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REVIEW

Apoptosis in the intestinal epithelium: Its relevance in normal and pathophysiological conditions

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Abstract Apoptosis is now recognized as an important process responsible for maintenance of the cellular balance between proliferation and death. Apoptosis is distinct from necrosis in that it is a programmed form of cell death and occurs without any accompanying inflammation. This form of cell death can be induced by a wide range of cellular signals, which leads to activation of cell death machinery within the cell and is characterized by distinct morphological changes. Apoptosis is especially relevant in the gastrointestinal tract, as the mammalian intestinal mucosa undergoes a process of continual cell turnover that is essential for maintenance of normal function. Cell proliferation is confined to the crypts, while differentiation occurs during a rapid, orderly migration up to the villus. The differentiated enterocytes, which make up the majority of the cells, then undergo a process of programmed cell death (apoptosis). Although apoptosis is essential for the maintenance of normal gut epithelial function, dysregulated apoptosis is seen in a number of pathological conditions in the gastrointestinal tract. The cellular mechanisms regulating this tightly regimented process have not been clearly defined and this topic represents an area of active investigation as delineation of this process will lead to a better understanding of normal gut mucosal growth.

Key words: apoptosis, intestinal epithelium.

INTRODUCTION

Types of cellular death processes

Cell death occurs in multicellular organisms by either of two well-characterized mechanisms, depending on the context and cause of death. Until approximately 25 years ago, cell death was considered harmful and detrimental to the body, as the clearly identified mechanism of cell death was necrosis. Cells that undergo necrotic death release their cytoplasmic and nuclear contents into the intercellular milieu, thus sparking inflammation. In the early 1970s, Kerr *et al.* coined the term apoptosis (derived from the Greek word for 'falling off') for a characteristic type of cell death observed morphologically.[1] The studies on the nematode worm, *Caenorhabditis elegans*, served to reawaken interest in this field after the discovery of specific genes that control the apoptotic process during development.[2] This indicated that apoptosis, unlike necrosis, was a genetically controlled form of cell death and the floodgates of information on this topic were opened. Apoptotic cell death has since been identified as a novel physiological process that is conserved during evolution and plays an important role in deleting senescent, damaged, redundant and deleterious cells from multicellular organisms. It is a form of 'cell suicide' and although induced by a wide range of signals, is a tightly controlled process occurring in a regulated and sequential manner. The various signals inducing apoptosis and the pathways by which the cellular components bring about this process have been under intense investigation in recent years. This has resulted in a burst of information on the intricate relationship between various components of the apoptotic cascade and insight into the functioning and regulation of the apoptotic pathway. The widespread occurrence of apoptosis has provoked efforts aimed at defining the cellular

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mechanisms and biochemical processes controlling it, which, in turn, may help in formulating new therapeutic strategies.

Apoptosis occurs following a specific sequence of events

Apoptosis can be induced when cells receive death-inducing stimuli; for example, lack of growth or survival factors, oxygen deficiency, metabolic defects, physical and chemical damage and/or binding of particular ligands to death receptors, like Fas, on the cell surface.[3,4] Once the cell surface death receptors (e.g. Fas receptors) are activated, their cytosolic domains bind to adapter proteins such as the Fas-associated death domain protein, which, in turn, links up with caspase-8 to form the death-induced signaling complex (DISC;[5] Fig. 1).

The initiation stage of apoptosis is followed by a signalling stage, where mitochondria are known to play a central role.[6] During apoptosis, mitochondria undergo characteristic changes including a non-specific increase in membrane permeability, which develops gradually,[7] and a reduction of mitochondrial membrane potential,[8] which is accompanied by oxidative stress.[9] Cytochrome *c*, a component of the mitochondrial respiratory chain residing exclusively in the intermembrane space of the mitochondria, is released into the cytosol during apoptosis.[10–12] This release of cytochrome *c* from mitochondria can be blocked by Bcl-2,[13] an anti-apoptotic protein localized in mitochondria,[14] while Bax, a pro-apoptotic member of the Bcl-2 family that translocates from the cytosol to the mitochondria during apoptosis,[15] induces cytochrome *c* release.[16,17] Recently, a mitochondrial apoptosis-inducing factor that translocates from mitochondria to nucleus during apoptosis was characterized.[18]

This signalling phase is followed by the execution phase of apoptosis, during which the activated apoptotic machinery acts on multiple cellular targets. The final executioners of apoptosis are the caspases, which are cysteine-containing aspartate-specific proteases, activated during apoptosis in almost all cell types.[19] These proteases are synthesized as proenzymes and when activated by proteolytic cleavage during apoptosis,[19,20] specifically degrade a number of cellular substrates such as poly(ADP-ribose) polymerase, lamin B and DNA topoisomerases I and II.[21] Caspase-8, once activated in the multiprotein DISC complex, has two major functions. It can directly activate further down-stream caspases[22] which bring about the final morphological features of apoptosis[20,23] and also induce release of cytochrome *c* from the mitochondria.[24] The released cytochrome *c* binds to an apoptosis activating factor-1 (Apaf-1), which in turn activates caspase-9 and amplifies the proteolytic cascade.[14] Bcl-2 could play a role here too, by regulating Apaf-1 and preventing activation of down-stream caspases.[14]

The caspases thus bring about degradation of cellular components and activation of specific endonucleases which cleave DNA,[25,26] resulting in degradation of chromatin. Cross-linking of proteins by transglutaminase, an enzyme whose activity is modulated by Ca^{2+}, guanosine triphosphate (GTP), S-nitrosylation and polyamines,[27] keeps the apoptotic bodies intact during fragmentation of the cell.[28] Following this, the interactions of the individual cell with its neighbours become disorganized[29] and membrane changes are initiated in the

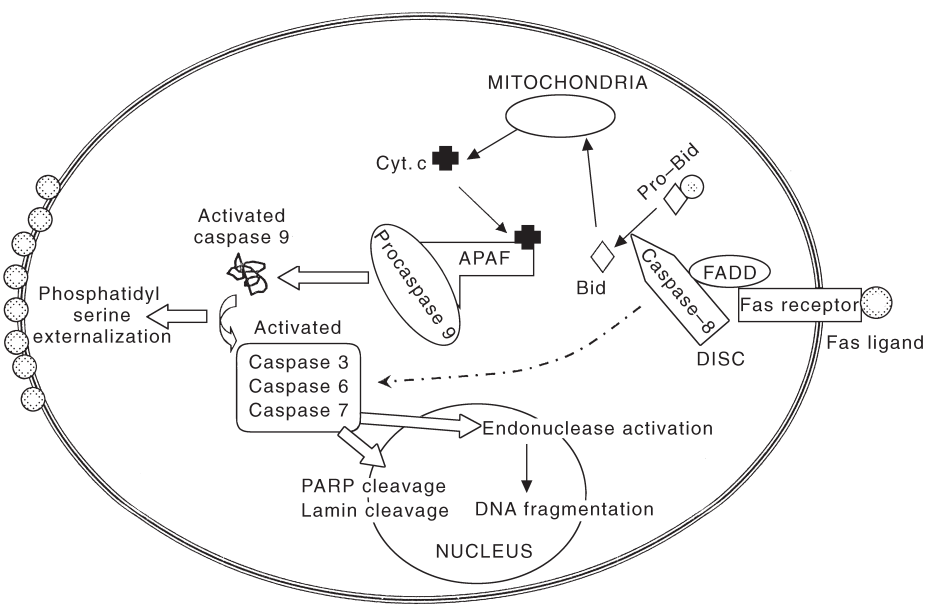

Figure 1 The signalling cascade during apoptosis. The attachment of Fas with its receptor on the cell surface leads to its binding with adapter proteins, such as the Fas-associated death domain (FADD). This complex then binds to and activates caspase-8, forming a multiprotein complex called the death-induced signalling complex (DISC). Caspase-8 can then either directly activate other members of the caspase family, or cleave pro-Bid to form Bid. Bid translocates to mitochondria and induces release of cytochrome *c*, which, along with the apoptosis activating factor 1 (Apaf-1) activates caspase-9. Caspase-9 can then activate down-stream caspases. The activated caspases bring about the final morphological features of apoptosis, such as DNA fragmentation and phosphatidylserine externalization. Cyt. c, cytochrome *c*.

apoptotic cell,[21] which enable apoptotic cell uptake by macrophages.[30,31] Thus, apoptosis occurs without leakage of intracellular macromolecules,[32] and without any inflammation.

