Helicobacter pylori induced apoptosis

Apoptosis, like Helicobacter pylori, has a long history, extending back into the 19th Century. Apoptosis was ignored or forgotten, just like H pylori, only to reemerge relatively recently. However, with the current exponential increase in the number of publications concerning H pylori, and in those written about apoptosis, it was only a matter of time before the influence of H pylori on apoptosis was investigated. We shall review the recent evidence that indicates that H pylori is capable of inducing apoptosis in gastric epithelial cells, and explore the mechanisms and major implication of this finding—that an alteration of gastric epithelial apoptosis may relate to the outcome of chronic H pylori colonisation.

Apoptosis in the stomach

The morphological changes occurring in a cell undergoing non-necrotic cell death were termed apoptosis by Kerr and coworkers in 1972. Although apoptosis is often used interchangeably today with the term “programmed cell death”, implying gene transcription and energy expenditure, in some instances the morphology of apoptosis may be achieved without activating the programmed cell death machinery. Semantic differences notwithstanding, apoptosis is defined by a highly characteristic sequence of morphological changes, resulting in the death of a cell without lysis. This apoptotic cell loss occurs in the absence of inflammation, and in those written about apoptosis, it was only a matter of time before the influence of H pylori on apoptosis was investigated. We shall review the recent evidence that indicates that H pylori is capable of inducing apoptosis in gastric epithelial cells, and explore the mechanisms and major implication of this finding—that an alteration of gastric epithelial apoptosis may relate to the outcome of chronic H pylori colonisation.

H pylori and gastric epithelial cell apoptosis

Evidence for the induction of apoptosis by H pylori has been obtained recently from two types of study—the identification of apoptotic cells in tissue sections from H pylori infected individuals, and the induction of apoptosis in cultured gastric epithelial cells in vitro. Each type of study has methodological advantages and disadvantages and these will now be considered.

Electron microscopy is often considered the gold standard for the determination of cellular apoptosis, but it is not practical for quantifying apoptotic cells in tissue sections. In the absence of inflammation, apoptotic cells may be recognised after staining with haematoxylin and eosin, although this is a highly labour intensive process. However, when lymphocytes and neutrophils are present, the distinction between the nuclei of apoptotic epithelial cells and of normal inflammatory cells by standard light microscopy is not feasible. For these reasons, the terminal deoxynucleotidyl nucleotide nick-end labelling (TUNEL) assay has been used as a surrogate marker to detect apoptotic cells in gastric biopsy samples. TUNEL is an in situ histochemical method that identifies cells containing fragmented DNA, a hallmark of apoptosis. It is somewhat capricious, and is prone to many of the same caveats as all histochemical quantification (and maybe some unique ones), yet several studies by independent groups have all shown that the H pylori colonised stomach contains more apoptotic epithelial cells than normal. Furthermore, the increased numbers of apoptotic epithelial cells decrease to normal following eradication of H pylori, suggesting that the bacterium or the associated inflammatory response is responsible for the increased apoptosis. Subsequently, it has been suggested that increased apoptosis may be restricted to those individuals colonised with H pylori which do not carry the cagA pathogenicity island, and further studies are in progress to determine whether the site of infection within the stomach or the extent of inflammation are related to the extent of apoptosis.

Although currently all determinations of apoptosis in the stomach in vivo have been by TUNEL staining, more dynamic methods to measure cellular turnover in biopsy...
samples are needed. Whether other surrogate markers of apoptosis will be useful in this regard remains to be seen, but such methods could include immunostaining for the proteins involved in the process of apoptosis, such as the Bcl-2 family, or specific proteases, for example.

In parallel with these investigations of biopsy material, the effect of *H pylori* on gastric epithelial cell apoptosis has been studied in cell culture too. This line of research has the advantage of examining the effect of *H pylori* in vitro, without the associated inflammatory process. But there are some disadvantages too, such as only being able to examine changes over a relatively short time period, and most studies of this type have been performed in gastric cancer cell lines which have in general been selected for their ability to survive in plastic dishes. By virtue of their isolation from the normal three dimensional interaction with other cells and the extracellular matrix, these cancer cell lines may not be representative of non-transformed cells. Nevertheless, the apoptotic responses of these gastric cancer cell lines to *H pylori* appear similar to those of short term primary cultures of normal gastric epithelial cells, and so co-culture of various clinical and laboratory strains of *H pylori* with gastric epithelial cells of different types is likely to provide useful information. Using such systems, several investigators have shown that *H pylori* is undoubtedly capable of inducing apoptosis of epithelial cells in vitro.\(^\text{10-12}\)

That *H pylori* can induce apoptosis readily in these cells may explain the apparent paradox that in vivo, *H pylori* is associated with increased numbers of proliferating gastric epithelial cells, whereas in vitro adding whole *H pylori* or extracts thereof, inhibits the growth curve of gastric epithelial cells.\(^\text{13-15}\)

