretreated with 0, 10, or 50 μM z-VAD-fmk for 30 min and then exposed to anti-Fas antibody (20 ng/ml) or UV radiation (60 mJ/cm²) for an additional 8 h. Cell volume and intracellular K⁺ levels were determined using flow cytometry as described under “Materials and Methods.” Results shown are the mean ± S.E. from three independent experiments. cells, reflecting a partial contribution of caspase-8 to the caspase cascade.

Fig. 4. Efficacy of z-VAD-fmk as a caspase inhibitor. A, Jurkat cells were pretreated with 0, 10, or 50 μM z-VAD-fmk for 30 min and then left untreated (control) or exposed to anti-Fas antibody (20 ng/ml) or UV radiation (60 mJ/cm²). After an additional 8 h, caspase activities in cell extracts were determined using Ac-IETD-pNA, Ac-LEHD-pNA, and Ac-DEVD-pNA as substrates, and the results shown are mean ± S.E. of at least three separate experiments. B, cells treated as described above were fixed and stained with PI for flow cytometric analysis of DNA content. The cell number versus PI fluorescence histograms show the distribution of cells with normal DNA (to the right of the red line) and sub-diploid DNA content (left of the red line). Representative results are shown from three separate experiments.

Fig. 5. Effect of z-VAD-fmk on anti-Fas- and UV-induced apoptotic volume decrease and K⁺ efflux. Jurkat cells were pretreated with 0, 10, or 50 μM z-VAD-fmk for 30 min and then exposed to anti-Fas antibody (20 ng/ml) or UV radiation (60 mJ/cm²) for an additional 8 h. Cell volume and intracellular K⁺ levels were determined by flow cytometry as described under “Materials and Methods.” Results shown are the mean ± S.E. from three independent experiments.
irradiation, the caspase-8-deficient cells became shrunken in a
time-dependent manner, along with a loss of intracellular K⁺ ions and viability (Fig. 8). These responses were reduced by
~10–30% relative to wild-type cells and were similar to the
lower level of caspase activities observed in Fig. 7. Thus,
caspase-8 may contribute to the UV apoptotic response but is
not necessary for cell shrinkage and K⁺ efflux.

Caspase-9 Is Critical for UV- but Not Fas-induced Cell
Shrinkage, K⁺ Efflux, and Cell Death—Although caspase-8
does not play an obligatory role in the UV response, it is
conceivable that an alternate initiator caspase may play a
prominent role. One candidate is caspase-9, an initiator
caspase of the cytochrome c/Apaf-1-mediated pathway of
caspase activation (19–22). To examine a possible involvement
of caspase-9 in the UV response, we generated Jurkat cells
stably expressing a caspase-9-DN mutant (41). Extracts de-
rived from the caspase-9-DN cells displayed an additional iso-
form of caspase-9 that migrates as a distinct upper band rela-
tive to the wild-type endogenous counterpart (Fig. 9
A). This
band corresponds to the FLAG-tagged caspase-9-DN mutant
(data not shown). Upon Fas activation, the processing of
caspase-9, 8-, and -3 was either unaltered or marginally atten-
In the Fas pathway, the dynamics of the apoptotic volume decrease (Fig. 1) correlated with the initial phases of cleavage and activation of caspase-8 and -9 (Fig. 3). Results from inhibitory experiments further show that the shrunken morphology during Fas-induced apoptosis is highly dependent on the death-inducing stimulus.

We have shown previously that physical cell shrinkage by hyperosmotic stress is sufficient to initiate apoptosis in lymphoid cells (5). This apoptotic volume decrease is associated with the efflux of K⁺ ions, and only the shrunken cells exhibit DNA degradation, disruption of mitochondrial membrane potential, and activation of effector caspases (5–7), suggesting that the loss of cell volume is a necessary element of the apoptotic program. Consistent with this idea, Maeno and co-workers (4) have recently demonstrated that, in different models of cell death, the apoptotic volume decrease occurs prior to DNA degradation, cytochrome c release, and activation of caspase-3-like enzymes. We have now examined the role of initiator caspases in regulating the characteristic loss of cell volume and K⁺ efflux in both intrinsic and extrinsic signaling pathways of apoptosis. We show that the functional involvement of specific caspases in the apoptotic volume decrease is highly dependent on the death-inducing stimulus.

Although Fas receptor ligation represents one way to activate a caspase cascade, an alternate mechanism exists through the release of mitochondrial cytochrome c and subsequent association with Apaf-1 and pro-caspase-9 (19–22), which activates downstream effector caspases (23–25). This caspase cascade can be induced by UV radiation (42). Fig. 3 shows that treatment of Jurkat cells with UV radiation led to proteolytic cleavage and activation of multiple caspases. Additional studies with the caspase-9-DN mutant reveal an important role for caspase-9 in the UV-activated caspase cascade and apoptosis progression (Figs. 9 and 10), consistent with the results of gene knockout studies (42, 43). Interestingly, several groups have proposed UV/ionizing radiation may utilize the Fas pathway to induce apoptosis (36–39, 44). We show here that inhibition of the Fas pathway at the point of caspase-8 only marginally attenuated but did not prevent the UV-induced caspase activation, apoptotic volume decrease, K⁺ efflux, and loss of cell viability. These data are consistent with other reports demonstrating that caspase-8 is not required for UV-induced cell death (40, 45). Thus, caspase-8 may contribute to some levels of the apoptotic machinery, possibly for amplification of the caspase cascade, but is not necessary for the apoptotic response to UV.