Reactive-oxygen species are important physiological effectors of apoptosis

Reactive-oxygen species (ROS) such as superoxide anion, hydrogen peroxide, organic peroxides and radicals are generated by cells as by-products of normal metabolism and these compounds have now been implicated as signalling molecules in apoptosis. Studies have demonstrated that apoptosis appears to be the major mode of cell death when cells experience a lethal oxidative insult from exposure to ROS.[33] Hydrogen peroxide from hepatocytes has been shown to induce apoptosis in sinusoidal endothelial cells in the liver[34] and H_2O_2 also mediates neutrophil apoptosis.[35] Hydrogen peroxide-mediated and iron-catalysed lysosomal rupture causes decompartmentalization of lysosomal enzymes, which may play a role in modulating the apoptotic process.[36] Oxidized low-density lipoproteins and lipid hydroperoxides have also been shown to induce apoptosis.[37,38]

Other than oxidants from the external medium, ROS generated within the cell itself can modulate the apoptotic process. Reactive-oxygen species generated at the ubiquinone site of the mitochondrial respiratory chain have been implicated as mediators in ceramide-induced apoptosis.[39] Fas ligation induces ROS production, which could be prevented by an NADPH oxidase inhibitor, implicating this oxidase system in generation of ROS during apoptosis.[40] Superoxide may be produced from mitochondria due to a switch over from the normal four-electron reduction of O_2 to a one-electron reduction when cytochrome *c* is released from the mitochondria. Bcl-2 is able to prevent this cytochrome *c* release as well as the superoxide generation.[33] Bcl-2 has also been shown to protect against oxidant-induced cell death by increasing the capacity of mitochondria to store calcium.[41] Oxidants have been implicated in p53-mediated apoptosis also[42] and p53 probably induces synthesis of specific proteins that increase the cellular ROS content, leading to mitochondrial damage and further down-stream events.[43] The biochemical mechanisms that can be activated by ROS during induction of apoptosis, may be seen at several central points in the cellular apoptosis regulatory systems and recent data also implicate ROS in a dual role in apoptosis: first, as a facultative signal during the induction phase; and second, as a common consequence of mitochondrial permeability transition, leading to final destruction of the cell.[44]

APOPTOSIS IN THE GASTROINTESTINAL TRACT

Epithelial cells in the gastrointestinal tract have a high turnover and as it is essential to maintain a normal cell balance, cell death, especially apoptosis, is crucial for maintenance of normal morphology and function. Despite being inconspicuous in histological material, apoptosis probably accounts for the bulk of cell loss in the gut and is a central feature of the regulation of cell numbers in adult tissues.[45] Hence, it is interesting to understand the role of apoptosis, its inducers and regulation, with reference to the gastrointestinal (GI) tract. The intestinal villi consist of differentiated absorptive cells that originate from the intestinal crypts. The crypt stem cells proliferate to produce the epithelial cells, which migrate upward and proliferate several times before reaching villi as differentiated epithelial cells (Fig. 2). Given the existence of this dynamic system, where cell proliferation has to be counterbalanced by cell death, the gastrointestinal tract offers an ideal system to study the apoptotic process *in vivo*. Apoptosis maintains the structure of the colonic crypts by providing a balance to the rate of cell proliferation. Defective apoptosis, which results in the failure of cells to die in response to premalignant damage, may allow the progression of disease and maintain the resistance of colon cancer cells to cytotoxic therapy,[46] again illustrating the importance of apoptosis in the GI tract. Thus, it is of interest to know the mechanism and the various physiological inducers of apoptosis, as well as the role apoptosis plays in pathological states of the gastrointestinal tract.

Physiological occurrence of apoptosis in the gastrointestinal tract

The intestinal mucosa has cells at various stages of differentiation from the immature crypt stem cells to the differentiated cells of the villus. Feedback signals from the villus to crypt cells regulate proliferation and these seem to be mediated by growth factors, such as epidermal growth factor, transforming growth factor-alpha and -beta3 (TGF-α, TGF-β3), mainly in the crypts.

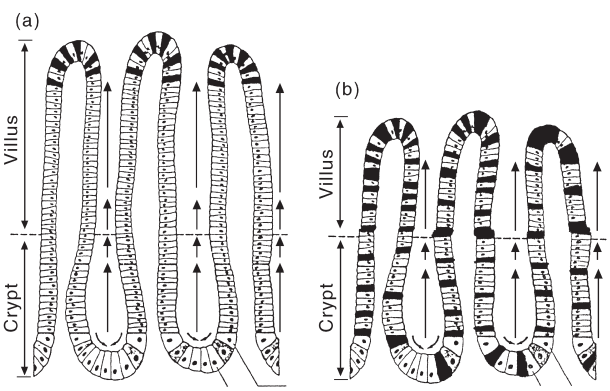

Figure 2 Schematic representation of the intestinal epithelium in (a) normal and (b) pathological conditions. Normally, the differentiated villus cells at the tip undergo apoptosis (■) in order to maintain homeostasis as the rapidly proliferating cells are at the crypt. In pathological conditions, excessive apoptosis of villus cells can cause shortening of the villi.

Although TGF-α stimulates, and TGF-β inhibits proliferation,[47] TGF-β resistant cells were found to lose contact inhibition and develop resistance to butyrate-induced apoptosis.[48] An autocrine TGF-β loop has been proposed to mediate the anti-proliferative effect of interleukin (IL)-2 in the intestinal epithelial cell (IEC) line, IEC-6.[49] Given the presence of cells at different stages of maturation along the crypt–villus axis, it seems logical that the differentiated villus cells are likely to die by apoptosis and be extruded into the lumen or taken up by macrophages and removed, but demonstration of this phenomenon has been riddled with controversy. Although studies have shown apoptosis in crypt stem cells,[50] work from our laboratory and others indicates that apoptosis occurs predominantly in the villus tip cells.[51,52] Apoptosis has been shown to occur in the rat small intestinal mucosa, predominantly at the villus tip, forming a prominent 'apoptotic cuff' of cells, while all cells in the crypt expressed Bcl-2 activity.[53] Immunoreactivity for Bcl-2 or Bax was found to be intense in cells that were prone to becoming apoptotic next in the course of cell turnover, but not in cells in an advanced apoptotic state.[54] In chicken caecal epithelium, apoptotic cells with intense DNA fragmentation were seen at the villus tip, suggesting that these cells were exfoliated into the lumen after the induction of apoptosis, a process probably mediated by lymphocytes.[55] Macrophages and lymphocytes have been implicated in the removal of apoptotic cells from the villus tip.[56] Transmission electron microscopy studies have shown protruding enterocytes at the villus tip, with nuclear chromatin condensation, immediately before being exfoliated into the lumen. The intercellular spaces beneath these protruding cells are often occupied by large lymphocytes,[57] which probably do not engulf aged enterocytes, but induce their apoptosis.[58]

Another cytokine with an important role in apoptosis in the GI tract is tumour necrosis factor (TNF). Tumour necrosis factor has been shown to induce apoptosis and detachment of enterocytes of the villus tip in mice,[59] probably by induction of caspase activity.[60] Tumour necrosis factor-α has also been shown to induce apoptosis in IEC-66 cells, although the cells died by necrosis when the caspase cascade was inhibited.[61] It has also been suggested that, in order to ensure rapid epithelial renewal of the villi and maintenance of a functional barrier, IEC eliminate infected and senescent cells through a combination of cytotoxicity, interferon (IFN)-γ and TNF release.[59] Tumour necrosis factor probably acts on its receptor 1 (TNFR1) to induce apoptotic detachment of the enterocytes from the tip of the villi, while increasing expression of p53 via either TNFR1 or TNFR2 in the crypt, to inhibit apoptosis.[62] Human tumour (HT)-29 cells were found to display a dual response to TNF-α and Fas antigen ligation: when in combination with interferon (IFN)-γ, HT-29 cells underwent apoptosis, whereas independently these factors stimulated secretion of IL-8.[63]

Although a large amount of data on the mechanisms and inducers of apoptosis are available, most of the work has been on cell lines and little information is available on the mechanisms and the inducers involved in apoptosis of the enterocyte *in vivo*. Studies from our laboratory have demonstrated a possible role of oxidative stress in the monkey normal villus epithelial cell apoptosis.[64] It was seen that there was an imbalance between oxidants and anti-oxidants, which can activate apoptosis in the villus cells while the crypt stem cells were normal. The increased apoptosis in the villus enterocytes were also associated with increased efflux of glutathione[51] and low activity of superoxide dismutase compared with crypt stem cells.[64] This compromised anti-oxidant defence may affect the neutralization of ROS in the villus cells and may facilitate their apoptosis. Our studies further indicated structural and functional changes in the mitochondria associated with apoptosis of the villus tip cells.[65] It was observed that villus mitochondria had a lower respiratory control ratio compared with crypt cells and they also showed an increase in the mitochondrial permeability transition. Mitochondrial lipid analysis indicated activation of a phospholipase D enzyme in the apoptotic villus cells, which probably facilitated the functional alterations.[65] Phospholipase D activation was found to occur in a time-dependent manner during apoptosis and this activation, as well as apoptosis, was effectively suppressed by caspase inhibitors.[66] The secretory phospholipase A2 inhibitor *p*-bromophenacyl bromide was also seen to produce a dose-dependent increase in apoptotic ratios in both IEC-6 and colon cancer cell line WB-2054-M4.[67]

Detachment-induced cell death can occur in a number of cell types, and although it is a recognized form of apoptosis in IEC,[68] whether the cells detach before actually dying is still controversial. A number of studies support this form of apoptosis and it is said to be one of the mechanisms by which IEC die as they reach the villi of the intestine before being extruded into the lumen or taken up by macrophages. DNA fragmentation was observed 90 min after detachment of human IEC and was preceded by the sequential activation of preformed members of the CPP32 family of caspases.[69] Apoptosis was seen in mouse intestinal cells 90 min after detachment and this was prevented by caspase inhibitors,[70] indicating that activation of caspases plays a role in the apoptosis of these cells. Although a recent report indicated that DNA fragmentation could precede cell elimination by days in the small intestinal villus,[71] the technique used for detection of apoptotic cells in the study, namely *in situ* end labelling, is prone to false results.

