Sequential and rapid activation of select caspases during apoptosis of normal intestinal epithelial cells

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Grossmann, Johannes, Susanne Mohr, Eduardo G. Lapetina, Claudio Fiocchi, and Alan D. Levine. Sequential and rapid activation of select caspases during apoptosis of normal intestinal epithelial cells. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G1117–G1124, 1998.—Detachment-induced cell death (DICD) is considered to be one of the means by which intestinal epithelial cells (IEC) die of apoptosis as they reach the lumen and are shed. Caspases, a family of cysteine proteases, play a central role in initiating, amplifying, and executing apoptosis; however, the pattern of caspase activation in response to distinct apoptotic stimuli remains unknown. We investigated the kinetics of caspase activation during DICD in freshly isolated human IEC. DNA fragmentation is observed 90 min after detachment and is preceded by the sequential activation of preformed members of the CPP32 family of caspases. Activation of caspase 6 and cleavage of the endogenous caspase substrate poly(ADP-ribose) polymerase (EC 2.4.2.30) are detected within 15 min of detachment, 30–45 min before caspase 3 activation. Caspase 1 and caspase 10 are present as proenzymes, yet they remain inactive in response to this trigger of apoptosis. Human IEC are primed to rapidly undergo detachment-induced apoptosis involving the selective and sequential activation of preformed caspases. This study may enhance our understanding of physiological events occurring as IEC are shed. Their rapid apoptotic response to detachment may facilitate the high turnover of cells and ensure homeostasis in the intestinal epithelium.

cysteine proteases; programmed cell death

APOPTOSIS, the programmed execution of cell death, is vitally important for development and maintenance of tissue homeostasis and is a tightly regulated process (68). Intestinal epithelial cells (IEC) derive from stem cells at the base of the crypts, migrate along the crypt-villus axis, and reach the lumen after a life span of only 3–5 days (50, 51). At the luminal surface, they die of apoptosis, lose anchorage, detach, and are shed (18, 61). Various intrinsic factors, including the expression of Bcl-2/Bax family members, as well as environmental factors, such as cell-to-cell contact, cell-to-extracellular matrix attachment, extracellular matrix composition, integrin expression, short-chain fatty acids, and cytokines, may contribute to the induction of apoptosis at the luminal surface (5, 7, 8, 27, 29, 44, 52, 55, 57). Despite the wealth of information on factors that may influence the events occurring at the luminal surface, the molecular basis of physiological epithelial cell apoptosis is poorly understood. Detachment-induced cell death (DICD) is a recognized form of apoptosis in freshly isolated human IEC (21, 62) and various other anchorage-dependent cell types, such as bronchial epithelial cells, mammary epithelial cells, renal epithelial cells, and endothelial cells (3, 9, 17, 42, 53).

The activation of cysteine proteases plays a pivotal role in the execution of apoptosis but has not been characterized during the programmed cell death of IEC. This family of proteolytic enzymes, recently classified as caspases (2), consists of 10 members forming three subfamilies based on their homology. Caspase 1 (ICE), caspase 4 (ICH-2, ICE-rel-I), and caspase 5 (ICE-rel-III) form the ICE family, whereas caspase 3 (CPP32), caspase 6 (Mch2), caspase 7 (Mch3, ICE-LAP3), caspase 8 (FLICE, Mch5), and caspase 10 (Mch4) form the CPP32 family. Caspase 2 (Ich-1) and caspase 9 (ICE-LAP6, Mch6) constitute the Ich-1 family (16, 69). Ectopic expression of caspase cDNAs by transfection induces apoptosis in the absence of an environmental stimulus (14, 43, 45). However, mice lacking the caspase 1 gene develop normally, although their thymocytes exhibit decreased susceptibility to Fas-mediated apoptosis and they are resistant to endotoxic shock (33, 36). In contrast, caspase 3-deficient mice exhibit severely disrupted brain development, have low birth weight, and die prematurely at 1–3 wk of age (34). No intestinal abnormalities were reported in these caspase-deficient animals, although caspase 3 is abundantly expressed in enterocytes (31). After apoptosis is triggered, caspases are activated by the cleavage of proenzymes (zymogens) into distinct subunits that rearrange to form the active cysteine proteases. Caspases transduce and augment the apoptotic signal by activation of other caspases. They are thought to execute cell death by proteolysis of important functional and structural intracellular proteins, such as poly(ADP-ribose) polymerase (PARP; EC 2.4.2.30) (10) and lamin A (35, 64). PARP, an enzyme involved in DNA repair and genome surveillance, is cleaved by members of the CPP32 family, whereas lamin A is a caspase 6-specific substrate (48).

