Desmoglein-2: A Novel Regulator of Apoptosis in the Intestinal Epithelium

Porfirio Nava,*† Mike G. Laukoetter,*†† Ann M. Hopkins,§ Oskar Laur,* Kirsten Gerner-Smith,* Kathleen J. Green,‖ Charles A. Parkos,* and Asma Nusrat*

*Epithelial Pathobiology Research Unit, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA 30322; †Department of General Surgery, University of Muenster, D-48149 Muenster, Germany; §UCD School of Medicine and Medical Science, University College, Dublin 4, Ireland; and ‖Northwestern University Feinberg School of Medicine, Chicago, IL 60611

Submitted May 9, 2007; Revised July 19, 2007; Accepted August 27, 2007
Monitoring Editor: M. Bishr Omary

Intestinal epithelial intercellular junctions regulate barrier properties, and they have been linked to epithelial differentiation and programmed cell death (apoptosis). However, mechanisms regulating these processes are poorly defined. Desmosomes are critical elements of intercellular junctions; they are punctate structures made up of transmembrane desmosomal cadherins termed desmoglein-2 (Dsg2) and desmocollin-2 (Dsc2) that affiliate with the underlying intermediate filaments via linker proteins to provide mechanical strength to epithelia. In the present study, we generated an antibody, AH12.2, that recognizes Dsg2. We show that Dsg2 but not another desmosomal cadherin, Dsc2, is cleaved by cysteine proteases during the onset of intestinal epithelial cell (IEC) apoptosis. Small interfering RNA-mediated down-regulation of Dsg2 protected epithelial cells from apoptosis. Moreover, we report that a C-terminal fragment of Dsg2 regulates apoptosis and Dsg2 protein levels. Our studies highlight a novel mechanism by which Dsg2 regulates IEC apoptosis driven by cysteine proteases during physiological differentiation and inflammation.

INTRODUCTION

Mucosal epithelial barriers are dependent upon the association of neighboring cells with each other through adhesive multiprotein complexes at sites of cell–cell contacts. Simple epithelial cells (such as those lining the intestine, lungs, and kidneys) associate through a series of intercellular junctions that maintain epithelial integrity, regulate paracellular movement of solutes, and restrict access of luminal antigens/pathogens from underlying tissue compartments. Intercellular junctions include tight junctions (TJs), adherens junctions (AJs), desmosomes (DMs), and in some epithelia gap junctions (Cereijido et al., 1978; Gonzalez-Mariscal et al., 2003). DMs have been visualized as punctate “spot welds” that hold cells together and provide mechanical strength to epithelial tissues by forming stable cell–cell contacts that are anchored to the keratin intermediate filaments (Getios et al., 2004b). Transmembrane proteins in DMs include the cadherin superfamily members desmoglein (Dsg 1-4) and desmocollin (Dsc 1-3) (Getios et al., 2004b; Kotke et al., 2006) that mediate calcium-dependent cell–cell adhesion. Simple epithelia such as in the intestine, express Dsg2 and Dsc2, which affiliate with underlying keratin intermediate filaments via the armadillo family of proteins and desmoplakin (DSP) (Bornslaeger et al., 1996; Hatzfeld, 1999; Jonkman et al., 2005).

Epithelial cells differentiate as they migrate toward the lumen along the intestinal crypt villus axis where they detach from the basement membrane and undergo apoptosis (Dufour et al., 2004). This cycle of progenitor crypt cell proliferation, migration, differentiation, and apoptosis is vital for maintaining integrity of the epithelium and therefore mucosal barrier function. The survival of most normal intestinal epithelial cells (IECs) requires cell–cell and cell–matrix adhesion, and loss of such cell adhesion induces epithelial cell death that is, in part, mediated by apoptosis (Dzierzewicz et al., 2003; Dufour et al., 2004; Fouquet et al., 2004). Pathologically, proinflammatory cytokines such as interferon IFN)-γ promote apoptosis in the inflamed epithelium. Apoptosis is regulated by cysteine proteases such as caspases, which are known to be responsible for a series of well defined morphological and biochemical changes in apoptotic cells, including loss of cell–cell contact, rounding of the cell body, loss of cytoskeletal integrity, and nuclear condensation (Jilling et al., 2004; Wenzel and Daniel, 2004).

Recently, our understanding of cellular signaling has been expanded by discoveries that key signaling proteins can congregate together in subcellular membrane compartments to execute their functions in an integrated manner. Lipid rafts, cholesterol- and glycosphingolipid-enriched plasma membrane microdomains (Smart et al., 1995; Song et al., 1996; Nusrat et al., 2000b), have emerged as particularly important reservoirs of cellular signaling proteins (Shaul and Anderson, 1998; Simons and Toomre, 2000). Some membrane rafts contain the structural proteins caveolin 1-3, and they are especially enriched in signaling components of...
several diverse families (Everson and Smart, 2001; Razani et al., 2002) in addition to performing distinct endocytic functions (Schnitzer, 2001). Both TJ (Nusrat et al., 2000b; Bruewer et al., 2004) and gap junction (Schubert et al., 2002a) proteins have recently been shown to coexist with lipid rafts. Here, we report that a monoclonal antibody (mAb) against lipid raft-enriched fractions of human model intestinal epithelial cells, recognizes an antigen (~165 kDa) that is localized in epithelial lateral membranes at sites of cell–cell contacts. The AH12.2 antigen was identified as human desmoglein-2. Furthermore, we observed that the cytoplasmonic tail of Dsg2 but not another DSC1 cadherin, Dsc2, is cleaved by cysteine proteases during the onset of stimulus-induced apoptosis. Short interfering RNA (siRNA)-mediated down-regulation of Dsg2 protected epithelial cells from apoptosis, supporting its role in facilitating apoptotic removal of cells after compromise of cell–cell adhesion.

MATERIALS AND METHODS

Cell Culture, IFN-γ, and Tumor Necrosis Factor (TNF)-α Incubation

T84 human colonic epithelial cell lines (American Type Culture Collection, Manassas, VA) were cultured as described previously (Hopkins et al., 2003). Growth to confluence was assessed by monitoring transepithelial electrical resistance by using an EndOhm/STX-II electrode system (WPI, Sarasota, FL). For transfection studies, SK-CO15 colonic epithelial cells were cultured in DMEM (Mandel et al., 2005). TNF-α (10 ng/ml; Genzyme, Cambridge, MA) and IFN-γ (10 U/ml; a kind gift from Genentech, South San Francisco, CA) were added basolaterally to monolayers for 72 h. Control (CTRL) monolayers were incubated with cell culture medium only.