The link between differentiation and apoptosis has also been demonstrated in the intestine derived cell lines and these were found to occur simultaneously in Caco-2 cells, suggesting that apoptosis may be linked to enterocyte differentiation. The induction of $p21^{Waf1/Cip1}$ and $p27^{Kip1}$ and the down-regulation of Bcl-2 and Bcl-XL further suggest a link between the cell cycle mechanisms regulating enterocyte differentiation and apoptosis.[72] Interferon-γ, depending on its concentration, has both differentiation- and apoptosis-inducing effects on the colon derived HT-29 cells; low concentrations induce differentiation while higher concentrations induce apoptosis.[73] Another study using

the colorectal adenoma cell line PC/BH/C1 showed that butyrate-induced apoptosis was preceded by the induction of two markers of colonic differentiation, namely alkaline phosphatase activity and E-cadherin, which were not seen in TGF-β1-induced cell death. The study concluded that sodium butyrate induced apoptosis via differentiation, but that TGF-β1 induced apoptosis by a differentiation-independent mechanism.[74]

Another inducing factor for apoptosis in the GI tract is lack of nutrients for cellular metabolism. Glutamine is the most abundant amino acid in the blood and is the preferred oxidative fuel for IEC. Glutamine deprivation has been shown to induce apoptosis in IEC,[75] suggesting that glutamine serves as a specific survival factor for enterocytes. Butyrate is the preferred fuel for colonic epithelial cells and removal of butyrate induces increased expression of bax proteins, paralleled by rapid apoptosis of colonocytes *in vitro*.[76]

Apoptosis in pathological states of the gastrointestinal tract

Apoptosis plays an important role in a number of pathological conditions of the gastrointestinal tract and this could be due either to an increase or decrease in the rate of cell death. For example, enterocyte apoptosis was found to be greatly increased in coeliac disease, indicating that increased apoptosis may be responsible for the villous atrophy seen in this disease.[77] Loss of epithelial cells in active ulcerative colitis (UC) occurs mainly by apoptosis in crypts of the involved and adjacent uninvolved areas[78] and this unscheduled apoptosis in the UC crypts is probably mediated by autocrine or paracrine Fas–Fas ligand (FasL) interaction.[79] Soluble FasL-mediated epithelial cell apoptosis in UC may also lead to the breakdown of epithelial barrier function, facilitating the invasion of pathogenic microorganisms,[80] a phenomenon also seen in apoptosis induced by doxorubicin, where increased apoptosis and increased bidirectional permeability of the intestinal barrier occur simultaneously.[81] Decreased apoptosis has been implicated in the pathogenesis of gastric carcinoma by the finding that microsatellite mutations in the *Bax* gene are common in these patients, suggesting that Bax may be an important apoptotic inducer and tumour suppressor in the stomach.[82]

Ischaemia–reperfusion as an inducer of apoptosis in the gastrointestinal tract

Ischaemia–reperfusion is a well-characterized clinical entity, which is known to induce tissue damage, especially during conditions such as transplantation. The small intestine is highly sensitive to ischaemia–reperfusion and we have previously shown that the rat intestinal mitochondria was irreversibly damaged by long periods of ischaemia or ischaemia followed by reperfusion, while the damage induced by short periods of ischaemia was reversible.[83] Studies have now shown that apoptosis in the rat small intestine is induced by ischaemia of the gut and this process is exacerbated by reperfusion.[84] Apoptosis after ischaemia–reperfusion was also demonstrated in transplantation of intestinal grafts in the rat.[85]

Cancer of the gastrointestinal tract and the role of Bcl-2 family proteins in dysregulated apoptosis

Maintenance of normal cell numbers in tissue requires a balance between the rate of cell division and cell death. Uncontrolled cell proliferation seen in malignancy could be the result of an increased proliferation and/or decreased apoptosis. In normal colonic mucosa, Bcl-2-positive epithelial cells tend to be localized to the base of the crypt, while Bax- and Bak-positive epithelial cells are located at the luminal surface.[86] Bcl-2 is an important anti-apoptotic protein, which can even prevent caspase-independent cell death.[87] Many colorectal tumours have elevated levels of Bcl-2, suggesting that the deregulated Bcl-2 expression may be important in tumour development.[88] It is possible that nutritional stress conditions in colonic tumours may contribute to Bcl-2 up-regulation[89] and Bcl-2 may play an important role in the early stage of the adenoma–carcinoma sequence, down-regulation of Bcl-2 being associated with risk of malignant transformation for colorectal adenoma.[90] Although the Bcl-2 oncoprotein inhibits apoptosis in adenoma and adenocarcinoma, mutations in another important tumour suppressor, the p53 oncoprotein might block apoptosis in adenocarcinoma.[91] p53 also plays a role in 5-fluorouracil-induced apoptosis in crypts of both the small intestine and midcolon,[92] and is accompanied by changes in RNA metabolism, which probably initiate events culminating in the expression of p53.[93] But again, p53 is not frequently found to be mutated in colorectal carcinomas with the microsatellite mutator phenotype, where frame-shift mutations within the gene for a pro-apoptotic member of the Bcl-2 family, *Bax*, are frequently found. This suggests that mutations in *Bax*, rather than mutations in p53, may contribute to the adenoma–carcinoma transition in hereditary non-polyposis colorectal cancer tumorigenesis.[94] *Bax* expression may be an additional prognostic marker in colorectal carcinomas[86] and butyrate deprivation increased expression of *Bax* in guinea-pig proximal colon, accompanied by oligonucleosomal DNA fragmentation and massive apoptosis of colonocytes.[95] Expression of *Bax* was strong in colonic adenocarcinomas[96] and more than 50% of human microsatellite mutator phenotype colon adenocarcinomas were found to have frame-shift mutations in the *Bax* gene, suggesting that wild type *Bax* plays a suppressor role in a p53-independent pathway for colorectal carcinogenesis.[97,98] Another member of the Bcl-2 family is *Bak*, which is an important regulator of apoptosis in normal IEC[99,100] and has been found to be down-regulated in a high proportion of colorectal tumours.[101] Activation of the oncogene *Ras*, induces the down-regulation of *Bak* in human IEC and this is probably important for the transforming ability of the *Ras* oncogene.[102]

Apoptosis and inflammatory bowel disease

A number of immune system-mediated bowel disorders, including coeliac disease, Crohn's disease and UC are characterized by accelerated epithelial cell turnover and apoptosis, leading to altered crypt/villus morphology. These changes, and the accompanying functional alterations of the bowel epithelium, are probably mediated by the cytokines released from infiltrating inflammatory cells, as well as from enterocytes themselves, acting in an autocrine fashion.[103] Polymorphonuclear neutrophil (PMN) apoptosis may be delayed under the influence of soluble mediators, especially granulocyte-colony stimulating factor, in the microenvironment of inflammatory bowel disease (IBD)-affected mucosa, thus providing a possible mechanism for tissue accumulation of PMN in IBD.[104] Activated macrophages are derived from circulating monocytes and appear to play a major role in the pathogenesis of IBD and are an important mechanism for loss of resident macrophages from normal mucosa in apoptosis.[105] T cells isolated from areas of inflammation in Crohn's disease, UC and other inflammatory states manifest decreased apoptosis and studies of cells from inflamed Crohn's disease tissue indicate that this defect is accompanied by elevated Bcl-2 levels.[106] The Bcl-2/Bax ratio is significantly higher in Crohn's disease and lower in UC, indicating that Crohn's disease may represent a disorder where the rate of cell proliferation exceeds the rate of cell death and insufficient T cell apoptosis may result in inappropriate T cell accumulation, causing changes in tolerance and chronic inflammation.[107] Fas ligation induces apoptosis of colonic epithelial cells and is implicated in the epithelial damage seen in UC, whereby Fas–FasL interactions in the intestinal mucosa may lead to complex signal transduction cascades and gene regulation that culminate in apoptosis.[108] Fas is expressed constitutively by colonic epithelial cells, and FasL is expressed by CD3 lymphocytes infiltrating into UC lesions, suggesting that Fas–FasL induced apoptosis participates in the mucosal damage of UC.[109] Accelerated epithelial cell turnover caused by chronic inflammation and epithelial cell damage might predispose the mucosa to DNA damage, resulting in an elevated risk of mutation in line with dysplasia and carcinoma development in patients with UC.[110] Colon cancer cells have been found to express functional FasL,[111] suggesting that this has a role in bestowing immune privilege on human tumours and that these cells are insensitive to the anti-FasL agonistic monoclonal antibody. This indicates that cancer cells resist Fas-mediated cytotoxicity but express functional FasL, an apoptotic death signal to which activated T cells are inherently sensitive.[112]