In this study we employ a new isolation protocol, specifically designed to characterize the molecular mechanism of DICD in freshly isolated human IEC (22). We demonstrate that DICD is rapidly executed in IEC within 2 h in the absence of de novo protein synthesis. Caspase 1 and 10 are expressed in IEC but remain inactive during DICD, whereas preformed caspase 6 and 3 are sequentially activated, associated with the cleavage of the intracellular substrates PARP and lamin A. The rapid apoptotic response of IEC after detachment in vitro may model the exfoliation of mucosal epithelial cells at the luminal surface.
EXPERIMENTAL PROCEDURES

Isolation of IEC. IEC were isolated, as previously described (22), from surgical specimens obtained from patients undergoing large bowel resection for colon cancer. Macroscopically normal tissue, at least 10 cm from the tumor margins, was studied. All specimens were processed within 1 h of the resection. Briefly, mucosal strips were dissected and washed for 30 min in 10 mM dithiothreitol (Fisher Biotech, Fair Lawn, NY). After the mucosa were treated for 60 min in 1 mM EDTA (Sigma Chemical, St. Louis, MO) at 4°C, the IEC were detached as intact crypts by 10 vigorous shakes of the vessel. The crypts were immediately harvested on an 80-µm mesh and backwashed with 20 ml serum-free medium [keratinocyte serum-free medium; Gibco-BRL; 2.5% penicillin-streptomycin-cytochrome (BioWhittaker); 1% gentamicin (BioWhittaker)]. After detachment, the intact intestinal epithelial crypts were kept in suspension at 37°C in a polypropylene tube. At the indicated time, cells were harvested by centrifugation at 4°C and further analyzed. To assess the role of de novo protein synthesis, mucosal strips were preincubated with cycloheximide (1–10 µg/ml) before treatment with EDTA. To determine that loss of anchorage rather than the method of isolation induces apoptosis, IEC were also isolated by mechanical disruption and enzymatic digestion, employing well-established, previously described protocols (19, 40).

DNA extraction and electrophoresis. DNA was extracted as described previously (56). Briefly, IEC were lysed with 0.4 ml of extraction buffer [10 mM Tris·HCl, pH 8.0 (Boehringer Mannheim), 0.1 M EDTA, pH 8.0, 0.5% SDS (Boehringer Mannheim)]. Proteinase K (200 µg/ml; Gibco-BRL) was added before overnight incubation at 42°C. DNA was purified with sequential phenol (Gibco-BRL) and chloroform-isomyl alcohol (Sigma Chemical) extractions. After incubation with RNase (50 µg/ml; Sigma Chemical), DNA was precipitated with ethanol, separated by electrophoresis on a 1.5% agarose gel, and visualized with ethidium bromide (0.5 µg/ml; Sigma Chemical) under ultraviolet light.

Ethidium bromide-acridine orange stain for apoptosis. Ethidium bromide-acridine orange staining was performed, as previously described (11). A combined dye of acridine orange and ethidium bromide (100 µg/ml; Sigma Chemical) was added to the cells, and apoptosis was rapidly assessed by ultraviolet fluorescence microscopy (Olympus-BH2; Olympus, Lake Success, NY). The apoptotic index (%) was determined by multiplying the number of apoptotic cells by 100 and then dividing by the total number of cells scored (200).