Dissection of the mechanisms underlying *H pylori*’s induction of apoptosis is just beginning, but preliminary evidence suggests that *H pylori* may influence apoptosis in a number of subtle ways. Adherence seems to be important as the induction of apoptosis can be largely prevented by a physical barrier separating *H pylori* from epithelial cells.\(^\text{14}\)

However, soluble extracts of *H pylori* or high doses of purified *H pylori* lipopolysaccharide\(^\text{15-21}\) can also induce apoptotic pathways, implying that there may be no requirement for whole bacteria. In Kato-3 cells, apoptosis is partially inhibited by blocking class II MHC antigen requirement for whole bacteria. In Kato-3 cells, apoptosis is accompanied by increased expression of an important Bcl-2 homologue Bak (“Bcl-2 associated killer”), with little change in expression of other Bcl-2 family members, suggesting that, as in the colon, Bak may be an important mediator of apoptosis in the stomach.\(^\text{22}\)

In support of this idea, the immunohistochemical expression of Bak was found to parallel apoptosis in human gastric biopsy specimens.\(^\text{27}\)

Most work on the mechanisms of *H pylori* associated apoptosis has examined the direct effects of the organism or of components of the inflammatory response on the gastric epithelial cell. However, the apoptosis of epithelial cells observed in vivo may occur through more indirect mechanisms. For example, *H pylori* may affect apoptosis through altering the expression of certain growth factors, such as tumour growth factor β (TGF-β),\(^\text{28}\) or by changing circulating concentrations of gastric regulatory peptides\(^\text{16}\) and thereby modulating epithelial apoptosis.

*H pylori* may be capable of inducing apoptosis in non-epithelial cells too. Most investigators have focused upon the ability of *H pylori* to induce apoptosis in epithelial cells, but the interaction of *H pylori* with cells of the immune system is also important in understanding the pathogenesis of this organism. Perhaps the induction of apoptosis in specific lymphocyte subsets or antigen presenting cells may be a mechanism by which *H pylori* switches the cell mediated immune response from Th2 to Th1.\(^\text{30}\)

**Implications of *H pylori* induced apoptosis**

The induction of apoptosis by *H pylori* in vivo may be the stimulus for the associated hyperproliferative response.\(^\text{18}\)

Alternatively, apoptosis may be viewed as the response to hyperproliferation in an attempt to reduce tissue growth; hyperplastic changes in *H pylori* infection are rare. Whether apoptosis is the primary or secondary event is not clear, but extrapolation from the data derived in cell culture would suggest that apoptosis is the initial epithelial cell response. Thus, the induction of excessive apoptosis by *H pylori* could induce a secondary hyperproliferative response in an attempt by the mucosa to maintain cell mass. Once hyperproliferation is established, then perhaps the increased rate of cell cycling predisposes gastric epithelial cells to genotoxic damage and an altruistic cell death. If this altruistic pathway fails, then unrestrained tissue growth may result.

In examining proliferation and apoptosis, understanding which is the chicken and which is the egg is not easy. Attempting to distinguish physiological from altruistic cell death and examining the compartmental distribution of these phenomena within the gastric gland may be helpful, as may longitudinal studies in the various animal models of *Helicobacter* infection.

For the clinician, a major question is whether alterations in the ratio of apoptosis to proliferation, associated with *H pylori* infection, are a factor in determining the clinical
outcome. Again, it will be helpful to study these changes over time in animal models. The hypothesis that atrophy results from excessive apoptosis needs to be tested in humans. The converse theory, that reduced apoptosis relative to proliferation may contribute to the development of cancer, is supported by a single immunohistochemical study—a high proliferating to apoptotic cell ratio was reported in poorly differentiated gastric cancers. Further evidence implicating abnormally regulated apoptosis in the pathogenesis of gastric carcinoma comes from the observation that microsatellite mutations in the Bcl-2 antagonist Bax gene are common in gastric cancer, suggesting that Bax may be an important tumour suppressor gene in the stomach. 

*H pylori* has changed our thinking about many aspects of gastrointestinal disease. The formerly heretical idea that this organism is responsible for peptic ulcer disease is now commonly accepted, so that it may be easier for conservative thinkers to entertain the possibility that other gastrointestinal diseases, such as inflammatory bowel disease, are also infectious diseases associated with helicobacter-like organisms. However, in considering the effects of *H pylori* on the epithelial cell, we should be aware that bacteria have devised a variety of methods to manipulate host cell apoptosis to their advantage, including toxin-mediated macrophage killing (pseudomonas, *Bacillus anthracis*), activating the host’s apoptotic execution protease (shigella) and even inhibiting the apoptosis of macrophages by intracellular organisms (leishmania). As *H pylori* has learned how to survive in its specialised intragastic niche over millions of years, we should not be surprised if the effects of this unique organism on gastric cell apoptosis prove to be subtle and complex.

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