Bacterial infection and apoptosis

Epithelial cells that line the human intestinal mucosa are the initial site of host invasion by bacterial pathogens. Human colon epithelial cells undergo apoptosis following invasion with invasive enteric pathogens such as *Salmonella* or entero-invasive *Escherichia coli*. This apoptosis occurs 6 h after bacterial infection and TNF-α and nitric oxide (NO) are important mediators in this process.[113] Verotoxin-producing, attaching and effacing *E. coli* also induce apoptosis in infected enterocytes.[114] Shiga toxin from *Shigella dysenteriae* and Shiga-like toxin from enterohaemorrhagic *E. coli* cause apoptosis in the mature, differentiated absorptive villus epithelium of rabbit intestine.[115]

Shigella species cause bacillary dysentery in humans by invading epithelial cells of the colonic mucosa, which leads to colonic epithelial cell destruction and inflammation. The acute phase of shigellosis inflammation is characterized by increased cell turnover in the lamina propria and the epithelium, and apoptosis of the lamina propria macrophages.[116,117] Invasion of the intestinal barrier by *Shigella flexneri* involves complex interactions with epithelial and phagocytic cells[118] and initial bacterial entry occurs essentially via M cells of the follicular-associated epithelium. It then causes apoptosis of macrophages located in the follicular dome, inducing release of IL-1β, which in turn initiates inflammation and destabilization of epithelial structure.[119,120]

Toxigenic strains of the anaerobic bacterium *Clostridium difficile* produce at least two large, single-chain protein exotoxins involved in the pathogenesis of antibiotic-associated diarrhoea and colitis. Intestinal cells exposed to toxin B underwent apoptosis with nuclear fragmentation and chromatin condensation[121] and exposure to toxin A induced epithelial cell rounding, detachment and apoptosis in organ cultures of human colonic biopsy specimens.[122] Exposure of HT-29 colon carcinoma cells to *Clostridium difficile* toxin A for 24 h also induced IL-8 production.[122]

The bacterium, *Helicobacter pylori* has been under intense scrutiny recently, having been implicated in the aetiology of various conditions. *Helicobacter pylori* colonization of the stomach has been found to result in more apoptotic cells than normal ones[123,124] and this reverted back to normal on eradication of the organism.[123] *Helicobacter pylori* is also capable of inducing apoptosis in epithelial cells *in vitro*.[124] Although the exact mechanism by which *H. pylori* induces apoptosis is not known, the available data indicate that it may be through various mediators. Monochloramine (NH_2Cl) is known to be one of the virulence factors in *H. pylori*-associated gastric mucosal injury and monochloramine was found to induce DNA fragmentation, one of the characteristic features of apoptosis, in the gastric cell line MKN45.[125] Adherence also seems to be necessary for apoptosis induction,[126] but high doses of purified *H. pylori* lipopolysaccharide can also induce apoptosis[124] and major histocompatibility class II molecules have also been implicated in the process.[127] The immune response associated with infection may also influence apoptosis after *H. pylori* infection. Inflammatory mediators, such as IFN-γ and TNF-α, augment apoptosis induced by *H. pylori*[128,129] and IFN-γ has been shown to up-regulate the Fas receptor on gastric epithelial cells.[129] Consequent to *H. pylori* infection, elevated expression of Fas antigen was seen in mucosal cells, concurrent with Fas ligand expressing lymphocytes. Thus, apoptosis in *H. pylori*-associated gastric and duodenal ulcer disease may be mediated by the Fas pathway.[130] Apoptosis induced by *H. pylori* in gastric epithelial cells was also accompanied by increased

expression of *Bak*, with little change in expression of other Bcl-2 family members. The expression of *Bak* was also increased in gastric biopsies from patients colonized by *H. pylori*. It appears that *H. pylori* induces gastric epithelial cell apoptosis by a *Bak*-dependent pathway.[126]

Radiation-induced apoptosis

The radiosensitivity of proliferating crypt epithelial cells makes the gut a major limiting factor in the use of radiotherapy for treatment of abdominal cancers. As the post-mitotic epithelial cells migrate from the small intestinal crypts to the base of adjacent villi they rapidly lose their ability to undergo apoptosis in response to ionizing radiation and, hence, it is the crypt cells that are more susceptible to radiation-induced apoptosis.[131] Various studies have shown an increase in apoptosis after radiotherapy, especially in the crypts. Very small doses of radiation can elevate the level of apoptosis in the crypt stem cells and the oncoprotein p53 is involved in recognizing radiation-induced damage and initiating apoptosis. This apoptosis plays an important role in the gut in terms of homeostasis and its protection against carcinogenesis by removal of potentially carcinogenically damaged cells.[132] The level of apoptosis was dramatically elevated 3–6 h following ionizing radiation exposure, most frequently in the crypt-associated stem cells,[133] but loss of p53 essentially rendered epithelial cells, from both the small intestine and colon, radioresistant.[134] p53 function seems to be essential for the normal response to gamma-irradiation-induced DNA damage in intestinal mucosal cells, and p53 deficiency permits a population of cells bearing DNA damage to escape the normal process of deletion.[135] However, other studies have indicated the existence of a delayed onset, p53-independent apoptotic mechanism, probably by mitotic catastrophe, at radiation doses of 8 Gy or more.[136] Differences have also been noted in the occurrence of radiation-induced apoptosis in the small and large intestine. Thus, it was seen that 3 h after whole-body irradiation of 8 Gy, the expression of p53 protein, as well as apoptosis, was much higher in the stem cell compartment of small intestine than in the colon.[134] Also, in the small intestine, peak levels of radiation-induced apoptosis appeared earlier than in the large intestine.[137] Small but statistically significant differences in radiation survival curves were observed along the length of the small intestine, but no difference was observed between the different regions of the large intestine.[138] Analysis of $(CcS\text{-}7 \times BALB/cHeA)F_2$ hybrid mice revealed linkage of susceptibility to radiation-induced apoptosis of colon crypt cells to two loci on chromosomes 9 and 16.[139] Studies have also shown that IL-11 can exert a potent effect on the recovery of small intestinal mucosa after treatment with combined chemotherapy and radiation by its combined effects on the proliferation and apoptosis of crypt cells.[140]

Nitric oxide and apoptosis in the gastrointestinal tract

Nitric oxide is an important molecule with diverse roles, now being studied with great interest due to its role in modulation of apoptosis. It has been shown to induce apoptosis in cells such as macrophages and T84 tumour cells,[141,142] but prevent apoptosis in others.[143] Nitric oxide has an apoptosis-inhibiting effect mediated through the inhibition of caspases by S-nitrosylation[144,145] and NO also inhibits Fas-induced apoptosis.[146] Nitric oxide was found to reversibly inhibit the respiration of IEC and elevate the levels of Bcl-2. As Bcl-2 is an anti-apoptotic protein, this elevation may protect enterocytes against toxic effects of NO and, thus, NO might effectively exhibit its antibacterial action in the anaerobic intestinal lumen without inducing apoptosis of Bcl-2 enriched mucosal cells.[147] Nitric

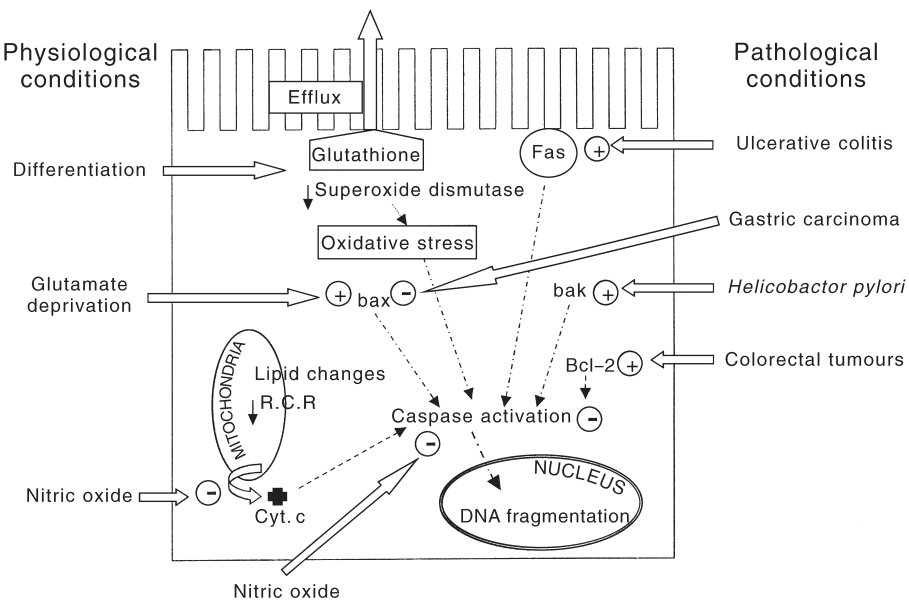

Figure 3 Epithelial cell apoptosis in the gastrointestinal tract. Apoptosis can be induced in the epithelial cell under both physiological and pathophysiological conditions and these are mediated by various effector molecules in the cell. RCR, respiratory control ratio; Cyt. c, cytochrome *c*.

