

## Leading article

# *Helicobacter pylori* induced apoptosis

Apoptosis, like *Helicobacter pylori*, has a long history, extending back into the 19th Century.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> Semantic differences notwithstanding, apoptosis is defined by a highly characteristic sequence of morphological changes, resulting in the death of a cell without inflammatory sequelae, unlike necrotic cell death.

Cell death by apoptosis has recently been calculated to account for most, if not all, cell loss in the gastrointestinal tract,<sup>4</sup> in contrast to earlier views that cells were shed passively into the lumen. It is thought that all cells have a default pathway ending in apoptosis, unless inhibited by specific external constraints, such as the interaction of a cell with its neighbour and/or the extracellular matrix. The phenomenon of the inevitable apoptosis of isolated cells has been termed anoikis or “death by homelessness”.<sup>5</sup> This time dependent pathway, or “senescent” apoptosis at the end of a cell’s natural lifespan, is probably the major physiological way cells are lost in the stomach, as in other regions of the gut. However, it is also important to recognise another route to apoptosis. As championed by Potten and colleagues, an altruistic or self-defensive form of apoptotic cell death may occur in response to severe DNA damage—such a mechanism was suggested to explain the induction of apoptosis in the intestinal crypts of irradiated rodents.<sup>6</sup> It is thought that this altruistic apoptosis serves to eliminate mutated cells before they can proliferate to form a potentially neoplastic clone, and before the end of their expected lifespan. Currently, we know little about the relative contributions of these two forms of apoptosis to normal cell loss in the stomach or, indeed, in other regions of the gut. There are currently no good markers to differentiate these two pathways to apoptosis, although p53 probably plays a role more in altruistic than in senescent apoptosis.

In recent years, interest in apoptosis has intensified, with increasing knowledge of the genes involved, and the realisation that it is a highly conserved process, from the lower invertebrates through to humans. This is of more than just

scientific interest, because understanding apoptosis may be of clinical relevance, too. How is tissue homeostasis achieved in organs which undergo rapid cellular turnover, such as the gastrointestinal tract? A constant tissue mass is now believed to be maintained by a balance between the rate of new cell production by proliferation and an equal rate of cell loss by apoptosis. Disturbances in this balance between apoptosis and proliferation would be predicted to lead to disease states. For example, atrophy when cell loss is excessive compared with proliferation, and neoplasia when the converse occurs.<sup>7</sup> As *H pylori* can cause very diverse clinical outcomes, including neoplasms in some individuals, in others atrophy, and in most an unaltered tissue mass, attention has recently been paid to examining the effect of *H pylori* on the balance between gastric epithelial cell apoptosis and proliferation.

### *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<sup>8</sup>), yet several studies by independent groups have all shown that the *H pylori* colonised stomach contains more apoptotic epithelial cells than normal.<sup>9-11</sup> 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.<sup>9</sup> 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,<sup>12</sup> 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 those of short term primary cultures of normal gastric epithelial cells,<sup>13</sup> 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.<sup>13-17</sup> 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 cells,<sup>18</sup> whereas in vitro adding whole *H pylori* or extracts thereof, inhibits the growth curve of gastric epithelial cells.<sup>13 19 20</sup> 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.<sup>14</sup> However, soluble extracts of *H pylori* or high doses of purified *H pylori* lipopolysaccharide<sup>13 15 21</sup> 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 expression with antibody, suggesting a role for these molecules in the induction process.<sup>22</sup> In the intact human stomach, the degree of apoptosis induced may not only depend upon bacteria and bacterial products, but also on the associated inflammatory response. The inflammatory mediators interferon  $\gamma$  (IFN- $\gamma$ ) and tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) augment apoptosis induced by *H pylori*.<sup>13 15 16</sup> A postulated mechanism for their interaction is through the upregulation of the Fas receptor on the gastric epithelial cell by IFN- $\gamma$ ,<sup>15</sup> and the interaction of Fas with closely related TNF- $\alpha$  receptors.<sup>23</sup> The ligand for the Fas receptor is normally expressed by activated T lymphocytes and natural killer cells, so that increased Fas receptor expression would be predicted to increase the susceptibility of gastric epithelial cells to T cell killing. However, epithelial cells may also be capable of expressing Fas ligand on their surface, so that in addition to lymphocyte mediated apoptosis, the gastric epithelial cells may be capable of fratricide. Further work using animal models with loss-of-function mutations in the Fas and Fas-ligand genes (*lpr* and *gld* mice respectively) may help clarify this issue. Other potential priming signals from *H pylori* or the inflammatory response to induce apoptosis include ammonia, urease, oxygen radicals, and ceramide; but these have not been examined in detail.

Following the initial priming stimulus, relatively little is known about the downstream pathways, the decision step (whether or not to activate the effector apoptotic pathways

after the initial stimulus), and details of the execution phase. Neither are the signal transduction events well characterised—for example, is inhibition of NF $\kappa$ B necessary, as in many other systems?<sup>17 24</sup> Whether *H pylori* normally induces a physiological, senescent type of cell death in normal but aging cells or an altruistic apoptosis in response to DNA damage, is also not clear from the experiments to date. In general, wild type p53 expression is increased during altruistic apoptosis, but whether this occurs with *H pylori* infection needs to be investigated further. We have recently investigated the role of the Bcl-2 family in the decision step of *H pylori* induced apoptosis as Bcl-2 and its related family members control a key downstream common cell cycle checkpoint, beyond which apoptosis is inevitable.<sup>25</sup> In cell culture, *H pylori* induced 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,<sup>14</sup> suggesting that, as in the colon, Bak may be an important mediator of apoptosis in the stomach.<sup>26</sup> In support of this idea, the immunohistochemical expression of Bak was found to parallel apoptosis in human gastric biopsy specimens.<sup>27</sup>

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 means. For example, *H pylori* may affect apoptosis through altering the expression of certain growth factors, such as tumour growth factor  $\beta$  (TGF- $\beta$ ),<sup>28</sup> or by changing circulating concentrations of gastric regulatory peptides<sup>29</sup> 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.<sup>30</sup>

### Implications of *H pylori* induced apoptosis

The induction of apoptosis by *H pylori* in vivo may be the stimulus for the associated hyperproliferative response.<sup>18</sup> 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 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.<sup>7</sup> 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.<sup>31</sup> Further evidence implicating abnormally regulated apoptosis in the pathogenesis of gastric carcinoma comes from the observation that microsatellite mutations in the Bcl-2 antagonistic Bax gene are common in gastric cancer,<sup>32</sup> 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.<sup>33</sup> 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).<sup>34</sup> As *H pylori* has learned how to survive in its specialised intragastric 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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