Helicobacter pylori and Apoptosis

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In an attempt to understand the diverse effects of infection with Helicobacter pylori on epithelial mucosal mass and consequent clinical outcome, the relationship between H. pylori infection and gastric epithelial cellular turnover has been investigated. Our results indicate that H. pylori increases epithelial cell proliferation and apoptosis in vivo, but that infection with bacteria of the cagA genotype leads to relatively more proliferation than apoptosis. This review explores the causes of the induction of apoptosis in gastric epithelial cells by H. pylori and the consequences of alterations in apoptosis to the maintenance of gastric mucosal homeostasis.

INTRODUCTION

How does infection by Helicobacter pylori produce such diverse clinical outcomes? Most of the world's population are chronically infected with this organism, yet fewer than one fifth develop ulcers during their lifetime and only a very small percentage, perhaps one to two percent, will develop cancer or lymphoma. The remainder continue to have a chronic gastritis over decades, with progression to chronic atrophy in about one half of these and intestinal metaplasia in around a tenth. However, most people infected are unlikely to develop any clinical consequences of their infection and die ultimately of an unrelated cause [1, 2, 3].

In recent years, much attention has focused on the role of possible bacterial pathogenesis factors in determining clinical outcome. These bacterial virulence factors include the vacuolating cytotoxin, vacA, and more lately, cagA, together with associated genes on the cagA pathogenicity island. However, although there is an association between vacA and cagA, and the incidence of ulcers and cancer in many populations, it is clear that the presence of these proteins does not account for all the disease associations. For example, although patients with ulcers are in general infected with cagA positive/vac A positive strains, many individuals harboring bacteria with this phenotype do not have ulcers, and in some parts of the world where the incidence of gastric cancer is very high, almost everyone is infected with cagA positive strains yet cancer is not universal [4]. Recent work in animal models has demonstrated that host factors may be just as important in determining outcome. For example, inbred strains of mice respond quite differently to the same infection with H. felis. Some strains had an intense inflammatory response, while only one of three strains developed erosions, a phenomenon only partially related to the MHC haplotype [5].

We have been interested in exploring the possibility that the outcome of infection is determined not only by the bacterium but by the interaction between bacterium and epithelium. Thus, interactions leading to epithelial cell loss would be expected to cause atrophy or ulceration, while interactions leading to excessive epithelial cell growth may

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predispose to cancer. Such models are based upon the recent recognition of the importance of programmed cell death (apoptosis) in the maintenance of tissue homestasis in organs undergoing cellular turnover, such as the gastrointestinal tract.

EPITHELIAL APOPTOSIS IN THE GASTROINTESTINAL TRACT

It is being increasingly appreciated that tissue mass is dependent not only upon new cell production by cell proliferation. To maintain tissue integrity and homeostasis, the rate of cell proliferation needs to be balanced by the rate of cell loss [6]. It is now believed that in the normal gastrointestinal tract, cells are lost not by passive shedding into the lumen, but by an active energy-requiring cell suicide program termed “apoptosis.” A renewed interest in examining cell loss in the gastrointestinal tract has occurred in part because of our increasing ability to recognize cells undergoing apoptosis. Although histologists had been familiar with nuclear pyknosis and karyorrhexis for decades, the phenomenon of non-necrotic cell death and the use of the term apoptosis to describe it are much more recent. In retrospect, it is clear that what pathologists were in many cases describing in a variety of different organs were apoptotic cells. For example, Councilman bodies in the liver, karyolytic bodies in intestinal crypts and so on [7]. Among the earliest papers looking at apoptosis in the gastrointestinal tract are those of Elmes [8], and Potten and co-workers, who examined the effect of DNA damaging agents on apoptosis in the small and large intestines of rodents [9]. Until very recently, however, apoptosis in tissues could only be assessed by light microscopy of H&E stained sections or by electron microscopy. Both have their drawbacks, the former related to the extreme difficulty of detecting apoptotic cells in the presence of inflammation and the latter being non-quantitative. The development of an in situ histochemical assay to stain cells carrying DNA strand breaks, one of the major hallmarks of apoptosis, by Gavrieli and colleagues in 1992 [10], led to the appreciation that apoptosis was a common event in the gastrointestinal tract [11]. The recognition that apoptosis is an important physiological process in the gut, as in other tissues, together with the ability to identify apoptotic cells has led to considerable interest in the possibility that alterations in apoptosis may be important in the pathogenesis of gastrointestinal disease [12].