oxide has also been found to protect B lymphocytes from antigen-mediated apoptotic death, probably by preventing the drop in the expression of the proto-oncogene Bcl-2,[148] and inhibit spontaneous apoptosis in cultured ovarian follicles via a c-guanidine monophosphate (cGMP)-dependent mechanism.[149] It has been shown that NO prevents apoptosis in hepatocytes by inhibiting caspase-3-like protease activation through a cGMP-dependent mechanism and by direct S-nitrosylation of caspase-3.[150] Kim *et al*. have shown that NO inhibits cytochrome *c* release by preventing Bcl-2 cleavage by caspase-3.[11] Recently, NO was shown to inhibit execution of apoptosis at two distinct ATP-dependent steps up-stream and down-stream of mitochondrial cytochrome *c* release. The release of cytochrome *c* from mitochondria was delayed and the processing of procaspase 3 and 7 to the active protease was prevented even after cytochrome *c* was released.[151] Our studies showed that NO prevented damage induced by intestinal ischaemia–reperfusion[152] and our recent studies have indicated that NO can protect against hypoxia-induced apoptosis in HT-29 colon carcinoma cells by inhibiting cytochrome *c* release from mitochondria.[153]

In conclusion, it appears that the apoptotic process plays an important role in the normal dynamic process of cell turnover in the intestinal epithelium. The apoptotic process in the IEC is a complex process involving a number of mediators (Fig. 3). As the mammalian intestinal mucosa undergoes a process of continuous cell turnover, where cell proliferation is confined to the crypts and differentiation occurs during a rapid, orderly migration up to the villus, the increased proliferation has to be counterbalanced by a similar rate of cell death. The differentiated enterocytes, which make up the majority of cells in the intestinal epithelium, probably undergo a process of apoptosis at the villus tips to maintain tissue homeostasis. Increased apoptosis also plays an important role in damage induced by ischaemia–reperfusion injury and IBD. Apoptosis is also important for removal of damaged cells after radiation injury and bacteria use apoptosis to induce death of host cells and enable infection. A decreased rate of apoptosis has been implicated in carcinoma, where uncontrolled proliferation leads to disease.

REFERENCES

1 Kerr JFR, Wyllie AH, Currie AR. Apoptosis: A basic biological phenomenon with wide ranging implications in tissue kinetics. *Br. J. Cancer* 1972; **26**: 239–57.
2 Shaham S, Horvitz HR. Developing *caenorhabditis elegans* neurons may contain both cell-death protective and killer activities. *Genes Dev*. 1996; **10**: 578–91.
3 Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immunol. Today* 1994; **15**: 7–10.
4 Nagata S, Goldstein P. The Fas death factor. *Science* 1995; **267**: 1449–55.
5 Muzio M, Chinnaiyan AM, Kischkel FC *et al*. FLICE, a novel FADD-homologous ICE/CED-3-like protease is recruited to the CD95 (Fas/Apo-1) death-inducing signaling complex. *Cell* 1996; **85**: 817–27.
6 Susin SA, Zamzami N, Kroemer G. Mitochondria as regulators of apoptosis: Doubt no more. *Biochem. Biophys. Acta* 1998; **1366**: 151–165.
7 Lemasters JJ, Nieminen AL, Qian T *et al*. The mitochondrial permeability transition in cell death: A common mechanism in necrosis, apoptosis and autophagy. *Biochim. Biophys. Acta* 1998; **1366**: 177–96.
8 Zamzami N, Marchetti P, Castedo M *et al*. Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death *in vivo*. *J. Exp. Med.* 1995; **181**: 1661–72.
9 Backway KL, McCulloch EA, Chow S, Hedley DW. Relationships between the mitochondrial permeability transition and oxidative stress during ara-C toxicity. *Cancer Res*. 1997; **57**: 2446–51.
10 Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: Requirement for dATP and cytochrome *c*. *Cell* 1996; **86**: 147–57.
11 Kim YM, Kim TH, Seol DW, Talanian R, Billiar TR. Nitric oxide suppression of apoptosis occurs in association with an inhibition of bcl-2 cleavage and cytochrome *c* release. *J. Biol. Chem*. 1998; **273**: 31 434–41.
12 Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a bcl-2 interacting protein, mediates cytochrome *c* release from mitochondria in response to activation of cell surface death receptors. *Cell* 1998; **94**: 481–90.
13 Yang J, Liu X, Bhalla K *et al*. Prevention of apoptosis by bcl-2: Release of cytochrome *c* from mitochondria blocked. *Science* 1997; **275**: 1129–32.
14 Cory S, Adams JM. The Bcl-2 protein family: Arbiters of cell survival. *Science* 1998; **281**: 1322–6.
15 Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG, Youle R. Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.* 1997; **139**: 1281–92.
16 Jurgensmeier JM, Xie Z, Deveraux Q, Ellerby L, Bredesen D, Reed JC. Bax directly induces release of cytochrome *c* from isolated mitochondria. *Proc. Natl. Acad. Sci. USA* 1998; **95**: 4997–5002.
17 Eskes R, Antonsson B, Osen-Sand A *et al*. Bax-induced cytochrome *c* release from mitochondria is independent of the permeability transition pore but highly dependent on Mg^{2+} ions. *J. Cell Biol*. 1998; **143**: 217–24.
18 Susin SA, Lorenzo HK, Zamzami N *et al*. Molecular characterization of mitochondrial apoptosis inducing factor. *Nature* 1999; **397**: 441–5.
19 Kidd VJ. Proteolytic activities that mediate apoptosis. *Ann. Rev. Physiol*. 1998; **60**: 533–73.
20 Martin SJ, Green D. Protease activation during apoptosis: Death by a thousand cuts? *Cell* 1995; **82**: 349–52.
21 Casiano CA, Martin SJ, Green D, Tan EM. Selective cleavage of nuclear autoantigens during CD95 (Fas/AOP-1)-mediated T cell apoptosis. *J. Exp. Med.* 1996; **184**: 765–70.
22 Srinivasula SM, Ahmad M, Fernandes-Alnemri T, Litwack G, Alnemri ES. Molecular ordering of the Fas-apoptotic pathway: The Fas/Apo-1 protease Mch5 is a CrmA-inhibitable protease that activates multiple ced-3/ICE-like cysteine proteases. *Proc. Natl Acad. Sci. USA* 1996; **93**: 14 486–91.
23 Porter AG, Janicke RU. Emerging roles of caspase 3 in apoptosis. *Cell Death Differ.* 1999; **6**: 99–104.

24 Kuwana T, Smith JJ, Muzio M, Dixit V, Newmeyer DD, Kornbluth S. Apoptosis induction by caspase-8 is amplified through the mitochondrial release of cytochrome *c*. *J. Biol. Chem*. 1998; **273**: 16 589–94.

25 Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNAse that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 1998; **391**: 43–50.

26 Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 1980; **284**: 555-6.

27 Melino G, Piacentini M. Tissue transglutaminase in cell death: A downstream or a multifunctional upstream effector? *FEBS Lett*. 1998; **430**: 59–63.

28 Fesus L, Thomazy V, Falus A. Induction and activation of tissue transglutaminase during programmed cell death. *FEBS Lett*. 1987; **224**: 104–8.

29 Ueda N, Shah SV. Apoptosis. *J. Lab. Clin. Med*. 1994; **124**: 169–77.

30 Fadock VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidyl serine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol*. 1992; **148**: 2207–16.

31 Nakano T, Ishimoto Y, Kishino J *et al*. Cell adhesion to phosphatidyl serine mediated by a product of growth arrest specific gene 6. *J. Biol. Chem*. 1997; **272**: 29 411–14.

32 Wyllie AH. Apoptosis. *Br. J. Cancer* 1993; **67**: 205–8.

33 Cai J, Jones DP. Superoxide in apoptosis: Mitochondrial generation triggered by cytochrome *c* loss. *J. Biol. Chem*. 1998; **273**: 11 401–4.

34 Motoyama S, Minamiya Y, Saito S *et al*. Hydrogen peroxide derived from hepatocytes induces sinusoidal endothelial cell apoptosis in perfused hypoxic rat liver. *Gastroenterology* 1998; **114**: 153–63.

35 Labelle ER, Grange MJ, Elhim C, Marquetty C, Pocidalo MAG, Pasquier C. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Rad. Biol. Med.* 1998; **24**: 563–72.

36 Hellquist HB, Svensson I, Brunk UT. Oxidant induced apoptosis: A consequence of lethal lysosomal leak? *Redox Report* 1997; **3**: 65–70.

37 Escargueil I, Salvayre NA, Pieraggi MT, Salvayre R. Oxidized low density lipoproteins elicit DNA fragmentation of cultured lymphoblastoid cells. *FEBS Lett*. 1992; **305**: 155–9.