Fluorogenic substrate assays. Synthetic tetrapeptide substrates, which are cleaved by activated caspases, were added to IEC extracts as described previously (66). N-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (DEVD-AMC; Biomol, Plymouth Meeting, PA) is preferentially cleaved by members of the CPP32 family. N-acetyl-Tyr-Val-Ala-Asp-4-methylcoumaryl-7-amide (YVAD-AMC; Biomol) is cleaved by ICE family caspases. Z-Val-Glu-Ile-Asp-7-amino-4-trifluoromethyl coumarin (Z-VAD-AFC; Enzyme Systems Products, Livermore, CA) is cleaved by caspase 6. We incubated 20 µg of cell extract and 50 µM of DEVD-AMC, YVAD-AMC, or Z-VAD-AFC for 30 min at 37°C and quantified liberated fluorescence by fluorospectrophotometry (PerkinElmer LS-3) at wavelengths recommended by the manufacturer. BSA (20 µg) served as negative control; activated recombinant caspase 1 (kindly provided by N. Thornberry, Merck Research Laboratories, Rahway, NJ) and the monocytic cell line U-937, treated with lipopolysaccharide (100 µg/ml) to activate caspase 1, served as positive controls for ICE family caspases. Standards containing 0–5,000 pmol of AMC or 0–2,000 pmol of AFC were used to determine the amount of fluorochrome released. Measurements were recorded over the linear range of the assay. To assess whether caspase inhibitors could prevent or delay apoptosis, we incubated mucosal strips with the CPP32 family-specific inhibitor N-acetyl-Asp-Glu-Val-Asp-aldehyde (DEVD-CHO; Biomol) and the broad spectrum caspase inhibitors Z-Val-Ala-Asp(Ome)-fluoromethyl ketone (Z-VAD-FMK; Enzyme Systems Products) at the maximum recommended concentrations of 200 and 100 µM, respectively (4, 30). Various experimental conditions were tested to facilitate penetration of these inhibitors into IEC (incubation for 2, 4, and 6 h before EDTA treatment at both 4°C and 37°C). Inhibition of apoptosis was determined by DNA electrophoresis, and caspase inhibition was assessed by Western blot for caspase 3, a known target of both inhibitors. Caspase activity was inhibited in whole cell extracts by the addition of 150 nM DEVD-CHO and 150 nM N-acetyl-Tyr-Val-Ala-Asp-aldehyde (YVAD-CHO; Biomol), a selective inhibitor for ICE family caspases, to extracts before addition of the fluorogenic substrates.

Western blot analysis. Cell extracts (15–50 µg protein) were fractionated by SDS-PAGE and electrotransferred to Immobilon P15 membrane (Millipore, Bedford, MA). The membrane was blocked with 5% milk in 0.1% Tween 20-PBS (Fisher) for 120 min and incubated for 60 min at room temperature with the indicated primary antibody. Anti-caspase 3 was purchased from Transduction Laboratories (Lexington, KY), and anti-caspase 1 was kindly provided by D. K. Miller (Merck Research Laboratories). Anti-PARP was kindly provided by N. A. Berger (Case Western Reserve University, Cleveland, OH), and anti-caspase 6, anti-caspase 10, and anti-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-lamin A was kindly provided by B. Burke (University of Calgary, Canada). The membranes were washed six times with 0.1% Tween 20-PBS and then incubated for 1 h with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz), washed again, and incubated with chemiluminescent substrate (Super Signal; Pierce, Rockford, IL) for 5 min. The membranes were then exposed to film (Amer sham, Arlington Heights, IL).

RESULTS

Rapid execution of apoptosis in IEC after detachment. To investigate the kinetics of DICD, freshly isolated intact intestinal crypts were incubated at 37°C. Samples of IEC were removed from the suspension at the indicated times after detachment and assessed for evidence of apoptosis by the appearance of DNA fragmentation. As shown in Fig. 1, purified IEC exhibited no evidence of apoptosis immediately after detachment. DNA fragmentation was detected after 90 min of detachment. To quantify the rate of apoptosis, IEC were stained with ethidium bromide-acridine orange dye at the indicated times after detachment (Fig. 2). In agreement with the findings of DNA electrophoresis, <2% of IEC were apoptotic immediately after detachment. The apoptotic index rapidly increased, and by 3 h >90% of the cells were apoptotic. Epithelial cells isolated in the presence of calcium by mechanical disruption or enzymatic digestion showed similar results, displaying apoptotic indexes of 87 ± 7% (n = 3) and 85 ± 8% (n = 3), respectively, 3 h after isolation.
Detachment-induced apoptosis of IEC does not require de novo protein synthesis. The rapid onset of apoptosis in these cells suggested that execution of cell death may not require de novo protein synthesis. Before initiation of DICD, mucosal strips were preincubated with either cycloheximide, a known inhibitor of protein synthesis, or vehicle alone. IEC were then isolated and analyzed for evidence of apoptosis 2 and 4 h after detachment, times when initial and maximal DNA fragmentation were observed. Inhibiting protein synthesis with cycloheximide did not alter the kinetics of DNA fragmentation (Fig. 3). The percentage of apoptotic cells as quantified with ethidium bromide-acridine orange staining was unchanged (data not shown).