Generation of Antibodies to Lipid Raft-enriched Epithelial Junction Proteins

Triton X-100-insoluble lipid rafts were prepared from confluent T84 cells and characterized as described previously (Nusrat et al., 2000b). To generate antibodies, BALB C mice were immunized intraperitoneally with lipid raft-enriched preparations. Splenocytes of mice with high titters of anti-epithelial antibodies fused with P3U1 myeloma cells according to standard procedures (Parkos et al., 1996a,b). Hybridoma supernatants were screened by immunofluorescence for binding to intercellular junctions of T84 epithelial monolayers on 96-well plates. Antibodies were purified from cell culture supernatants by using protein A-Sepharose (Sigma-Aldrich, MO), dialyzed against normal saline-HEPES buffer (150 mM NaCl and 10 mM HEPES pH 7.4), and stored in aliquots at ~80°C.

Immunofluorescence

Confluent intestinal epithelial monolayers were fixed/permeabilized in 3.7% paraformaldehyde (10 min at room temperature [RT]) followed by 0.5% Triton X-100 in HBSS (30 min at RT) or in ice-cold absolute ethanol (20 min at ~20°C). Frozen sections (6 μm) of normal human colonic mucosa were fixed in absolute ethanol. Nonspecific protein binding was blocked in 5% normal goat serum or 3% bovine serum albumin in Hanks’ balanced salt solution (HBSS)+ (1 h at RT) and incubated with primary antibodies (1 h at RT), washed in HBSS++, and subsequently labeled with secondary antibodies (1 h at RT). Nuclei were stained using To-Pro-3-iodide (Invitrogen, Carlsbad, CA) in HBSS++ (10 min at RT). Monolayers were mounted in 1:1:0.1 vol/vol/vol phosphate-buffered saline (PBS)/glycerol–phenylenediamine, and subsequently labeled with secondary antibodies from A. S. Yap, University of Queensland, Australia), and desmocollin-2 antibodies were obtained from HECD-1 hybridoma supernatant (a kind gift from A. S. Yap, University of Queensland, Australia), and desmocollin-2 antibodies were from hybridoma 7G6 (Kowalczyk et al., 1994a). Fluorescein-conjugated secondary antibodies were obtained from Invitrogen (Alexa dye series). Sigma-Aldrich (mouse anti-Flag), and Abcam (rabbit anti-myc).

SDS-Polyacrylamide Gel Electrophoresis (PAGE)/Western Blotting

T84 confluent monolayers were scraped into lysis buffer (100 mM KCl, 3 mM NaCl, 3.5 mM MgCl₂, 10 mM HEPES, pH 7.4, and 1% Triton X-100) containing protease and phosphatase inhibitors (Sigma-Aldrich). Cell suspensions were sonicated, and the nuclei were removed by centrifugation (10,000 × g for 5 min at 4°C). Postnuclear lysates were mixed with Laemmli sample buffer with 20 mM dithiothreitol. Equivalent protein concentrations were separated by SDS-PAGE and immunoblotted with antibodies mentioned above. Densitometry was performed using the UN-SCAN-IT automated digitizing system (Silk Scientific, Orem, UT).

Immunoprecipitation

Confluent T84 cell monolayers were washed in HBSS+ and scraped into lysis buffer (as described above). Postnuclear fractions were precleared for 1 h at 4°C. The antigen recognized by AH12.2 was immunoprecipitated (overnight at 4°C) by using antibody coupled to Sepharose beads. Other antigens of interest were immunoprecipitated using 5 μg of mAb or isotype-matched control IgG antibody, and they were retrieved by rotation with protein G-Sepharose (5 h at 4°C). All beads were washed, boiled in reducing Lammeli sample buffer, and subjected to SDS-PAGE and Western blot analysis.

Mass Spectrometry and Identification of AH12.2 Antigen

T84 cells (~10⁶ cells) were used to perform large-scale immunoprecipitations of the antigen recognized by AH12.2 antibody (as described above). Immunoprecipitates were electrophoresed on 7.5% polyacrylamide gels and stained with Coomassie Blue. The protein band corresponding to AH12.2 antigen was excised and trypsin-digested, and the peptides were extracted and desalted using C18ZipTip (Millipore, Billerica, MA). Tryptic peptides were analyzed in the Emory Microchemical Facility by matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF)-mass spectrometry (MS) and nano-liquid chromatography-tandem mass spectrometry (LC-MS/MS); and in the HarVard Microchemistry Facility by microparticle reverse-phase high-performance liquid chromatography (HPLC) nano-electrospray tandem mass spectrometry (μLC-MS/MS) on a Finnigan LCQ DECA XP Plus quadrupole ion trap mass spectrometer (Thermo Electron, MA).

siRNA Experiments

siGENOME SMARTpool siRNA for Human Dsg2, Dsc2, Lamin A/C, and a scramble control were purchased from Dharmacon RNA Technologies (Lafayette, CO). SK-CO15 cells cultured to 60–70% confluence were transfected with the SMARTpool reagents at a final concentration of 20 nM by using Lipofectamine 2000 (Invitrogen). The cells were incubated for an additional 3 d after the transfection to allow for sufficient knockdown of the targeted protein. Confluent epithelial cultures were analyzed. Each transfection was performed at least three times.

Expression of Dsg2 Constructs

The DNA fragments encoding the desmoglein cytoplasmic tail tagged with the Flag epitope were generated by polymerase chain reaction (PCR) with the following primers: for Cter (siRNA)-Flag (encompassing the full-length C terminus, 405 amino acids [aa]), forward 5'-ACCCGTCACTCAGCAATCGCCATCGGCGAAGGCGGCC-3', and reverse 5'-CCGAAATCTCCGACATTGCCGACAGAGAAATGAACTCGCAAT3’-; for Cter≌Flag (encompassing the last 217 aa), forward 5’-CCGAAATCTCCGACATTGCCGACAGAGAAATGAACTCGCAAT3’-; and reverse 5’-GGGTCTGCACAAAGCTTCACCTCTACATCAGGG-3’. DNA was ligated into the pIRES2-EGFP expression vector (Clontech, Mountain View, CA), which is readily transcribed with an added 3' untranslated region (3'UTR) that can be repressed by translation inhibition. The media were replaced after 6 h with high-glucose DMEM, and cells were cultured for 2 d before apoptosis analysis.