38 Sandstrom PA, Tebbey PW, Van Cleave S, Buttke TM. Lipid hydroperoxides induce apoptosis in T cells displaying a HIV associated glutathione peroxidase deficiency. *J. Biol. Chem*. 1994; **269**: 798–801.

39 Mary AQ, Jaffrezou JP, Mansat V, Bordier C, Naval J, Laurent G. Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis. *J. Biol. Chem*. 1997; **272**: 21 388–95.

40 Suzuki Y, Ono Y, Hirabayashi Y. Rapid and specific reactive oxygen generation via NADPH oxidase activation during Fas-mediated apoptosis. *FEBS Lett*. 1998; **425**: 209–12.

41 Ichiyama M, Chang SH, Liu H, Berezesky IK, Trump BF, Amstad PA. Effect of Bcl-2 on oxidant-induced cell death and intracellular Ca^{2+} mobilization. *Am. J. Physiol*. 1998; **275**: C832–9.

42 Johnson TM, Yu ZX, Ferrans VJ, Lowenstein RA, Finkel T. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc. Natl Acad. Sci. USA* 1996; **93**: 11 848–52.

43 Polyak K, Xia Y, Zweler JL, Kinzler KW, Vogelstein B. A model for p53 induced apoptosis. *Nature* 1997; **389**: 300–5.

44 Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochem. Pharmacol*. 1999; **57**: 231–45.

45 Hall PA, Coates PJ, Ansari B, Hopwood D. Regulation of cell number in the mammalian gastrointestinal tract: The importance of apoptosis. *J. Cell Sci*. 1994; **107**: 3569–77.

46 Butler LM, Hewett PJ, Fitridge RA, Cowled PA. Deregulation of apoptosis in colorectal carcinoma: Theoretical and therapeutic implications. *Aust. NZ J. Surg*. 1999; **69**: 88–94.

47 Ruifrok AC, Mason KA, Lozano G, Thames HD. Spatial and temporal patterns of expression of epidermal growth factor, transforming growth factor alpha and transforming growth factor beta 1–3 and their receptors in mouse jejunum after radiation treatment. *Radiat. Res.* 1997; **147**: 1–12.

48 Sheng H, Shao J, O'Mahony CA *et al*. Transformation of intestinal epithelial cells by chronic TGF-betal treatment results in downregulation of the type II TGF-beta receptor and induction of cyclooxygenase-2. *Oncogene* 1999; **18**: 855–67.

49 Taheri A, Lavagna C, Rampal P. Effect of interleukin-2 on the proliferation of two intestinal cell lines and on the expression of TFG-beta gene and Jun/Fos oncogenes. *Gastroenterol. Clin. Biol.* 1993; **17**: 925–31.

50 Potten CS, Loeffler M. Stem cells: Attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* 1990; **110**: 1001–20.

51 Benard O, Madesh M, Anup R, Balasubramanian KA. Apoptotic process in the monkey small intestinal epithelium. 1. Association with glutathione level and its efflux. *Free Rad. Biol. Med*. 1999; **26**: 245–52.

52 Moss SF, Holt PR. Apoptosis in the intestine (Letter). *Gastroenterology* 1999; **111**: 567–8.

53 Westcarr S, Farshori P, Wyche J, Anderson WA. Apoptosis and differentiation in the crypt-villus unit of the rat small intestine. *J. Submicrosc. Cytol. Pathol*. 1999; **31**: 15–30.

54 Aschoff AP, Ott U, Funfstuck R, Stein G, Jirikowski GT. Colocalization of Bax and Bcl-2 in small intestine and kidney biopsies with different degrees of DNA fragmentation. *Cell Tissue Res*. 1999; **296**: 351–7.

55 Takeuchi T, Kitagawa H, Imagawa T, Uehara M. Apoptosis of villous epithelial cells and follicle associated epithelial cells in chicken cecum. *J. Vet. Med. Sci*. 1999; **61**: 149–54.

56 Iwanaga T. The involvement of macrophages and lymphocytes in the apoptosis of enterocytes. *Arch. Histol. Cytol*. 1995; **58**: 151–9.

57 Shibahara T, Sato N, Waguri S *et al*. The fate of effete epithelial cells at the villus tips of the human small intestine. *Arch. Histol. Cytol.* 1995; **58**: 205–19.

58 Iwanaga T, Hoshi O, Han H, Takahashi-Iwanaga H, Uchiyama Y, Fujita T. Lamina propria macrophages involved in cell death (apoptosis) of enterocytes in the small intestine of rats. *Arch. Histol. Cytol*. 1994; **57**: 267–76.

59 Guy-Grand D, DiSanto JP, Henchoz P, Malassis-Seris M, Vassalli P. Small bowel enteropathy: Role of intraepithelial lymphocytes and of cytokines (IL-12, IFN-gamma, TNF) in the induction of epithelial cell death and renewal. *Eur. J. Immunol.* 1998; **28**: 730–44.

60 Piguet PT, Vesin C, Donati Y, Barazzone C. TNF-induced enterocyte apoptosis and detachment in mice: Induction of caspases and prevention by a caspase inhibitor, ZVAD-fmk. *Lab. Invest.* 1999; **79**: 495–500.

61 Ruemmele FM, Dionne S, Levy E, Seidman EG. TNFα-induced IEC-6 cell apoptosis requires activation of ICE caspases whereas complete inhibition of the caspase cascade leads to necrotic cell death. *Biochem. Biophys. Res. Commun.* 1999; **260**: 159–66.

62 Piguet PT, Vesin C, Guo J, Donati Y, Barazzone C. TNF-induced enterocyte apoptosis in mice is mediated by the TNF receptor 1 and does not require p53. *Eur. J. Immunol.* 1998; **28**: 3499–505.

63 Abreu-Martin MT, Vidrich A, Lynch DH, Targan SR. Divergent induction of apoptosis and IL-8 secretion in HT29 cells in response to TNF-α and ligation of Fas antigen. *J. Immunol.* 1995; **155**: 4147–54.

64 Madesh M, Benard O, Balasubramanian KA. Apoptotic process in the monkey small intestinal epithelium. 2. Possible role of oxidative stress. *Free Rad. Biol. Med.* 1999; **26**: 431–8.

65 Madesh M, Anup R, Benard O, Balasubramanian KA. Apoptosis in the monkey small intestinal epithelium: Structural and functional alterations in the mitochondria. *Free Rad. Biol. Med.* 1999; **26**: 836–43.

66 Kasai T, Ohguchi K, Nakashima S *et al.* Increased activity of oleate-dependent tyrosine phospholipase D during actinomycin D-induced apoptosis in Jurkat T cells. *J. Immunol.* 1998; **161**: 6469–74.

67 Longo WE, Grossmann EM, Erickson B, Panesar N, Mazuski JE, Kaminski DL. The effect of phospholipase A2 inhibitors on proliferation and apoptosis of murine intestinal cells. *J. Surg. Res.* 1999; **84**: 51–6.

68 Grossman J, Fiocchi C, Levine AD. Extracellular matrix (ECM) orchestrates death and survival of human intestinal epithelial cells (IEC). *Gastroenterology* 1997; **112**: A987.

69 Grossman J, Mohr S. Lapetina EG, Fiocchi C, Levine AD. Sequential and rapid activation of select caspases during apoptosis of normal intestinal epithelial cells. *Am. J. Physiol.* 1998; **274**: G1117–24.

70 Scheving LA, Jin WH, Chong KM, Gardner W, Cope FO. Dying enterocytes downregulate signaling pathways converging on Ras: Rescue by protease inhibition. *Am. J. Physiol.* 1998; **274**: C1363–72.

71 Pompeiano M, Hvola M, Chun J. Onset of apoptotic DNA fragmentation can precede cell elimination by days in the small intestinal villus cells. *Cell Death Differ.* 1998; **5**: 702–9.

72 Litvak DA, Evers M, Hwang KO, Hellmich MR, Ko TC, Townsend CM. Butyrate induced differentiation of Caco 2 cells is associated with apoptosis and early induction of $p21^{Waf1/Cip1}$ and $p27^{Kip1}$. *Surgery* 1998; **124**: 161–70.

73 Veldman RJ, Klappe K, Hoekstra D, Kok JW. Interferon γ induced differentiation and apoptosis of HT29 cells: Dissociation of (glucosyl) ceramide signaling. *Biochem. Biophys. Res. Commun.* 1998; **247**: 802–8.

74 Butt AJ, Hague A, Paraskeva C. Butyrate, but not TGF B1-induced apoptosis of colorectal adenoma cells is associated with increased expression of the differentiation markers E-cadherin and alkaline phosphatase. *Cell Death Differ.* 1997; **4**: 725–32.

75 Papaconstantinou HT, Hwang KO, Rajaraman S, Hellmich MR, Townsend Jr CM, Ko TC. Glutamine deprivation induces apoptosis in intestinal epithelial cells. *Surgery* 1998; **124**: 152–9.