Activation of selected caspases during detachment-induced apoptosis of IEC. The kinetics of activation for distinct caspase families during DICD were determined using synthetic tetrapeptide fluorogenic substrates. Cleavage of the substrate YVAD-AMC measures ICE family caspase activation. DEVD-AMC and VEID-AFC are cleaved by activated members of the CPP32 family of cysteine proteases. YVAD-AMC was not cleaved when incubated with the extracts of IEC kept in suspension, demonstrating that ICE family caspases are not activated during DICD (Fig. 4A). For a positive control, the substrate YVAD-AMC was cleaved by recombinant caspase 1 (33.3 pmol·min⁻¹·μg⁻¹) and an extract of lipopolysaccharide-stimulated U-937 cells (116.7 pmol·min⁻¹·μg⁻¹). The negative control, BSA, did not cleave the substrates (<2 pmol·min⁻¹·μg⁻¹). ICE-like enzymatic activity present in the positive control was inhibited by 98% with the addition of the ICE family inhibitor YVAD-CHO (150 nM).

In contrast, cell extracts isolated from IEC maintained in suspension exhibited a time-dependent increase in enzymatic activity for the cleavage of both VEID-AFC and DEVD-AMC (Fig. 4A). During the first 30 min after detachment, 20% of maximal VEID-AFC cleavage activity was detected and remained unchanged, while DEVD-AMC cleavage was undetectable. This was followed by a second phase of rapid amplification, resulting in a parallel increase of both VEID-AFC and DEVD-AMC cleavage activity within 90 min. Cleavage activity for both substrates reached a plateau at 2 h. Cleavage of DEVD-AMC was not due to nonspecific activation of proteases, since addition of DEVD-CHO (150 nM) to extracts blocked 98% of the cleavage activity for this substrate. As a control, human intestinal fibroblasts (HIF), another type of adherent cell, did not undergo apoptosis when kept in suspension for 6 h after detachment (data not shown). As expected, cell extracts of HIF maintained in suspension showed no evidence of caspase activation (Fig. 4B).

Caspase 1 remains inactive during detachment-induced apoptosis of IEC. The absence of YVAD-AMC cleavage in Fig. 4A demonstrates no proteolytic activity...
of ICE family members during DICD. Yet, it is likely that human IEC express caspase 1, since these cells undergo Fas-mediated apoptosis (63), a pathway thought to lead to caspase 1 activation (12). Therefore, the expression of caspase 1 was determined in freshly isolated IEC by immunoblot. The proenzymatic form of caspase 1 (p45) was abundantly expressed in IEC (Fig. 5). As the cells undergo DICD, neither the p10 nor p20 subunit indicative of caspase 1 activation was detected, in agreement with the fluorogenic assay. The active subunits of caspase 1 (p10 and p20) and the processing intermediates (p24 and p26) of activated recombinant caspase 1 served as a positive control.

Sequential activation of CPP32 family caspases during detachment-induced apoptosis of IEC. The fluorogenic assay using DEVD-AMC and VEID-AFC as substrates demonstrated the activation of CPP32 family members during DICD. The expression and kinetics of activation for distinct members of this caspase family, specifically caspase 3, caspase 6, and caspase 10, were assessed by Western blot (Fig. 6). Nonapoptotic, freshly isolated IEC contain abundant amounts of caspase 3 in its proenzymatic form (p32) after detachment, as the p32 signal decreased. Appearance of the p17 subunit was maximal by 60 min and declined over the following 3 h. Caspase 10 is another member of the CPP32 family. Only the proenzymatic form (p39) of caspase 10, not the p27 subunit of the activated protease, was detected, indicating that caspase 10 was expressed in IEC but remained inactive during DICD. Caspase 6, a third member of the CPP32 family, is cleaved to form a p20 subunit on activation. Figure 6 shows the presence of the p20 subunit of caspase 6 immediately after detachment and a marked increase in signal intensity 30 min later. The proenzymatic 34-kDa form of caspase 6 was not detectable using this reagent. The early detection of activated caspase 6 by immunoblot is consistent with the presence of VEID-AFC cleavage activity in the first 30 min after detachment, as seen in Fig. 4A. Equal loading of protein was confirmed by actin immunoblot.