Induction of Apoptosis, Preparation of Cell Lysates, and Apoptosis Assay

Apoptosis was induced in confluent monolayers cultured in six-well plates by adding 2 μg/ml camptothecin (Camp). Detached cells were harvested from the culture medium by centrifugation (310 × g for 10 min), and they were pooled with adherent cells followed by incubation in lysis buffer (as described above). The total protein concentration of the cell lysates was determined with a BCA protein assay system (Pierce Chemical, Rockford, IL). For inhibition studies, cells were cultured to 60 min with 2 μM Camptothecin (50 μM DnD Systems, Minneapolis, MN) and/or colcemid (52 nM; Calbiochem, San Diego, CA) before adding camptothecin. Cell viability measurements were performed as follows. SK-CO15 cells were loaded with 1 μM propidium
iodine (PI) in PBS (Sigma-Aldrich). After two washes, images of cells were captured using an inverted microscope (Carl Zeiss Microimaging). Cells with orange fluorescence were scored as nonviable and image analysis was performed using MetaMorph version 7.1 (Molecular Devices, Sunnyvale, CA). Detection of apoptotic cells in situ was performed as described previously (Bruewer et al., 2003). Caspase-3 activation was analyzed by Western blot using the Caspase-3 antibody (Cell Signaling Technology, Danvers, MA) according to the manufacturer’s instructions. All statistical analyses were carried out using GraphPad Prism version 3.0 for Windows (GraphPad Software Inc., San Diego, CA).

RESULTS

Characterization of a mAb Generated against Human Epithelial Lipid Rafts That Recognizes a Protein in Epithelial Intercellular Junctions

Because our previous findings suggested enrichment of AJC proteins in epithelial membrane rafts (Nusrat et al., 2000b; Bruewer et al., 2004), we immunized mice with a lipid raft fraction and screened for monoclonal antibodies that recognized proteins in lateral intercellular contacts. Using this approach, we identified a mAb, AH12.2, which labeled intercellular contacts of epithelial cells. The antibody was used in Western blots to probe lipid raft-enriched fractions obtained from isopycnic sucrose density gradients of T84 epithelial cells. As shown in Figure 1A, the AH12.2 antibody recognizes two protein bands of ~165 and ~100 kDa that were enriched in light-density fractions (~22 ± 2% sucrose) containing membrane raft proteins, flotillin and caveolin-1 (labeled Rafts). Most of the cellular actin fractionated in high-density fractions at the bottom of the gradient. To further confirm the membrane raft association of the AH12.2 antigen, we immunolocalized the AH12.2 antigen with caveolin-1. As shown in the en face confocal micrographs taken at the level of lateral intercellular contacts (Figure 1A), both AH12.2 and caveolin-1 colocalized at such sites of cell–cell contacts. The affiliation of the AH12.2 antigen with lipid rafts was additionally verified by incubating cells with the cholesterol-sequestering agent methyl-β-cyclodextrin to disrupt lipid rafts (Christian et al., 1997). The fractionation profile of the AH12.2 antigen in sucrose density gradients was then determined by Western blotting. As shown in Figure 1B, the AH12.2 antigen associated with high-density fractions at the bottom of the gradient after disruption of membrane rafts with cyclodextrin treatment. Immunofluorescence labeling and confocal microscopy additionally revealed reduced association of the AH12.2 antigen with lateral epithelial membranes (Figure 1B). Last, the AH12.2 antigen was localized in intercellular junctions of native human intestinal epithelial cells as highlighted by immunofluorescence labeling of intestinal mucosal frozen sections (Figure 1C).

Identification of Dsg2 as the Antigen Recognized by the mAb AH12.2

To identify the antigen recognized by the AH12.2 antibody, we performed immunofluorochromatography by using cell lysates from confluent monolayers of polarized T84 epithelial cells (~1,000 cm²; ~10⁸ cells). The immunopurified antigen consisted of the ~165- and ~100 kDa bands (as shown in Figure 1, A and B). Trypsin digestion and nano-electrospray tandem mass spectrometry revealed a strong identity between these two bands and the human desmosomal cadherin Dsg2 (Koch et al., 1990, 1991). These results were verified in multiple independent immunopurification preparations that were trypsin digested and analyzed by MALDI-TOF-MS and nano-LC-MS/MS at the Emory Microchemical Facility (data not shown). Thus, we defined the antigen recognized by AH12.2 as human Dsg2. Of the three Dsgs (Dsg1, Dsg2, and Dsg3), Dsg2 is the most widespread Dsg, because it is found in all desmosome-assembling tissues, including epidermis, gastrointestinal mucosa, heart, and meninges (Schafer et al., 1996), and Dsg2 expression has been observed in cancer cell lines (Chitaev and Troy...
brane where it does not colocalize with the TJ protein occludin. We examined whether the AH12.2 antigen colocalized with TJ, and our results indicated that the bottom ~100-kDa band (Figure 1, A and B) consists of a truncated variant of Dsg2 (tDsg2) that lacks the ICS domain, the intracellular proline-rich linker (IPL), the RUDs I-VI, and the C-terminal domain (TD) (Figure 2A) [Kowalczyk et al., 1994b].

To better define the localization of the AH12.2 antigen, we examined whether the AH12.2 antigen colocalized with TJ, AJ, and DM proteins in polarized T84 epithelial cells (Figure 2B). As shown in the en face confocal micrographs, the AH12.2 antigen is distributed along the basolateral membrane where it does not colocalize with the TJ protein occludin. Reconstructed confocal images in the xz plane confirmed localization of AH12.2 antigen immediately subjacent to punctate focal staining for occludin (data not shown). As can be seen, a significant pool of AH12.2 antigen colocalizes with the DM protein DSP. Additionally, AH12.2 also colocalized with E-cadherin, an AJ protein localized along the entire lateral membrane. Furthermore, double labeling of confluent epithelial monolayers with AH12.2 antibody, and a commercial antibody that recognizes an extracellular domain (6D8-Dsg2) of Dsg2 (Wahl, 2002; Ota et al., 2003), revealed perfect colocalization with the AH12.2 antigen (Figure 2C). To further establish that the protein recognized by the mAb AH12.2 was indeed hDsg2, anti-AH12.2 was used to immunoprecipitate this protein and the Western blots were probed with a commercial anti-hDsg2 antibody that recognizes an extracellular domain in Dsg2 (6D8-Dsg2) and vice versa. Mouse IgG immunoprecipitates were used as a negative control. As shown in Figure 2C, AH12.2 recognized two protein bands (~165 and ~100 kDa) that correspond to the antigen immunoprecipitated by 6D8-Dsg2 antibody. Analogous results were obtained when membranes were immunoblotted with 6D8-Dsg2. This antibody recognizes an ~165-kDa band, and it seems to have less affinity than AH12.2 for the band ~100 kDa (Supplemental Figure 2).

