Leading article

Necrosis and apoptosis in the gastrointestinal tract

Cell death, once dismissed as a chaotic phenomenon unworthy of serious study, now finds itself the subject of some of the most exciting biomedical research today. This transformation was brought about by the realisation that cell death is the result of distinct cellular events rather than random disintegration. Although various modes of cell death exist, only the processes of necrosis and apoptosis have been described in detail. Necrosis is a passive process resulting from the destruction of critical cellular structures, whereas apoptosis requires the active participation of the cell in its own death and can be prevented by inhibitors of RNA and protein synthesis.[1] Recently, it has been discovered that some of the genes that control apoptosis are mutated in colorectal cancer and other tumours. This has given credence to the notion that failure of cell death is an important factor in carcinogenesis and has catapulted apoptosis into the headlines of modern cell biology.

Necrosis is characterised morphologically by cellular swelling, dilatation of mitochondria and endoplasmic reticulum, and flocculation of nuclear chromatin. Later there is rupture of plasma, nuclear and organelle membranes, which leads to the destruction of the cell. Large contiguous populations of cells undergo necrosis simultaneously and an inflammatory response is evoked. Necrosis is caused by important changes to the cellular environment such as the presence of toxins, cytolysins, hypoxia, ischaemia or large changes in temperature. It is rarely seen as a part of normal embryogenesis or differentiation.[2]

Over the past decade the physicochemical correlates of these morphological changes have been defined. Particular attention has been paid to necrosis caused by reactive oxygen metabolites (hydrogen peroxide, hypochlorous acid, the superoxide anion, and the hydroxyl radical) released from granulocytes in tissue inflammation and the ischaemia/reperfusion reaction.[3] Recently, we have shown that hydrogen peroxide damages cells derived from human colonic epithelium.[4] A variety of cellular events have been found to occur, including increases in intracellular calcium,[5] destabilisation of the cytoskeleton,[6] peroxidation of membrane lipids,[7] proteolysis,[8] increases in cell volume, and membrane damage leading to plasma membrane blebbing and lysis.[9] The types of cellular damage vary depending on the oxidant being considered. For example, sulphydryl oxidation of membrane proteins is a more important mechanism of injury from hypochlorous acid than hydrogen peroxide. Lipid peroxidation is initiated more by the hydroxyl radical than the lipid insoluble superoxide anion.[10]

Depletion of cellular ATP plays a dominant part in determining the fate of the cell from hydrogen peroxide mediated damage. A number of mechanisms are responsible including inhibition of glycolysis at the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) step.[11] Repair of oxidant induced damage to DNA by poly-ADP-ribose polymerase (PARP) can cause a fall in nicotinamide adenine dinucleotide (NAD) with consequent inhibition of GAPDH and interference with cellular ATP synthesis.[12] This may be of therapeutic importance as PARP inhibitors have been shown to protect endothelial cells against hydrogen peroxide induced injury.[13] We have shown that the PARP inhibitors 3-aminobenzamide and nicotinamide protect HT29 cells, against necrosis induced by hydrogen peroxide.[14] This protection is only short lived and exposure of damaged epithelium to PARP inhibitors for prolonged periods of time is almost certainly toxic. Nevertheless inhibition of PARP could be useful in preventing damage from ischaemia/reperfusion reactions during surgery or organ transplantation. Oxidants also cause an increase in the permeability of the inner mitochondrial membrane to small ions causing loss of the mitochondrial membrane potential and uncoupling of oxidative phosphorylation, again inhibiting ATP synthesis.[15]

Apoptosis is distinct from necrosis. Apoptosis can be induced by a number of physiological receptor mediated events such as exposure of thymocytes to dexamethasone,[16] growth factor withdrawal from haemopoietic cells,[17] binding of anti-CD3 antibodies to the CD3/T cell receptor complex of autoreactive T cells[18] or reactive oxygen metabolites.[14] Excess or unwanted cell types are removed by apoptosis during embryogenesis and normal tissue growth.[19] Cells undergoing apoptosis are characterised morphologically by condensation of nuclear chromatin into caps at the edge of the nucleus and detachment from their neighbours. There is no mitochondrial swelling or early rupture of the plasma membrane as in necrosis. Blebs develop on the cell surface and the cell fragments into 'apoptotic bodies', which are recognised by macrophages and phagocytosed. Finally, there is destruction of remaining cellular elements; a phase sometimes confusingly termed 'secondary necrosis'. Unlike necrotic cells which swell, apoptotic cells characteristically shrink. In fact apoptosis was first called 'shrinkage necrosis' before the term apoptosis* was coined. Another cardinal feature of apoptosis is cleavage of DNA into fragments, which are multiples of 180–200 basepairs in size, by cleavage between nucleosomes by endonucleases.[14] This produces a characteristic ladder appearance when DNA is subjected to agarose gel electrophoresis. This is now known to be a late feature, which is often preceded by cleavage of DNA into large fragments 50 or 200 kilobases in size.[20]

