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.

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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. 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. 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. 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; Fig. 1).

The initiation stage of apoptosis is followed by a signalling stage, where mitochondria are known to play a central role. During apoptosis, mitochondria undergo characteristic changes including a non-specific increase in membrane permeability, which develops gradually, and a reduction of mitochondrial membrane potential, which is accompanied by oxidative stress. 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. This release of cytochrome c from mitochondria can be blocked by Bcl-2, an anti-apoptotic protein localized in mitochondria, while Bax, a pro-apoptotic member of the Bcl-2 family that translocates from the cytosol to the mitochondria during apoptosis, induces cytochrome c release. Recently, a mitochondrial apoptosis-inducing factor that localizes from mitochondria to nucleus during apoptosis was characterized.

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. These proteases are synthesized as proenzymes and when activated by proteolytic cleavage during apoptosis, specifically degrade a number of cellular substrates such as poly(ADP-ribose) polymerase, lamin B and DNA topoisomerases I and II. Caspase-8, once activated in the multiprotein DISC complex, has two major functions. It can directly activate further down-stream caspases which bring about the final morphological features of apoptosis and also induce release of cytochrome c from the mitochondria. The released cytochrome c binds to an apoptosis activating factor-1 (Apaf-1), which in turn activates caspase-9 and amplifies the proteolytic cascade. Bcl-2 could play a role here too, by regulating Apaf-1 and preventing activation of down-stream caspases.

The caspases thus bring about degradation of cellular components and activation of specific endonucleases which cleave DNA, resulting in degradation of chromatin. Cross-linking of proteins by transglutaminase, an enzyme whose activity is modulated by Ca²⁺, guanosine triphosphate (GTP), S-nitrosylation and polyamines, keeps the apoptotic bodies intact during fragmentation of the cell. Following this, the interactions of the individual cell with its neighbours become disorganized and membrane changes are initiated in the

![Diagram](image-url)

**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, which enable apoptotic cell uptake by macrophages. Thus, apoptosis occurs without leakage of intracellular macromolecules 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. Hydrogen peroxide from hepatocytes has been shown to induce apoptosis in sinusoidal endothelial cells in the liver and H$_2$O$_2$ also mediates neutrophil apoptosis. Hydrogen peroxide-mediated and iron-catalysed lysosomal rupture causes decompartmentalization of lysosomal enzymes, which may play a role in modulating the apoptotic process. Oxidized low-density lipoproteins and lipid hydroperoxides have also been shown to induce apoptosis.

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. Fas ligation induces ROS production, which could be prevented by an NADPH oxidase inhibitor, implicating this oxidase system in generation of ROS during apoptosis. 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. Bcl-2 has also been shown to protect against oxidant-induced cell death by increasing the capacity of mitochondria to store calcium. Oxidants have been implicated in p53-mediated apoptosis and p53 probably induces synthesis of specific proteins that increase the cellular ROS content, leading to mitochondrial damage and further down-stream events. 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.

**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. 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 epithelium. 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, 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-$\alpha$, TGF-$\beta$3), 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.](image-url)
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.\(^53\) Immunoactivity 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,\(^59\) most probably by induction of caspase activity.\(^60\) Tumour necrosis factor-α also has 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\(^61\) 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 ρ-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.

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, 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 areas78 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-regulation89 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 colon,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 carcinomas95 and butyrate deprivation increased expression of Bax in guinea-pig proximal colon, accompanied by oligonucleosomal DNA fragmentation and massive apoptosis of colonocytes.96 Expression of Bax was strong in colonic adenocarcinomas96 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 IEC99,100 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. 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. 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. 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/Bax ratio is significantly high 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. 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. 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. 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. Colon cancer cells have been found to express functional FasL, 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.

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. Verotoxin-producing, attaching and effacing E. coli also induce apoptosis in infected enterocytes. 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. 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. Invasion of the intestinal barrier by Shigella flexneri involves complex interactions with epithelial and phagocytic cells 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.

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 and exposure to toxin A induced epithelial cell rounding, detachment and apoptosis in organ cultures of human colonic biopsy specimens. Exposure of HT-29 colon carcinoma cells to Clostridium difficile toxin A for 24 h also induced IL-8 production.

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 and this reverted back to normal on eradication of the organism. Helicobacter pylori is also capable of inducing apoptosis in epithelial cells in vitro. 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 (NH2Cl) 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. Adherence also seems to be necessary for apoptosis induction, but high doses of purified H. pylori lipopolysaccharide can also induce apoptosis and major histocompatibility class II molecules have also been implicated in the process. 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 and IFN-γ has been shown to up-regulate the Fas receptor on gastric epithelial cells. 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. 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.144 Also, in the small intestine, peak levels of radiation-induced apoptosis appeared earlier than in the large intestine.157 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.158 Analysis of (Ccs-7 × BALB/cHeA)F2 hybrid mice revealed linkage of susceptibility to radiation-induced apoptosis of colon crypt cells to two loci on chromosomes 9 and 16.159 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-nitrosylation144,145 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

![Figure 3](image-url)

**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,\textsuperscript{148} and inhibit spontaneous apoptosis in cultured ovarian follicles via a c-guanidine monophosphate (cGMP)-dependent mechanism.\textsuperscript{10} 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.\textsuperscript{150} Kim \textit{et al.} have shown that NO inhibits cytochrome c release by preventing Bcl-2 cleavage by caspase-3.\textsuperscript{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.\textsuperscript{151} Our studies showed that NO prevented damage induced by intestinal ischaemia–reperfusion\textsuperscript{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.\textsuperscript{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.

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