Thus, the detection of these two protein bands with both antibodies confirms that tDsg2 is a variant of hDsg2. The tested monoclonal antibodies revealed low cross-reactivity with a band below 100 kDa that is in the IgG immunoprecipitate control (Figure 2C). Last, we observed that the AH12.2 antibody recognizes an intracellular epitope on Dsg2 as revealed by the lack of labeling of nonpermeabilized versus strong labeling of Triton X-100-permeabilized monolayers with AH12.2 and DSP (cytoplasmic plaque protein) (Figure 2D). Together, the above-mentioned results highlighted an ~100-kDa Dsg2 fragment and the recognition of an intracellular epitope suggest that the AH12.2 antibody recognizes a cytoplasmic epitope located in the intracellular anchor domain of Dsg2 (Figure 2E).

**Generation of tDsg2**

To analyze the nature of tDsg2, we first examined the possibility that tDsg2 could be generated by alternative splicing. Analysis of the intron–exon organization of the Dsg2 gene revealed that there are at least 15 exons in the gene. The last exon is the largest and includes all six tandem Dsg2 repeats at the C terminus of the protein. The peptide closest to the tDsg2 C terminus that was found by mass spectrometry analysis corresponds to the sequence encoded in the last exon of human Dsg2. Given that splicing is not commonly observed in the center of an exon, these observations suggest that alternative splicing is likely not a mechanism for generation of tDsg2. We therefore tested whether tDsg2 represents a proteolytically cleaved product of full-length Dsg2. Interestingly, it has recently been reported that cleavage of cadherin family members can be mediated by cysteine proteases during biological events such as apoptosis. Cadherin and Dsg1 cleavage by cysteine proteases has been shown to occur in the vicinity of the transmembrane domain to generate an ~100-kDa fragment. Furthermore, the extracellular domain has been reported to be released from the cell surface by action of metalloproteinase(s) (Steinhusen et al., 2001; Weiske et al., 2001; Weiske and Huber, 2005; Bech-Serra et al., 2006; Dusek et al., 2006). To determine whether tDsg2 is a cleavage product of Dsg2 as a consequence of spontaneous apoptosis (Sinicrope et al., 1996; Gitter et al., 2000), IECs were exposed to camptothecin, a drug that damages DNA and induces apoptosis. Cell lysates containing pooled floating and adherent cells were collected at time points from 0 to 24 h. The fate of Dsg2, Dsc2, and DSP was analyzed by Western blotting. We found that Dsg2 was cleaved within 24 h after camptothecin-induced apoptosis and a fragment with apparent molecular mass of ~100 kDa was increased in detergent extracts after 3 h of apoptosis induction (Supplemental Figure 3). The generation of this fragment correlated with the activation of caspase-3 and an increase in the cleavage of poly(ADP-ribose) polymerase (PARP), which are characteristic markers for apoptotic cells (Figure 3A). Stimulation of apoptosis was confirmed biochemically in analyses for caspase-3 and PARP cleavage products (Figure 3A). Analysis of the fate of Dsc2 after induction of apoptosis suggested that Dsc2 is also proteolytically targeted in apoptotic cells. Interestingly, Dsc2 and the scaffold protein DSP protein levels were decreased after 24 h of apoptosis induction (Figure 3A and Supplemental Figure 3), and no changes were detected during the onset of apoptosis. Interestingly, a Dsc2 cleavage product ~80 kDa was observed after 24 h of camptothecin treatment, suggesting that this protein is targeted during the late stage of apoptosis. Because cleavage of Dsg2 removes the cytoplasmic domain required for its localization and binding to DM plaque proteins, immunolocalization of Dsg2 and the desmosomal plaque protein DSP were performed after apoptosis induction. Initially, Dsg2 and DSP were distributed at sites of cell–cell contact (Figure 3B), and their expression was reduced after 24 h of apoptosis induction, consistent with the Western blot data.

Because proinflammatory cytokines such as IFN-γ have been shown to be physiologically relevant stimuli of the apoptotic pathway in IECs (Madara and Stafford, 1989; Adams et al., 1993; Youakim and Ahdieh, 1999; Bruewer et al., 2003), we examined the influence of IFN-γ on epithelial apoptosis. IFN-γ treatment did not induce irreversible cell necrosis (Bruewer et al., 2004). As has been reported previously, incubation with IFN-γ-induced proapoptotic markers in cells exposed to this cytokine compared with CTL monolayers or monolayers incubated with TNF-α alone (data not shown). Analysis of Dsg2 revealed an increase in tDsg2 after exposure of monolayers to IFN-γ compared with controls and TNF-α treatment. Although this effect is observed within 24 h of IFN-γ exposure, it is especially prominent at 72 h at which time point significant apoptosis is noted (Figure 3C). In contrast, Dsc-2 expression was indistinguishable from control monolayers at all time points (Figure 3C). IFN-γ treatment results suggest that specific cleavage of the Dsg2 cytoplasmic tail is observed not only after camptothecin treatment but also in response to another physiological
Figure 2. AH12.2 antigen recognizes an intracellular epitope in human Dsg2. (A) Predicted protein domain structure of tDsg2 in comparison to the full-length Dsg2. AH12.2 antigen was immunoprecipitated from T84 cells, concentrated and resolved by electrophoresis. AH12.2 antigen was identified by microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry to be human Dsg-2 and tDsg-2. Transmembrane domain is underlined. Putative epitope recognized by AH12.2 is marked with dashed lines. Mass spectrometry analysis revealed peptide fragments up to amino acid 772 of full-length Dsg-2 (of a possible 1117 amino acids). Sequence data indicated that tDsg2 lacks the ICS, IPL, RUD I-VI, and DTD domains of full-length Dsg2. (B) Double-immunolabeling studies were performed in T84 cells to localize AH12.2 (red) antigen in relation to the TJ protein, Occludin (green), DM protein, DSP (green) and AJ protein, E-cadherin (green). As shown in the en face confocal micrograph, AH12.2 antigen colocalized with DSP and E-cadherin. Bar, 10 μm. (C) Immunofluorescence labeling and confocal microscopy using AH12.2 and a commercial antibody against the extracellular domain of Dsg2 (6D8-Dsg2) in human T84 epithelial cells. The AH12.2 antigen colocalizes with the desmosomal cadherin Dsg-2. Cell lysates were immunoprecipitated with AH12.2 or with a commercial anti-Dsg2 antibody (6D8-Dsg2). Mouse IgG was used as a control. Immunoprecipitated proteins were immunoblotted with AH12.2 and 6D8-Dsg2. Both antibodies recognize the full-length Dsg2 and tDsg2 protein. (D) Immunofluorescence localization of AH12.2 antigen (green) and DSP (red) in T84 cells in nonpermeabilized cells (+) TX-100 reveals that AH12.2 antibody recognizes an intracellular epitope of Dsg2. Bar, 10 μm. (E) Schematic representation of tDsg2 and epitope for 6D8 and putative epitope for AH12.2.
apoptotic stimulus. These results suggest a relationship between onset of apoptosis and Dsg2 cleavage in IECs.