76 Hass R, Busche R, Luciano L, Reale E, Engelhardt WV. Lack of butyrate is associated with induction of bax and subsequent apoptosis in proximal colon of guinea pig. *Gastroenterology* 1997; **112**: 875–81.

77 Moss SF, Attia L, Scholes JV, Walters JRF, Holt PR. Increased intestinal apoptosis in coeliac disease. *Gut* 1996; **39**: 811-17.

78 Iwamoto M, Koji T, Makiyama K, Kobayashi N, Nakane PK. Apoptosis of crypt epithelial cells in ulcerative colitis. *J. Pathol.* 1996; **180**: 152–9.

79 Iwamoto M, Makiyama K, Koji T, Nakane PK. Expression of Fas and Fas-ligand in epithelium of ulcerative colitis. *Nippon Rinsho* 1996; **54**: 1970–4.

80 Strater J, Wellisch I, Riedl S *et al.* CD95 (APO1/Fas)-mediated apoptosis in colon epithelial cells: A possible role in ulcerative colitis. *Gastroenterology* 1997; **113**: 160–7.

81 Sun Z, Wang X, Wallen R. The influence of apoptosis on intestinal barrier integrity in rats. *Scand. J. Gastroenterol.* 1998; **33**: 415–22.

82 Yamamoto H, Sawai H, Perucho M. Frameshift somatic mutations in gastrointestinal cancer of the microsatellite type. *Cancer Res.* 1997; **57**: 4420–6.

83 Madesh M, Bhaskar L, Balasubramanian KA. Enterocyte viability and mitochondrial function after graded intestinal ischemia and reperfusion in rats. *Mol. Cell. Biochem.* 1997; **167**: 81–7.

84 Noda T, Iwakiri R, Fujimoto K, Matsuo S, Aw TY. Programmed cell death induced by ischemia–reperfusion in rat intestinal mucosa. *Am. J. Physiol.* 1998; **274**: G270–6.

85 Shah KA, Shurey S, Green CJ. Apoptosis after intestinal ischemia-reperfusion injury. *Transplantation* 1997; **64**: 1393–7.

86 Ogura E, Senzaki H, Yamamoto D *et al.* Prognostic significance of Bcl-2, Bcl-XL/S, Bax and Bak expressions in colorectal carcinomas. *Oncol. Rep.* 1999; **6**: 365–9.

87 Okuno S, Shimizu S, Ito T *et al.* Bcl-2 prevents caspase independent cell death. *J. Biol. Chem.* 1998; **273**: 34 272–7.

88 Hague A, Mooghen M, Hicks D, Chapman M, Paraskeva C. Bcl-2 expression in human colorectal adenomas and carcinomas. *Oncogene* 1994; **9**: 3367–70.

89 Paraskeva C, Singh B. Bcl-2 as a possible sensor of nutritional stress inhibiting apoptosis and allowing cell survival during colorectal carcinogenesis. *Biochem. Soc. Trans.* 1998; **26**: 236–41.

90 Yang HB, Chow NH, Sheu BS, Chan SH, Chein CH, Su IJ. The role of Bcl-2 in the progression of the colorectal adenoma-carcinoma sequence. *Anticancer Res.* 1999; **19**: 727–30.

91 Sun B, Zhang N, Zhao X. Apoptosis and expression of its regulating genes in adenoma and adenocarcinoma of large intestine. *Chung Hua Ping Li Hsuch Tsa Chih* 1997; **26**: 137–40.

92 Pritchard DM, Potten CS, Hickman JA. The relationship between p53 dependent apoptosis, inhibition of prolifera-

tion and 5-fluorouracil-induced histopathology in murine intestinal epithelia. *Cancer Res*. 1998; **58**: 5453–65.
93 Pritchard DM, Watson AJM, Potten CS, Jackman AL, Hickman JA. Inhibition by uridine but not thymidine of p53 dependent intestinal apoptosis initiated by 5-fluorouracil: Evidence for the involvement of RNA perturbation. *Proc. Natl Acad. Sci. USA* 1997; **94**: 1795–9.
94 Yagi OK, Akiyama Y, Nomizu T, Iwama T, Endo M, Yuasa Y. Proapoptotic gene Bax is frequently mutated in hereditary nonpolyposis colorectal cancers but not in adenomas. *Gastroenterology* 1998; **114**: 268–74.
95 Hass R, Busche R, Luciano L, Reale E, Engelhardt WV. Lack of butyrate is associated with induction of bax and subsequent apoptosis in the proximal colon of guinea pig. *Gastroenterology* 1997; **112**: 875–81.
96 Hirose Y, Yoshimi N, Suzui M, Kawabata K, Tanaka T, Mori H. Expression of Bcl-2, Bax and Bcl-XL proteins in azoxymethane-induced rat colonic adenocarcinomas. *Mol. Carcinog*. 1997; **19**: 25–30.
97 Rampino N, Yamamoto H, Ionov Y *et al*. Somatic frameshift mutations in the Bax gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997; **275**: 967–9.
98 Iacopetta BJ, Soong R, House AK, Hamelin R. Gastric carcinomas with microsatellite instability: Clinical features and mutations to the TGF-beta type II receptor, IGF II receptor and Bax genes. *J. Pathol*. 1999; **187**: 428–32.
99 Moss SF, Agarwal B, Arber N *et al*. Increased intestinal Bak expression results in apoptosis. *Biochem. Biophys. Res. Commun*. 1996; **223**: 199–203.
100 Krajewski S, Krajewska M, Reed JC. Immunohistochemical analysis of *in vivo* patterns of Bak expression, a proapoptotic member of the Bcl-2 protein family. *Cancer Res*. 1996; **56**: 2849–55.
101 Krajewska M, Moss SF, Krajewski S, Song K, Holt PR, Reed JC. Elevated expression of Bcl-X and reduced Bak in primary colorectal adenocarcinomas. *Cancer Res*. 1996; **56**: 2422–7.
102 Rosen K, Rak J, Jin J, Kerbel RS, Newman MJ, Filmus J. Down regulation of the pro-apoptotic protein Bak is required for the Ras-induced transformation of intestinal epithelial cells. *Curr. Biol*. 1998; **8**: 1331–4.
103 Ruemmele FM, Seidman EG. Cytokine-intestinal epithelial cell interactions: Implications for immune mediated bowel disorders. *Chung Hua Min Kuo Hsiao Erh Ko I Hseuh Hui Tsa Chih* 1998; **39**: 1–8.
104 Ina K, Kusugami K, Hosokawa T *et al*. Increased mucosal production of granulocyte colony stimulating factor is related to delay in neutrophil apoptosis in inflammatory bowel disease. *J. Gastroenterol. Hepatol*. 1999; **14**: 46–53.
105 McAlindon ME, Galvin A, McKaig B, Gray T, Sewell HF, Mahida YR. Investigation of the expression of IL-1 beta converting enzyme and apoptosis in normal and inflammatory bowel disease (IBD) mucosal macrophages. *Clin. Exp. Immunol*. 1999; **116**: 251–7.
106 Boirivant M, Marini M, DiFelice G *et al*. Lamina propria T cells in Crohn's disease and other gastrointestinal inflammation show defective CD2 pathway-induced apoptosis. *Gastroenterology* 1999; **116**: 557–65.
107 Ina K, Itoh J, Fukushima K *et al*. Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *J. Immunol*. 1999; **163**: 1081–90.
108 Abreu-Martin MT, Palladino AA, Faris M, Carramanzana NM, Nel AE, Targan SR. Fas activates the Jnk pathway in human colonic epithelial cells: Lack of a direct role in apoptosis. *Am. J. Physiol*. 1999; **276**: G599–605.
109 Ueyama H, Kiyohara T, Sawada N *et al*. High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. *Gut* 1998; **43**: 48–55.
110 Arai N, Mitomi H, Chtani Y, Igarashi M, Kakita A, Okayasu I. Enhanced epithelial cell turnover associated with p53 accumulation and high $p21^{WAF1/CIP1}$ expression in ulcerative colitis. *Mod. Pathol*. 1999: **12**: 604–11.
111 Peduto EL, Guillou L, Saraga E *et al*. Fas and Fas ligand expression in tumor cells and in vascular smooth-muscle cells of colonic and renal carcinomas. *Int. J. Cancer* 1999; **81**: 772–8.
112 O'Connell J, O'Sullivan GC, Colins JK, Shanahan F. The Fas counterattack: Fas mediated T-cell killing by colon cancer cells expressing Fas ligand. *J. Exp. Med*. 1996; **184**: 1075–82.
113 Kim JM, Eckmann L, Savidge TC, Lowe DC, Witthoft T, Kagnoff MF. Apoptosis of human intestinal epithelial cells after bacterial invasion. *J. Clin. Invest*. 1998; **102**: 1815–23.
114 Wada Y, Mori K, Iwanaga T. Apoptosis of enterocytes induced by inoculation of a strain of attaching and effacing *Escherichia coli* and verotoxin. *J. Vet. Med. Sci*. 1997; **59**: 815–18.
115 Keenam KP, Sharpnack DD, Collins H, Formal SB, O'Brien AD. Morphologic evaluation of the effects of Shiga toxin and *E. coli* Shiga-like toxin on the rabbit intestine. *Am. J. Pathol*. 1986; **125**: 69–80.
116 Zychlinsky A, Prevost MC, Sansonetti PJ. *Shigella flexneri* induces apoptosis in infected macrophages. *Nature* 1992; **358**: 167–9.
117 Islam D, Veress B, Bardhan PK, Lindberg AA, Christensson B. *In-situ* characterization of inflammatory responses in the rectal mucosa of patients with Shigellosis. *Infect. Immun*. 1997; **65**: 739–49.
118 Sansonetti PJ, Tran Van Nhieu G, Egile C. Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clin. Infect. Dis*. 1999; **28**: 466–75.
119 Sansonetti PJ. Molecular and cellular mechanisms of invasion of the intestinal barrier by enteric pathogens. The paradigm of *Shigella*. *Folia Microbiol*. 1998; **43**: 239–46.
120 Bloom PD, Boedeker EC. Mucosal immune responses to intestinal bacterial pathogens. *Semin. Gastrointest. Dis*. 1996; **7**: 151–66.
121 Fiorentini C, Fabbri A, Falzano L *et al*. *Clostridium difficile* toxin B induces apoptosis in intestinal cultured cells. *Infect. Immun*. 1998; **66**: 2660–5.
122 Mahida YR, Makh S, Hyde S, Gray T, Borriello SP. Effect of *Clostridium difficile* toxin A on human intestinal epithelial cells: Induction of interleukin-8 production and apoptosis after cell detachment. *Gut* 1996; **38**: 337–47.
123 Moss SF, Calam J, Agarwal B *et al*. Induction of gastric epithelial apoptosis by *Helicobacter pylori*. *Gut* 1996; **38**: 498–501.
124 Wagner S, Beil W, Westermann J *et al*. Regulation of gastric epithelial cell growth by *Helicobacter pylori*: Evi-