To demonstrate that DICD is mediated by caspase activation, the ability of caspase inhibitors to block apoptosis in freshly isolated IEC was assessed. Incubation of mucosal strips with DEVD-CHO or Z-VAD-FMK under various conditions was unable to prevent or retard apoptosis, as measured by DNA fragmentation.
Fig. 6. Preformed CPP32 family caspases are sequentially and selectively activated during DICD. Lysates from IEC were prepared as described earlier and analyzed for expression and kinetics of activation for distinct CPP32 family members. Caspase 3: intact pro-caspase 3 (p32) is observed in nonapoptotic IEC (t = 0). The p17 subunit, indicative of active caspase 3, is detected within 45 min after detachment. Bands at p30 and p20 are intermediates in the processing of caspase 3. Caspase 10: zymogen of caspase 10 (p39) is expressed in nonapoptotic IEC and remains uncleaved during DICD. p27 cleavage product of activated caspase 10 is not detected. Caspase 6: p20 subunit of caspase 6, indicating caspase 6 activation, is detected immediately after detachment. Pro-caspase 6 is not detectable with this antibody. Actin: immunoblot for actin was performed on the same membrane as used for caspase 6 to control for equal loading of protein. Western blots are representative of 4 experiments.

and ethidium bromide-acridine orange stain. To determine the efficacy of the inhibitors, the kinetics of caspase 3 activation were followed by Western blot. The rate of caspase 3 activation was not altered by treatment with these inhibitors, demonstrating the lack of their uptake by IEC (data not shown).

Cleavage of PARP and lamin A during detachment-induced apoptosis of IEC. To demonstrate proteolytic activity of caspases within IEC undergoing DICD, the kinetics of endogenous substrate cleavage were determined. PARP is a known substrate of members of the CPP32 family, such as caspase 3, caspase 6, caspase 7, and caspase 8 (46, 48). Intact PARP (116 kDa) is specifically cleaved, yielding a signature fragment of 85 kDa. Whole cell extracts from IEC were isolated at the indicated times after detachment and analyzed for PARP cleavage by immunoblot. As shown in Fig. 7, freshly isolated IEC contained only the intact uncleaved protein. Fifteen minutes after detachment, PARP cleavage was initiated, as demonstrated by the appearance of the 85-kDa fragment. Within 45 min PARP cleavage was complete, before caspase 3 activation (Fig. 6). Lamin A, a 70-kDa protein, is specifically cleaved by caspase 6, yielding a 46-kDa fragment (48).

To further study the characteristics of caspase 6 activation, we followed the kinetics of lamin A cleavage during DICD by Western blot (Fig. 7). Lamin A remained intact during the initial 60 min of DICD, after which it was rapidly and completely cleaved. Because caspase 6 was active during these first 60 min (Figs. 4 and 6), these findings indicate that lamin A is a late target of this caspase.

**DISCUSSION**

In this study we characterize the sequential and selective activation of caspases in freshly isolated, nontransformed human IEC undergoing detachment-induced apoptosis. Programmed cell death in IEC is induced in vitro by the disruption of anchorage, leading to the dramatic execution of >90% of the cells within 3 h. In vivo IEC show evidence of apoptosis as they reach the luminal surface, where their short life span is terminated by exfoliation (18). However, the molecular mechanisms leading to the induction of IEC apoptosis at the luminal surface and detachment are poorly understood. Changes of integrin and extracellular matrix composition along the crypt-villus axis (8, 52, 54, 55) and the increase in transforming growth factor-β expression at the luminal surface (5, 6, 67) contribute to a milieu that promotes growth arrest, loss of anchorage, and apoptosis of the epithelium. Furthermore, the decreased expression of Bcl-2 and the concurrent increase in expression of Bax and Bak in IEC as they reach the luminal surface suggest that these cells become more susceptible to the proapoptotic environment of this region (32, 44, 57). The loss of anchorage as a trigger of apoptosis may therefore play an important role in the physiological life cycle of the intestinal epithelium.

Our studies demonstrate that IEC constitutively contain the machinery of cell death, which is rapidly activated on loss of anchorage, leading to caspase activation within minutes of detachment and to DNA fragmentation in less than 2 h. Comparing our observations with those from other reports, the kinetics of programmed cell death in epithelial cells vary considerably depending on the apoptotic trigger or cell type. Intestinal HT-29 tumor cells, primed with interferon-γ, require 24 h to undergo apoptosis when stimulated...
with tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and only 4 h when triggered via Fas (1). Butyrate induces apoptosis in intestinal tumor cell lines after 24–48 h (23, 24, 27), whereas butyrate deprivation leads to cell death within 45 min in nontransformed IEC (26). DICD in endothelial cells and the Madin-Darby canine kidney cell line requires 36 and 7 h, respectively (17, 53), yet in this study we demonstrate that detachment of nontransformed human IEC leads to apoptosis in >90% of the cells by 3 h. Together, these studies underscore that the sequence and pattern of caspase activation associated with different apoptotic stimuli or cell types vary.