Aside from caspase-8 and -9, it appears that z-VAD-sensitive caspases in general do not appear to play a primary role in volume and ionic regulation during UV-induced cell death. Evidence supporting this idea is shown in Fig. 5, where substantial cell shrinkage and K⁺ efflux still occurred in the presence of z-VAD-fmk. Similar z-VAD-insensitive pathways for the apoptotic volume decrease have been observed in Jurkat

**Fig. 8.** Role of caspase-8 in Fas receptor- and UV-mediated cell death. A, wild-type and caspase-8-deficient (Casp-8 (-)) Jurkat cells were treated with anti-Fas antibody (20 ng/ml) or UV radiation (60 mJ/cm²). At the indicated time, cells were directly analyzed by flow cytometry for cell volume (panel A), intracellular K⁺ level (panel B), or loss of cell viability as judged by PI uptake (panel C). Results shown are the mean ± S.E. from three independent experiments.
Fig. 9. Caspase activation and DNA degradation in caspase-9-DN mutant or wild-type cells during Fas- and UV-induced apoptosis. Jurkat cells stably transfected with a DN mutant of caspase-9 (DN-C9) or empty vector (Wt) were left untreated (Con) or treated with anti-Fas antibody (20 ng/ml) or UV radiation (60 mJ/cm²). A, at 6 h after treatment, cell extracts were prepared for immunoblotting using specific antibodies against caspase-9, -8, and -3. **Closed arrows** denote the position of pro-enzymes, whereas **open arrows** indicate the cleaved forms of each caspase. Asterisk denotes the band corresponding to the FLAG-tagged caspase-9-DN mutant. B, cell extracts prepared as described above were used for in vitro caspase activity measurements using LEHD-pNA, IETD-pNA, and DEVD-pNA as substrates. **Black** and **gray bars** represent results from vector (wild type) and caspase-9 DN mutant cells, respectively. Each data point represents the mean ± S.E. of six samples from three separate experiments. C, cells were fixed and stained with PI for flow cytometric analysis of DNA content. The cell number versus PI fluorescence histograms show the distribution of cells with normal DNA (to the right of the red line) and subdiploid or degraded DNA content (left of the red line). Representative results are shown from three separate experiments.
cells exposed to A23187 or thapsigargin (46), and staurosporine-treated U937 cells (4). However, cell shrinkage can be effectively blocked by z-VAD-fmk in other apoptosis models, including Fas-treated Jurkat cells (Fig. 5), ML-1 cells treated with etoposide (47), or B lymphocytes exposed to TGF-β (48).

These observations suggest that the regulation of apoptotic volume decrease is both cell type- and stimulus-dependent. One caveat in our work is that z-VAD-fmk (10 or 50 µM) completely inhibited caspase-9-like activities in UV-treated cell extracts (Fig. 4) but not the apoptotic volume decrease of intact cells (Fig. 5); yet this apoptotic feature was suppressed by a DN mutant of caspase-9 (Fig. 10). The basis for this paradox is unclear, although the efficacy of z-VAD-fmk observed in an in vitro assay for caspase activities may not recapitulate its effect in intact cells. Additionally, the sensitivity of caspase-9 to z-VAD-fmk in vivo could be regulated by accessory proteins such as Atp1. Additionally, z-VAD-fmk in living cells is capable of affecting non-caspase components (34, 35) that may modulate the UV-induced apoptotic volume decrease.

It is conceivable that the mechanism by which UV induces cell death is intrinsically complex, such that multiple pathways may function in concert to facilitate the demise of the cells. Although these pathways are being defined, our present work underscores the notion that the apoptotic volume decrease in Jurkat cells can be differentially regulated by distinct initiator caspases depending on the specific death signal. The maintenance of cell volume is orchestrated by a number of regulatory mechanisms that involve ion transport, osmolyte accumulation, cytoskeletal organization, and gene expression (49–52). Among these, the movement of monovalent ions has been demonstrated to be a critical and necessary element for the loss of cell volume and other apoptotic events (4, 6, 7, 53, 54). Furthermore, apoptosis is enhanced under conditions where the intracellular K⁺ concentration is diminished and inhibited when K⁺ efflux is prevented (5). Results presented here also illustrate that K⁺ movement is tightly coupled to cell volume, and factors that prevent K⁺ efflux also suppress the loss of cell volume and viability. Indeed, accumulating evidence indicates that the activity of one or more K⁺ channel(s) is likely an important regulatory component of apoptosis (4, 55–59). These studies implicate the presence of K⁺-sensitive mechanisms that are critical for the initiation of certain death programs. Our present work has advanced the understanding of ionic movement during apoptosis by demonstrating the differential involvement of initiator caspases in regulating the K⁺ efflux and the coupled apoptotic volume decrease.

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