To identify the cleavage site(s) in the Dsg2 cytoplasmic domain after induction of apoptosis, camptothecin-treated T84 cell extracts were examined by Western blotting by using an antibody that recognizes an epitope in the DTD domain of the C terminus of Dsg2 (Getios et al., 2004a) (Figure 3D and Supplemental Figure 4). Two distinct cleavage products of Dsg2 with apparent molecular masses of ~65 and 60 kDa were detectable 3 h after induction of apoptosis with the 4B2 mAb that recognizes the C terminus of Dsg1 (Dusek et al., 2006) and Dsg2 (Supplemental Figure 4) (Getios et al., 2004a). Minimal baseline cleavage of endogenous Dsg2 (Figure 3D) or ectopically expressed Dsg2.myc tagged (Figure 7A) was observed without any treatment, suggesting that Dsg2 cleavage is not a bystander event dur-
Dsg2 Regulates Apoptosis in the Intestine

Figure 4. Dsg2 is proteolytically cleaved by cysteine proteases during onset of apoptosis. (A) Schematic representation of tDsg2 and putative epitopes for 6D8, 4B2, and AH12.2. (B) To further analyze Dsg2 proteolysis during apoptosis, T84 cells were incubated for 60 min with Z-VAD(OMe)-FMK or calpeptin before apoptosis induction. In apoptotic T84 cells, generation of the lower cleavage fragment recognized by 4B2 was maximal at 6 h after camptothecin treatment. The smaller fragment in the doublet protein bands (~60 kDa) declined over the next 24 h, suggesting further degradation. Analogous to the 4B2 antibody results, further analyses of Dsg2 cleavage by using the AH12.2 antibody revealed two cleavage products of similar Mr (~100 kDa) after apoptosis induction (Figure 3E). These results are consistent with Dsg2 targeting by a second cleavage event during onset of apoptosis. Additional experiments revealed that treatment of T84 monolayers with IFN-γ to induce apoptosis increased the presence of such cleavage fragments (data not shown), in a manner analogous to the camptothecin treatment.

Although the role of caspases in apoptosis have been clearly established, recent studies have suggested that other proteases may also play an important role in apoptosis (Johnson, 2000). Cleavage of the cadherin family members during apoptosis induction is mediated by calpain and caspase-3 (Steinhansen et al., 2001; Weiske et al., 2001; Rios-Doria et al., 2003; Rios-Doria and Day, 2005; Dusek et al., 2006). Thus, to determine whether Dsg2 cleavage is mediated by caspase(s) and/or calpain (Figure 4B), we cultured cells before camptothecin treatment with the membrane-permeable irreversible caspase inhibitor Z-VAD(OMe)-FMK, the membrane-permeable calpain inhibitor calpeptin, and an irreversible inhibitor of cysteine proteinases E64. Cell lysates were analyzed by Western blotting with AH12.2 and 4B2 6 h after Camptothecin treatment. In the presence of Z-VAD(OMe)-FMK, we observed the generation of the ~65-kDa fragment recognized by 4B2 and the ~100-kDa band detected by AH12.2. However, the lower fragment detected by AH12.2 (~90 kDa) and the ~60-kDa band detected by 4B2 were partially inhibited with Z-VAD(OMe)-FMK treatment (Figure 4B). In contrast, in the presence of calpeptin, generation of the ~65-kDa and the upper ~100-kDa fragment detected by AH12.2 were inhibited, whereas formation of ~60 kDa and the lower fragment detected by AH12.2 was also reduced (Figure 4B). The presence of E64 an irreversible inhibitor of cysteine proteinases, in contrast, decreased the generation of all Dsg2 protein fragments (data not shown). These findings support our conclusion that Dsg2 is targeted by at least two cysteine proteases. Taken together, these results suggest that Dsg2 is targeted in the C-terminal cytodomain by caspase(s), calpain or another cysteine protease during apoptosis of IECs (Figure 4A).

Surprisingly, we observed that cleavage of Dsg2 by caspase (or cysteine proteases) does not diminish total Dsg2 protein levels. Indeed, analyses of Western blots over short developmental periods (Figure 5A and Supplemental Figure 3), suggested that Dsg2 protein levels were increased during the onset of apoptosis. Moreover, the presence of Dsg2 was difficult to detect in Western blots that had been developed for a short time. This is likely due to an abundance of the full-length protein. To exclude the possibility of nonspecific binding of AH12.2 antibody, a second analysis using the commercial antibody 6D8-Dsg2 (against the extracellular domain of Dsg2) was performed and the results were similar to those obtained with AH12.2 (Figure 5A). Paradoxically, the increase in full length Dsg2 protein was inhibited after treatment of monolayers with the caspase inhibitor Z-VAD(OMe)-FMK. Indeed, immunofluorescence labeling analyses using the same antibodies revealed that the increased Dsg2 protein is distributed in the membrane (Figure 5B). Analogous to our Western blotting results (Figure 5A), decreased intensity of Dsg2 staining was observed when...
cells were treated with the caspase inhibitor Z-VAD(OMe)-FMK (Figure 5B, bottom). Apoptosis-induced reorganization of Dsg2 was associated with an increase in the overall Dsg2 protein levels as determined by Western blotting. These findings support an overall up-regulation of Dsg2 rather than its relocation. In keeping with the Western blotting results (Figure 3, A and C), we did not observe any changes in Dsc2 and DSP localization and/or expression by immunofluorescence labeling during the onset of apoptosis (Supplemental Figure 5). Moreover, Z-VAD(OMe)-FMK treatment did not influence the overall expression of Dsc2 and DSP (data not shown).

**Dsg2 Knockdown in Intestinal Epithelial Cells Is Associated with Decreased Apoptosis**

To further examine whether the presence of Dsg2 is involved in sensitizing epithelial cells to apoptotic cell death, we decreased expression of desmosomal cadherins by using short interfering RNA (SMARTpool siRNA). Because T84 cells are difficult to efficiently transiently transfected under standard conditions, we used SK-CO15 cells, a transformed human colonic epithelial cell line that forms confluent monolayers with high transepithelial resistance and that is readily transfectable with siRNA. Furthermore, we have observed that this cell line is highly responsive to apoptotic stimuli. To investigate the role of Dsg2 in cells undergoing apoptosis, SK-CO15 cells transfected with Dsg2 siRNA were exposed to camptothecin. Western blotting and Immunofluorescence analyses (data not shown) of SK-CO15 cells confirmed specific and significant reductions in AH12.2 (Dsg2) antigen protein levels compared with controls (Figure 6A). Lamin A/C and Dsc2 siRNA-transfected controls showed significant reductions in Lamin A/C and Dsc2 protein levels, respectively, without influencing AH12.2 antigen protein levels (Figure 6A).