dence for a major role of apoptosis. *Gastroenterology* 1997; **113**: 1836–47.
125 Suzuki H, Seto K, Mori H, Suzuki M, Miura S, Ishii H. Monochloramine induced DNA fragmentation in gastric cell line MKN45. *Am. J. Physiol.* 1998; **275**: G712–16.
126 Chen G, Sordillo EM, Ramey WG *et al*. Apoptosis in gastric epithelial cells is induced by *Helicobacter pylori* and accompanied by increased expression of Bak. *Biochem. Biophys. Res. Commun*. 1997; **239**: 626–32.
127 Fan X, Crowe SE, Behar S *et al*. The effect of class II major histocompatibility complex expression on adherence of *Helicobacter pylori* and induction of apoptosis in gastric epithelial cells: A mechanism for T helper cell type 1 mediated damage. *J. Exp. Med*. 1998; **187**: 1659–69.
128 Rudi J, Kuck D, Krammer PH *et al*. Possible involvement of the APO-1/Fas (CD95) receptor/ligand system in *H. pylori*-induced apoptosis (Abstract). *Gastroenterology* 1997; **112**: A274.
129 Behar S, Van Houten N, Bamford K *et al*. *H. pylori* induces apoptosis in gastric epithelial cells which is enhanced by cytokines derived from Th1 cells (Abstract). *Gastroenterology* 1996; **110**: A862.
130 Houghton J, Korah RM, Condon MR, Kim KH. Apoptosis in *Helicobacter pylori*-associated gastric and duodenal ulcer disease is mediated via the Fas antigen pathway. *Dig. Dis. Sci.* 1999; **44**: 465–78.
131 Coopersmith CM, Gordon JI, Gamma-ray-induced apoptosis in transgenic mice with proliferative abnormalities in their intestinal epithelium: Re-entry of villus enterocytes into the cell cycle does not affect their radioresistance but enhances the radiosensitivity of the crypt by inducing p53. *Oncogene* 1997; **15**: 131–41.
132 Potten CS, Merritt A, Hickman J, Faranda A. Characterization of radiation-induced apoptosis in the small intestine and its biological implications. *Int. J. Radiat. Biol*. 1994; **65**: 71–8.
133 Potten CS, Grant HK. The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. *Br. J. Cancer* 1998; **78**: 993–1003.
134 Merritt AJ, Potten CS, Kemp CJ *et al*. The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53 deficient mice. *Cancer Res*. 1994; **54**: 614–17.
135 Clarke AR, Gledhill S, Hooper ML, Bird CC, Wyllie AH. p53 dependence of early apoptotic and proliferative responses within the mouse intestinal epithelium following gamma-irradiation. *Oncogene* 1994; **9**: 1767–73.
136 Merritt AJ, Allen TD, Potten CS, Hickman JA. Apoptosis in the small intestinal epithelium from p53-null mice: Evidence for a delayed, p53 independent G2/M-associated cell death after gamma-irradiation. *Oncogene* 1997; **14**: 2759–66.
137 Arai T, Kida Y, Harmon BV, Gobe GC. Comparative alterations in p53 expression and apoptosis in the irradiated rat small and large intestine. *Br. J. Cancer* 1996; **74**: 406–12.
138 Cai WB, Roberts SA, Bowley E, Hendry JH, Potten CS. Differential survival of murine small and large intestinal crypts following ionizing radiation. *Int. J. Radiat. Biol*. 1997; **71**: 145–55.
139 Mori N, Van Wezel T, Van Der Valk M, Sakuma S, Okumoto M, Demant P. Genetics of susceptibility to radiation-induced apoptosis in colon: Two loci on chromosomes 9 and 16. *Mamm. Genome* 1998; **9**: 377–80.
140 Orazi A, Du X, Yang Z, Kashai M, Williams DA. Interleukin 11 prevents apoptosis and accelerates recovery of small intestinal mucosa in mice treated with combined chemotherapy and radiation. *Lab. Invest*. 1996; **75**: 33–42.
141 Cul S, Reichner JS, Mato RB, Albina JE. Activated murine macrophages induce apoptosis in tumor cells through nitric oxide dependent and independent mechanisms. *Cancer Res*. 1994; **54**: 2462–7.
142 Sandoval M, Zhang XJ, Liu X, Mannick EE, Clark DA, Miller MJ. Peroxynitrite-induced apoptosis in T84 and RAW 264.7 cells: Attenuation by L-ascorbic acid. *Free Radic. Biol. Med*. 1997; **22**: 489–95.
143 Kim YM, De Vera ME, Watkins SC, Billiar TR. Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-α induced apoptosis by inducing heat shock protein 70 expression. *J. Biol. Chem*. 1997; **272**: 1402–11.
144 Mohr S, Zech B, Lapetina EG, Brune B. Inhibition of caspase-3 by *s*-nitrosylation and oxidation caused by nitric oxide. *Biochem. Biophys. Res. Commun*. 1997; **238**: 387–91.
145 Melino G, Bernassola F, Knight RA, Corasaniti MT, Nistico G, Finazzi-Agro A. S-nitrosylation regulates apoptosis. *Nature* 1997; **388**: 432–3.
146 Mannick JB, Miao XQ, Stamler J. Nitric oxide inhibits Fas induced apoptosis. *J. Biol. Chem*. 1997; **272**: 24 125–8.
147 Nishikawa M, Takeda K, Sato EF, Kuroki T, Inoue M. Nitric oxide regulates energy metabolism and bcl-2 expression in intestinal epithelial cells. *Am. J. Physiol*. 1998; **274**: G797–801.
148 Genaro AM, Hortelano S, Alvarez A, Martinez AC, Bosca L. Splenic B lymphocyte programmed cell death is prevented by nitric oxide release through mechanisms involving sustained Bcl-2 levels. *J. Clin. Invest*. 1995; **95**: 1884–90.
149 Chun SY, Eisenhauer KM, Kubo M, Huseh AJW. Interleukin-1β suppresses apoptosis in rat ovarian follicles by increasing nitric oxide production. *Endocrinology* 1995; **136**: 3120–7.
150 Kim YM, Talanian RV, Billiar TR. Nitric oxide prevents apoptosis by preventing increases in caspase-3 like activity via two distinct mechanisms. *J. Biol. Chem*. 1997; **272**: 31 138–48.
151 Leist M, Single B, Naumann H *et al*. Nitric oxide inhibits execution of apoptosis at two distinct ATP-dependent steps upstream and down stream of mitochondrial cytochrome *c* release. *Biochem. Biophys. Res. Commun*. 1999; **258**: 215–21.
152 Madesh M, Ramachandran A, Pulimood A, Vadranam M, Balasubramanian KA. Attenuation of intestinal ischaemia–reperfusion injury with sodium nitroprusside. *Biochem. Biophys. Acta* (2000) in press.
153 Madesh M, Anup R. Balasubramanian KA. Nitric oxide prevents anoxia-induced apoptosis in colonic HT29 cells. *Arch. Biochem. Biophys*. 1999; **366**: 240–8.