Caspase 10 (FLICE/2/Mch4) is assumed to be activated early during Fas/TNF-\(\alpha\)-mediated apoptosis, since it contains a Fas-associated death domain, enabling its binding to the Fas/TNF-\(\alpha\)-receptor complex (13, 46, 47). The activation of this caspase during Fas/TNF-\(\alpha\)-mediated apoptosis, however, has not been reported in a whole cell system. Our findings demonstrate that human colonic IEC express caspase 10 zymogen, indicating that IEC possess the machinery to undergo Fas/TNF-\(\alpha\)-mediated apoptosis. However, caspase 10 is not activated during DICD, pointing to a specific role for this protease in other pathways of programmed cell death.

Caspase 1 (ICE) is also thought to be activated during Fas-mediated apoptosis (12, 33, 37); however, its role in this form of apoptosis is controversial (25, 58). IEC have been shown to be susceptible to Fas-mediated apoptosis (1, 63), suggesting that caspase 1 may be involved in the execution of programmed cell death within these cells. In this study we demonstrate that IEC express preformed caspase 1 zymogen, which remains inactive during DICD. However, caspase 1 in IEC extracts could be autoactivated with recombinant caspase 1 in vitro (data not shown), indicating that it may have a functional role in other pathways of IEC apoptosis. Further studies are necessary to determine the role for caspase 1 and caspase 10 in response to other triggers of apoptosis. Our results suggest that the execution of DICD in IEC does not require the activation of all caspases expressed in the cell and that the protease cascade is selective and highly regulated.

Caspase 6 is activated very early during DICD and 45 min before caspase 3, suggesting that caspase 6 is upstream of caspase 3, an observation confirming previous studies using recombinant caspases in a cell-free system (49).

In agreement with studies on other cell types, the endogenous caspase 6 substrates PARP and lamin A are not cleaved simultaneously (20, 35). PARP is cleaved rapidly, within 15 min of detachment and 45 min before lamin A cleavage. Although in vitro studies (64) indicate that caspase 6 has a higher affinity for lamin A than for PARP, other studies (35, 69) suggest that more than one cytosolic protease may be involved in the cleavage of lamin A, located in the nucleus. It is possible that the subcellular localization of lamin A delays its accessibility to these proteases (69). Caspase 3, which has a higher affinity for PARP than lamin A (62), does not cleave PARP during DICD of IEC. PARP is completely degraded before the activation of caspase 3, which remains as a zymogen for the initial 45 min of the apoptotic cascade. However, PARP is also a substrate of other CPP32 family caspases (46, 48, 65), including caspase 6, which is activated before PARP cleavage during DICD. Actin, another pivotal structural protein previously described to be a substrate of activated caspases (39, 41), is not cleaved during the first 4 h of DICD in IEC (see Fig. 6), confirming the findings of Song et al. (59). Our inability to prevent IEC apoptosis with caspase inhibitors may be attributed to the complex structure of the intact crypt or the production of a protective mucus layer prohibiting penetration of the inhibitors. Further studies with new inhibitors are needed.

In summary, we investigated the molecular mechanisms of DICD in nontransformed human IEC using a newly developed in vitro model, specifically designed to elucidate early events of the apoptotic cascade (22). IEC are primed with preformed caspases to initiate programmed cell death within minutes of detachment, resulting in DNA fragmentation in less than 2 h. The immediate activation of the death machinery may ensure mucosal homeostasis in light of the high turnover of IEC in vivo. Furthermore, this unique response may effectively prevent ectopic growth of IEC after exfoliation. Understanding the molecular mechanisms of DICD in normal human IEC may enable us to identify pathogenic factors that disrupt the delicate balance of life and death in the intestinal epithelium.

We thank N. Thornberry and D. K. Miller (Merck Research Laboratories, Rahway, NJ) for kindly providing us with recombinant caspase 1 and anti-caspase 1 antibodies, B. Burke (Department of Medicine, University of Calgary, Calgary, Canada) for kindly
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