As shown in the Figure 6, siRNA-mediated down-regulation of Dsg2, was associated with decreased cleavage of caspase 3 (Figure 6A) and PARP (data not shown) after induction of apoptosis as demonstrated by decreased intensity of protein staining bands in Western blots by using antibodies that recognize cleavage products of these proteins. Interestingly, knock-down of Dsg2 with siRNA did not inhibit the cleavage of caspase-3 to the same extent as Dsg2 knockdown after camptothecin treatment. Membrane permeability was evaluated by fluorescence microscopy using propidium iodine after the induction of apoptosis in SK-CO15 cells lacking Dsg2. Lamin A/C and Dsc2 siRNA were used as controls. As shown in Figure 6B, down-regulation of Dsg2 expression resulted in decreased cell death as demonstrated by PI stained cells compared with controls (Scramble, Lamin A/C, and Dsc2).

In contrast to Dsg2, we were not able to observe analogous regulation of apoptosis by the other DM cadherin, Dsc2. We do not observe changes in the localization, expression of Dsc2 in the same time frame as Dsg2 in the early phases of apoptosis. These observations imply a unique role of Dsg2 in regulation of onset of apoptosis in simple IECs. Perhaps, the large cytoplasmic tail of Dsg2 and its dissimilarity with Dsc2 accounts for the unique functional properties of these two proteins.

**Apoptosis Induction Is Associated with Increased Expression of Dsg2 and Presence of Its Cleaved Products**

Our results in T84 cells suggested that the C terminus of Dsg2 is proteolytically cleaved to yield a truncated Dsg2 and a cytoplasmic C-terminal fragment. As an initial approach to explore the cell biological functions of C-terminal of Dsg2, we transfected SK-CO15 cells with Flag-tagged full-length Dsg2 C terminus (Cter635–1117Flag) and the last 218 aa of Dsg2 C terminus (Cter218aa–1117Flag). Both constructs were Flag-tagged at the C terminus. We evaluated the effect of expressing both fragments on Dsg2 protein levels and survival of IECs. Immunofluorescence labeling was performed to examine the relative distributions of ectopic Dsg2,myc- Cter635–1117Flag-, and Cter218aa–1117Flag-tagged proteins. Dsg2,myc was organized in a punctate pattern (Figure 7A), colocalizing at cell–cell borders with the desmosomal plaque protein, plakoglobin (Pg) (data not shown), suggesting that the transfected protein was incorporated into desmosomes. In contrast the Cter635–1117Flag- and Cter218aa–1117Flag-tagged proteins were localized in the cytoplasm in a vesicular pattern (Figure 7A). As shown in the Western blots in Figure 7A, expression of Cter635–1117Flag and Cter218aa–1117Flag tag resulted in a protein product of ~60 and
Dsg2 Regulates Apoptosis in the Intestine

Figure 6. Down-regulation of Dsg2 expression inhibits epithelial cell apoptosis. A, SK-CO15 monolayers were transfected with lamin A/C, Dsg2, Dsc2 or Scramble, siRNA. Dsg2, Dsc2 and lamin A/C expression was determined by immunoblot and densitometric analysis after apoptosis induction. A significant decrease in AH12.2 antigen protein expression was detected in Dsg2 siRNA-transfected cells but not in lamin A/C-, Scramble-, or Dsc2 siRNA-transfected cells. Caspase-3 was analyzed after 24 h of apoptosis induction. Immunoblotting and densitometric analysis revealed decreased cleavage of caspase-3 in cells with Dsg2 knockdown compared with control siRNA-transfected cells (lamin A/C, Scramble control, or Dsc2). (B) Mean values for percentage of dead cells in lamin A/C-, Dsg2-, Dsc2-, or Scramble control-transfected cells by using propidium iodine staining. Decreased cell death was observed after inhibition of Dsg2 expression, supporting a resistance to apoptosis induction. The mean of four experiments is shown. Error bars are SEM **p < 0.0014.

As described above, Dsg2 C terminus was released from the membrane and localized in intracellular vesicular structures after transfection of the Dsg2 C-terminal constructs (Figure 7A). These findings suggest that cleavage of Dsg2 could be an early event in the disruption of desmosomes. To further determine this possibility, we examined the fate of Dsg2 C terminus relative to a desmosomal scaffold protein, Pg, by immunofluorescence labeling and confocal microscopy. For these experiments, IECs were treated with camptothecin for 6 h to induce apoptosis, and immunofluorescence labeling was performed using the 4B2 antibody that recognizes cleaved cytokeratin 18. Additionally, we evaluated the number of apoptotic cell nuclei per high-power field (X20). The percent of M30 staining cells and presence of apoptotic nuclei was ~twofold or greater in IECs transfected with the Dsg2 C-terminal constructs compared with control cells (Dsg2.myc and Mock) (Figure 7B). These findings support our hypothesis that cleavage of Dsg2 and generation of the C-terminal fragments of Dsg2 sensitizes cells to undergo apoptosis.

Induction of Apoptosis Induces Internalization of Dsg2 and Pg

As described above, Dsg2 C terminus was released from the membrane and localized in intracellular vesicular structures after transfection of the Dsg2 C-terminal constructs (Figure 7A). These findings suggest that cleavage of Dsg2 could be an early event in the disruption of desmosomes. To further determine this possibility, we examined the fate of Dsg2 C terminus relative to a desmosomal scaffold protein, Pg, by immunofluorescence labeling and confocal microscopy. For these experiments, IECs were treated with camptothecin for 6 h to induce apoptosis, and immunofluorescence labeling was performed using the 4B2 antibody that recognizes the intracellular domain of Dsg2. In cells incubated with media alone, Dsg2, DSP, and Pg largely localize in the lateral membrane with a small internalized component (Figure 8). In contrast, camptothecin treatment induces internalization of the Dsg2 C terminus (Figure 8). We also colabeled cells for Dsg2 and another plaque protein, DSP. After camptothecin treatment, increased internalization of Dsg2 was observed and Dsg2 does not colocalize with DSP (Figure 8). Interestingly, colabeling of Dsg2 with Pg revealed extensive colocalization of Pg with the internalized Dsg2 C terminus (Figure 8). However, the closely related armadillo family proteins β-catenin and p120, which localize predominantly