H. PYLORI, LIFE AND DEATH IN THE STOMACH

In the stomach, new cells are produced from the proliferative zone located at the base of gastric pits and migrate upward towards the lumen, where they differentiate and ultimately undergo apoptosis after about one week. There is also a downward, slower, migration of cells, ending in apoptosis and probably phagocytosis at the base of the glands after one to two months. Thus, cell proliferation and apoptosis are intimately interrelated, representing opposite ends of the cells’ life cycle. Increased rates of cell proliferation would, therefore, produce a greater mass of tissue, unless balanced by increased rates of apoptosis, and conversely, increased rates of cell death may be responsible for stimulating increased cell proliferation. A balanced, homeostatic relationship between proliferation and apoptosis allows the gastrointestinal tract to adjust for damage due to luminal insults and serves to prevent cell accumulation where cell proliferation is excessive. Although apoptosis is commonly viewed as the physiological endpoint of the cell life span, there is also evidence in the gastrointestinal tract, at least, that apoptosis may be activated early in the cell’s life span as a self-defense mechanism to eliminate cells carrying mutations. This “altruistic” cell death occurs after DNA damage induced by irradiation or mutagenic drugs, for example and is dependent upon p53 [13].
What is the relevance of gastric apoptosis to the pathogenesis of *H. pylori*? Increased cell proliferation rates have been noted in all stages of the gastric preneoplastic process, including atrophic gastritis, intestinal metaplasia, gastric dysplasia and gastric carcinoma [14]. Several recent studies have clearly demonstrated, using a variety of different cell proliferation markers, that the number of proliferating gastric epithelial cells is increased in the presence of *H. pylori* infection and that this can be normalized by the eradication of *H. pylori*, suggesting that the organism or the associated gastritis is responsible for this phenomenon [15-20]. We have confirmed this in a group of duodenal ulcer patients, measuring epithelial cell proliferation rate using the Mib-1 antibody to detect cells expressing the Ki-67 cell proliferation-associated antigen. In this study, patients were examined both before and after treatment with bismuth-based triple therapy, and thus served as their own controls (Figure 1). The eradication of *H. pylori* was associated with a marked reduction in the numbers of proliferating gastric epithelial cells (Figure 2). Since *H. pylori* increases cell proliferation wherever examined, why is there not exuberant gastric mucosal growth in patients infected by *H. pylori*?—could this be because *H. pylori*-increased proliferation is a response to increased apoptosis? Furthermore, although *H. pylori* increases cell proliferation *in vivo*, most studies have demonstrated that, *in vitro*, adding *H. pylori* to gastric epithelial cells in culture results in an inhibition of cell growth and proliferation [21-23]. Could this reflect the induction of apoptosis by *H. pylori*?

**THE EFFECT OF H. PYLORI ON GASTRIC APOPTOSIS**

To examine the effects of *H. pylori* on epithelial cell apoptosis *in vivo*, we used *in-situ* terminal deoxuryridine nucleotide nick end-labeling (the TUNEL method) to stain apoptotic cells in gastric antral biopsies. As for proliferation, we examined apoptosis in patients with duodenal ulcer disease, comparing the same patients before and after treatment [24]. Apoptotic cells could be identified in gastric antral biopsies and were normally located at the luminal end of the gastric gland. In *H. pylori* associated antral gastritis, the number of