Detailed studies by Potten and colleagues of epithelial cell kinetics in the mouse gastrointestinal tract have shown that apoptosis is rare in normal epithelium, one apoptotic body being seen every 5th crypt.[21] The paucity of apoptotic cells on histological section reflects the rapid kinetics of apoptosis and the removal of apoptotic cells by phagocytosis. This, together with an absence of inflammatory response probably explains why apoptosis went unnoticed for so long. Apoptosis in the crypt presumably serves to regulate the number of cells migrating up the crypt-villus axis. It is unclear if apoptosis is the mode of cell death responsible for the removal of epithelial cells at the villus tip.[21 22] Madara has described a process in which

*Apoptosis refers to falling leaves in autumn.

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Conditions of the gastrointestinal tract in which apoptosis may play a part

Excessive apoptosis	*Defective apoptosis*
Melanosis coli	Carcinogenesis
Shigella flexneri dysentary	
Graft versus host disease	
NSAID induced enteropathy	
AIDS	
?Inflammatory bowel disease	

? Indicates that a role for apoptosis is being proposed on theoretical grounds only.

cells are extruded from the villus tip with epithelial continuity being maintained by neighbouring cells extending processes to form new junctional elements behind the extruded cell.[23] Some investigators have observed DNA fragmentation in villus tip cells using the TdT mediated dUTP-biotin nick end labelling technique and have suggested this indicates apoptosis.[24] We have found labelling at the villus tip to be non-specific, however, and find specific labelling of DNA fragmentation confined to the base of the crypt.[25] More studies are required to resolve the fate of cells at the villus tip. It is possible, for example, that the classic morphological features of apoptosis do not appear until the cells have been shed into the lumen.

Apoptosis has been shown in a number of diseases of the gastrointestinal tract (Table). Most gastroenterologists have seen apoptosis though may not have been aware of it as the pigmentation of melanosis coli is a consequence of apoptosis. Anthraquinones induce apoptosis in colonic epithelial cells. The resulting apoptotic bodies, after phagocytosis by intraepithelial macrophages, are carried to the lamina propria where they are transformed into lipofuschin in macrophage heterolysosomes to produce the characteristic pigmentation.[26] *Shigella flexneri* kill phagocytes in great numbers to cause the characteristic mucosal abscesses found in dysentery.[27] Only the invasive strains of *Sh flexneri* possess this capacity to induce apoptosis, most other cytotoxic intestinal bacteria killing by the induction of necrosis. In both graft versus host disease[28] and non-steroidal anti-inflammatory drug induced intestinal injury,[29] apoptotic bodies are found in the intestinal epithelium and can be a helpful diagnostic feature. In neither case, however, is it clear whether the dying cells are enterocytes or lymphocytes. Nor is it known whether apoptosis is playing an important part in disease pathogenesis or whether apoptosis is merely part of the normal clearance of damaged cells. HIV-1 can induce apoptosis in $CD4^+$ lymphocytes and has been suggested to be an important mechanism of lymphocyte depletion in AIDS.[30] The mechanism by which HIV-1 induces apoptosis is poorly understood but recently the HIV-1 tat protein has been implicated.[31] As apoptosis has been shown in the rectal epithelial cells of patients with AIDS[32] it is tempting to speculate that HIV-1 induced apoptosis of epithelial cells contributes to the development of AIDS enteropathy.

In inflammatory bowel disease tissue damage is due mainly to the release of reactive oxygen metabolites, nitric oxide, and proteases from granulocytes, which induce necrosis in the gut epithelium. Evidence has also been found, however, for cytotoxic T cell mediated cell lysis in ulcerative colitis[33] though this result awaits confirmation.[34] Cytotoxic T cells release a calcium dependent pore forming protein called perforin, which polymerises and inserts into the plasma membrane resulting in the formation of small pores. These allow small ions to pass through the plasma membrane with resulting collapse of ion gradients and necrosis of the target cell by osmotic rupture. This mechanism is predominant when there is an excess of cytotoxic T cells in comparison with target cells with high concentrations of perforin. Under conditions found in vivo, however, where the ratio of cytotoxic T cells to cognate target cells is close to unity, the effects of perforin are less pronounced and cell death occurs instead via apoptosis.[35] Whether such a mechanism operates in inflammatory bowel disease is unknown.