~27 kDa, respectively. The size of Cter635–1117Flag was similar to the size of the fragment observed after cleavage of Dsg2 (Figure 3D) and ectopically expressed Dsg2.myc (Figure 7A). A ~70-kDa band was recognized by the anti-Flag antibody which was also identified in control nontransfected cells, suggesting that this band is nonspecific. Furthermore, comparison of SK-CO15 control cells and SK-CO15–trans-

fected cells revealed the appearance of a new fragment ~55 kDa that reflects nonspecific cross-reactivity of the antibody with an unknown polypeptide. To assess whether the protein levels of endogenous Dsg2 are affected by Dsg2 C-terminal expression, cell lysates of SK-CO15 cells transfected with Cter635–1117Flag and Cter699–1117Flag were Western blotted using the AH12.2 antibody. Endogenous Dsg2 protein levels increased severalfold in response to Dsg2 C-terminal transfection, whereas Dsg2 protein levels were relatively unchanged in control and even in full-length Dsg2.myc-transfected cells (Figure 7A). 6D8-Dsg2 antibody revealed similar results (data not shown). Dsg2 C-terminal transfection did not influence Dsc2 (Figure 7A) and DSP (data not shown) protein expression. These results suggest that the C terminus of Dsg2 generated in cells regulates the overall Dsg2 protein expression and that the last 218 aa (Cter699–1117Flag) comprises the minimal fragment required to regulate Dsg2 expression. To further explore the biological function of Dsg2 C terminus in apoptosis, we evaluated its role in IEC apoptosis. We analyzed the cleavage of Caspase-3 by Western blotting in cells transfected with Cter635–1117Flag and Cter699–1117Flag (48 h after transfection). Expression of Cter635–1117Flag and Cter699–1117Flag induced up-regulation of active Caspase3 compared with control cells (Figure 7A), implicating a role of Dsg2 C terminus in the onset of apoptosis. To further evaluate this possibility, SK-CO15 were transfected with constructs expressing the Dsg2 C-terminal region and cells were evaluated for apoptosis. Because caspase-3 activation in epithelial cells is associated with cytokeratin 18 cleavage (Caulin et al., 1997), cells were labeled with the M30 antibody that recognizes cleaved cytokeratin 18. Additionally, we evaluated the number of apoptotic cell nuclei per high-power field (X20). The percent of M30 staining cells and presence of apoptotic nuclei was ~twofold or greater in IECs transfected with the Dsg2 C-terminal constructs compared with control cells (Dsg2.myc and Mock) (Figure 7B). These findings support our hypothesis that cleavage of Dsg2 and generation of the C-terminal fragments of Dsg2 sensitizes cells to undergo apoptosis.

The inhibition of Dsg2 expression, supporting a resistance to apoptosis

Dsc2- or Scramble control-transfected cells by using pro-

Immunoblotting and densitometric analysis revealed decreased

cleavage of caspase-3 in cells with Dsg2 knockdown compared with

control siRNA-transfected cells (lamin A/C, Scramble control, or

Dsc2). (B) Mean values for percentage of dead cells in lamin A/C-, Dsg2-, Dsc2-, or Scramble control-transfected cells by using pro-

podium iodine staining. Decreased cell death was observed after

inhibition of Dsg2 expression, supporting a resistance to apoptosis

induction. The mean of four experiments is shown. Error bars are

SEM **p < 0.0014.
in adherens junctions, did not colocalize with the internalized Dsg2 C terminus (data not shown), suggesting that Pg is associated with the internalized Dsg2 C terminus. That the internalized Dsg2 C terminus colocalized with Pg, but not DSP, suggests that interactions between desmosomal plaque components are disrupted during the onset of apoptosis as shown in other desmosomal disassembly models (Calkins et al., 2006; Cirillo et al., 2008). In further support of a possible role of Pg in early phase of apoptosis, we also observed an increase in the TX-100–insoluble pool of Pg (data not shown). Interestingly, analogous to Dsg2, we observed an increase in the Pg protein levels after induction of apoptosis (data not shown). Pg is known to regulate apoptosis in several cell lines (Dusek et al., 2007) and epithelial homeostasis (Williamson et al., 2006).

In summary our data are consistent with the following series of events: during apoptosis of epithelial cells, desmoglein-2 is efficiently targeted by two distinct, presumably...
Dsg2 is targeted in the cytosol near the transmembrane domain by cysteine proteases, thereby generating a cytosolic fragment that colocalizes with Pg. The remaining fragment, including the extracellular and transmembrane domain, however, still seems to remain affiliated with the lateral membrane. The shedding of the Dsg-2 extracellular domain mediated by metalloproteinase would then eliminate residual adhesive activity and result in complete disruption of Dsg2-mediated cell–cell adhesion (Bech-Serra et al., 2006). We propose that regulated cleavage of Dsg2 (Figure 9) likely represent an important event during extrusion of apoptotic cells from epithelial cell layers and that it is important in regulating Dsg2, e.g., during release of enterocytes at the tips of intestinal villi or during involution of epithelial structures such as the prostate and mammary gland.

DISCUSSION

Here, we report the existence of a truncated form of human Dsg2 in simple intestinal epithelial cells that is increased after induction of apoptosis. Our results suggest that tDsg2 is generated through cysteine protease cleavage. Cadherin family members that are targets of proteases have been found in multiple adhesive junctions during apoptosis. Cleavage of these substrates is thought to contribute to changes in cell morphology and decreased cell–cell adhesion associated with programmed cell death (Schmeiser and Grand, 1999). Here, we show that the cleavage of Dsg2 could have important signaling functions.

Intercellular junctions are crucial for maintaining the polarized architecture and functional composition of epithelial barriers. We have reported previously that components of the TJ are enriched in epithelial lipid rafts (Nusrat et al., 2000a; Bruewer et al., 2004) and in specialized plasma membrane microdomains with a unique lipid composition that makes them more rigid than phospholipid-enriched bulk plasma membranes (Brown and London, 1998; Nusrat et al., 2000b). The functional association between TJs and lipid rafts is further supported by evidence that mice deficient in caveolin-1 (a structural component of caveolar lipid rafts) have significantly smaller TJs in lung endothelial vessels (Schubert et al., 2002b). Enrichment of epithelial gap junction proteins in lipid rafts has been shown recently by other groups (Schubert et al., 2002a; Girao et al., 2004). Thus, we generated monoclonal antibodies against intestinal epithelial lipid raft fragments, and we used them to identify new proteins at sites of cell–cell contact that are enriched in such membrane microdomains. Our AH12.2 antibody recognized Dsg2 in lipid raft fractions of cultured epithelial cells, and it was also identified in native human colonic epithelial cells.

Our proteomic studies of purified AH12.2 antigen suggest the existence of an ~100-kDa cleaved product of Dsg2 lacking the C-terminal domain. We have termed this protein tDsg2, and we demonstrate its increase after apoptosis induction. A significant decrease in tDsg2 after the treatment of the IECs with the caspase inhibitor Z-VAD(OMe)-FMK was observed. These findings and another report (Cirillo et al., 2008) support involvement of a caspase(s) in Dsg2 cleavage. Whether tDsg2 is a caspase-induced proteolytic product
in apoptosis remains to be determined. We did not identify a caspase cleavage motif in the C-terminal tail of Dsg2, suggesting that other proteases may participate in the generation tDsg2. Unexpectedly, our results show that the proteolytically processed C-terminal domain exerts a stimulatory effect on Dsg2 protein levels. This result raises the possibility that the C terminus of Dsg2 acts as a signaling molecule or can increase the stability of Dsg2. Thus, the Dsg2 C terminus could both alter DMs and potentiate apoptosis. Consequently, Dsg2 may be an important part of a positive-feedback pathway regulating both Dsg2 expression and apoptosis in the IECs. Epithelial cell apoptosis and extrusion without loss of barrier function represent normal physiological events in the gastrointestinal tract during anokis. Dsg2 targeting by cysteine proteases could thus be a regulatory mechanism in the regeneration of intestinal epithelia as reported for E-cadherin (Fouquet et al., 2004). Interestingly, truncated species of Dsg2 have been reported in a previous study (Bech-Serra et al., 2006). However, in contrast to our findings, studies in another epithelial cell line, A431 documents appearance of tDsg2 that is not sensitive to caspase-inhibitors as is shown here (Bech-Serra et al., 2006). Thus, it is possible that the mechanism responsible for the production of tDsg2 could be tissue specific or those different fragments of similar size (~100 kDa) can be generated by Dsg2 proteolysis and under different physiological conditions, including apoptosis. Additionally, a bystander effect on papain-like cysteine proteases observed with caspase inhibitors has been reported. Thus, participation of other cysteine proteases is possible (Rozman-Pungercar et al., 2003). In addition to Dsg2, it has also been reported that Dsg1 and E-cadherin are cleaved during apoptosis (Vallorosi et al., 2000; Steinhusen et al., 2001; Dusek et al., 2006). This suggests that C-terminal cleavage is a feature of cadherins and that other members of this family may also have similar effects. Our IECs exclusively express Dsg2 and not Dsg1; therefore, the proapoptotic effects are exclusively mediated by Dsg2. In our studies, we did not analyze the C-terminal domains of other cadherin family members expressed in IECs, but it is possible that the C-terminal domains of other cadherins may act in other types of cells or may be transcriptional repressors or regulators of signaling. This would be consistent with previous findings that have highlighted a role of the cadherin family members as regulators of several physiological functions, including apoptosis. In aggregate, all of these observations suggest that generation of tDsg2 (Bech-Serra et al., 2006) is a regulated event that may play an important role in the maintenance of DMs homeostasis. Future studies are required to answer these important questions.

Contrary to Dsg2, the other DM cadherin in IECs termed Dsc2 did not show cleavage at early time points after induction of apoptosis by camptothecin or IFN-γ. Such differential effects on Dsg2 cleavage in IEC cells is likely related to the early time points after induction of apoptosis that were the focus of our studies here. Studies analyzing UV induced apoptosis have demonstrated analogous caspase induced cleavage of another Dsg, Dsg1 in a human skin keratinocyte cell line (Dusek et al., 2006) suggesting an important role of Dsg1 in the induction of apoptosis after exposure to noxious stimuli such as UV radiation. Interestingly, recent reports have shown that cadherins are capable of promoting cell survival and inhibiting apoptosis (Kantak and Kramer, 1998; Carmeliet et al., 1999; Vallorosi et al., 2000). Our studies thus demonstrate that Dsg2 regulates stimulus induced apoptosis in IECs analogous to Dsg-1 in keratinocytes (Dusek et al., 2006). Moreover, our results suggest that cysteine protease-induced cleavage of Dsg2 may play an active role in promoting or potentiating apoptosis in IECs. Dsg2 association with lipid rafts, might facilitate interaction of Dsg2 with such proteases. As demonstrated by others, the onset of apoptosis may require lipid raft localization for efficient cell death signaling (Herirns et al., 2005; Furne et al., 2006).

Rapid turnover of epithelial cells is observed in the gastrointestinal tract and barrier properties are maintained by a balance of physiological processes that regulate cell proliferation, differentiation and apoptosis. Integrity of intercellular junctions is a vital process for maintaining epithelial differentiation and barrier properties. It is now evident that intercellular junction proteins also regulate epithelial apoptosis. Apoptotic processing of intercellular junction proteins such as Dsg2 likely play an important role in physiological loss of epithelial cells in addition to removal of such cells following exposure to proinflammatory/proapoptotic stimuli. Such events would ensure efficient removal of damaged cells. Future studies are necessary to address the mechanism by which cleavage of Dsg2 contributes to signaling events that eventuate in apoptosis and Dsg2 homeostasis.

In conclusion, these findings suggest that apoptosis of intestinal epithelial cells is triggered by molecular events that induce cell detachment through activation of cysteine proteases targeting Dsg2. Currently, we cannot exclude the possibility that other proapoptotic molecular events are triggered by detachment of intestinal epithelial cells, as has been described in other cell types (Gilmore et al., 2000; Puthalakath et al., 2001). However, we propose that DM proteins play an important role in the induction of apoptosis during remodeling of epithelial cells.

**ACKNOWLEDGMENTS**

We are grateful to Drs. Jan Pohl (Emory Microchemical Facility) and Bill Lane (Harvard Microchemistry Facility) for interpretations of mass spectrometry data. We also thank Dr. Susan Voss for expert cell culture assistance and L. Matthew Winfree, A’Drian Pineda, and G. Thomas Brown for help in rafts purification. This work was supported by a Cronin’s and Colitis Foundation of America fellowship award (to A.M.H. and P.N.D.), National Institutes of Health (NIH) grants DK-55679 and DK-99888 (to A.N.), NIH DK-72564 and DK-61379 (to C.A.P.), DK-6499 (NIH Digestive Disease Research Center tissue culture and morphology grant), RO1 CA-122151 (to K.G.), and the German Research Foundation (Deutsche Forschungsgemeinschaft) LA 2359/1-1 (to M.L.).

**REFERENCES**


Dsg2 Regulates Apoptosis in the Intestine