The main importance of apoptosis in intestinal disease lies in its role in carcinogenesis and hence the promise of treatment for cancer. Colorectal cancer is now believed to result from a series of mutations of specific oncogenes and tumour suppressor genes.[36] Among the function of some of these genes (c-myc, p53, and bcl-2) is the regulation of apoptosis. For example, overexpression of wild-type p53 induces apoptosis in human tumour colonic epithelial cells.[37] We have shown that radiation fails to induce apoptosis in the intestinal epithelium of mice lacking the p53 gene.[38] It has been postulated that wild type p53 interacts with other genes in a signal transduction pathway that controls cell cycle arrest at the G_1/S checkpoint following DNA damage, which allows time for DNA repair to take place.[39] If the repair of DNA is unsuccessful then the cell may be deleted by activation of cellular events, which lead to apoptosis. Mutations of p53 cause failure of this self destruction mechanism, permitting the proliferation of malignant clones. c-myc seems to drive both cell proliferation and apoptosis. These two functions seem contradictory but Evan and coworkers have proposed this to be a failsafe mechanism to prevent the development of cancer.[40] c-myc is only able to promote proliferation when apoptosis is actively inhibited, for example by bcl-2 expression. Thus deregulated c-myc expression alone is insufficient for oncogenesis without suppression of apoptosis by other independent mutations.

It is now realised that, in general, anticancer agents do not kill by necrosis but rather by causing sensitive cancer cells to commit suicide by the induction of apoptosis.[41] Specific genes and proteins must be expressed before apoptosis can occur. Some cancers are not able to undergo apoptosis because the genes required for apoptosis are mutant or their protein products are incorrectly expressed. This is one reason for therapeutic resistance. In the gastrointestinal tract ionising radiation and many commonly used chemotherapeutic agents can induce apoptosis in the intestinal epithelium.[42] High energy ionising radiation also causes necrosis. Like spontaneous apoptosis, chemotherapeutically induced apoptosis does not occur randomly but is focused on specific locations in the crypt. Following exposure to chemotherapeutic agents apoptosis is found in the ileal crypt at the level of the stem cell[43] whereas in the colon it occurs well above the stem cell region. Potten and coworkers have proposed that this difference may explain the low incidence of small bowel cancer. In the small intestine, stem cells with oncogenic mutations are deleted by apoptosis whereas in the large intestine there is a greater chance of mutated stem cells escaping deletion as apoptosis occurs higher in the crypt.[44] This difference in the tendency of small and large intestinal epithelium to undergo apoptosis can be partly explained by the expression of the anti-apoptosis gene bcl-2, which is expressed at the base of colonic crypts but is absent in the small intestine.[25] Moreover, radiation induced apoptosis is increased in crypts of bcl-2-deficient mice.[25]

Much more needs to be learnt about cell death in the gastrointestinal tract.[45] The factors determining the reversibility of the early stages of necrotic cell injury are not fully understood. Methods for enhancing endogenous protective mechanisms against necrosis need to be explored and may prove effective in the treatment of inflammatory diseases, intestinal ischaemia, and in the preservation of organs for transplantation. Study of apoptosis in the gastrointestinal tract has been hampered by lack of a good in vitro model. At present research is largely restricted to

histological techniques though a cell culture model has been described.[46] The development of a simple, reliable method of maintaining intestinal epithelial cells in primary culture would be an important step forward. Questions remain about the role of apoptosis in the pathogenesis of gastrointestinal disease. For example, is the remodelling of jejunal mucosa in coeliac disease due to immune mediated apoptosis of villous enterocytes? Does epithelial cell loss in inflammatory bowel disease occur only by necrosis? Finally, a major challenge is to fully understand the factors that regulate apoptosis and to develop therapies that can manipulate apoptosis for the treatment of cancer, for example by blockade of the effects of anti-apoptosis genes such as bcl-2.

The author is grateful to John Hickman, Anita Merritt, Chris Potten, Caroline Dive, Juliette Askew, and Mike Marsh for helpful discussions during the preparation of this manuscript. AJMW is supported by a grant from the British Digestive Foundation and the Medical Research Council.

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