![Figure 1. Proliferating cells in gastric antral mucosa, stained with the Mib-1 antibody against the Ki-67 antigen. Before eradication of *H. pylori*, numerous proliferating cells are present in an expanded proliferative zone (a, left), nuclear Ki-67 immunoreactivity can be appreciated better at higher power (b, middle). After eradication of *H. pylori* the number of proliferating cells is markedly decreased (c, right). Original magnification x 200 (a, c); x 500 (b).](image-url)
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Figure 2. Effect of eradication of *H. pylori* on gastric epithelial cell proliferation, measured by immunostaining with the Mib-1 antibody. Bars represent the mean values.

apoptotic epithelial cells was increased, and apoptotic cells could be seen spread throughout the entire gland. Furthermore, there were increased numbers of apoptotic cells in the inflammatory infiltrate, although the increased number of apoptotic epithelial cells in *H. pylori* gastritis was not related to the degree of inflammation. After the eradication of *H. pylori*, the increased number of apoptotic cells was reduced significantly and became similar to a group of *H. pylori*-negative dyspeptic controls (Figure 3). It should be noted, however, that although the mean number of apoptotic cells was increased in *H. pylori* gastritis; this was because around half of the patients had increased numbers of apoptotic cells, but half had numbers similar to those of controls. In fact, there was a poor correlation between the numbers of proliferating cells and apoptotic cells from serial sections of the same biopsies (r = .027, p = .07), with two apparent groups: one with high numbers of proliferation cells but few apoptotic cells, and another group with greatly increased number of both proliferating cells and apoptotic cells (Figure 4). In a further study in collaboration with Dr. Peek and colleagues at Vanderbilt University, we have evaluated the relationship between apoptosis and proliferation in a larger group of patients prospectively, and found that the two groups described above may represent the outcome of infection with *cagA* positive and *cagA* negative strains [25]. Proliferation was increased in both *cagA* positive and *cagA* negative strains, compared with normals but was greatest in the *cagA* positive infected mucosa. Apoptosis was increased only by *cagA* negative strains and in *cagA* positive cases was not significantly different to normal. A strong concordance was found between the presence of *cagA* positivity and the vacA s1a signal sequence, so that these relationships held when the data were reanalyzed for the vacA s1a signal sequence. Thus, *cagA*-negative *H. pylori* infection is associated with both increased apoptosis and increased proliferation, whereas in the *cagA* positive group there was an apparent imbalance between the two, with increased rates of cell proliferation unaccompanied by increased apoptosis. Ultimately, an imbalance between increased proliferation, but relatively normal apoptosis could lead to unrestrained tissue growth and even neoplasia.
Figure 3. Effect of eradication of *H. pylori* on gastric epithelial cell apoptosis, measured by the TUNEL assay. Bars represent the mean values. At right are the results (mean + standard deviation) of a group of 12 non-ulcer dyspeptic controls. From [24] with permission.

Figure 4. Relationship between apoptosis and proliferation in duodenal ulcer patients, before and after eradication therapy.
Figure 5. The regulation of apoptosis—a simplified schema. ECM = extracellular matrix; (○) = specific cell surface receptor. See text for details.

THE REGULATION OF APOPTOSIS

Apoptosis is a highly organized and regulated process requiring transcriptional regulation, new protein expression and the utilization of energy stores. The net effect of several stimulatory and inhibitory pathways determines whether apoptosis occurs, and examining the effect of *H. pylori* on these pathways may be helpful in understanding how *H. pylori* alters gastric epithelial cell apoptosis. Although our studies so far have indicated that bacterial factors may be important in determining the extent of apoptosis, it is possible that the effects we have seen are related not directly to bacteria but to the associated inflammatory response. Figure 5 summarizes some of the key determinants of apoptosis. The endogenous apoptosis pathways may be activated by the detection of DNA damage by wild type p53. In gastric carcinogenesis, where p53 is lost or mutated, the ability to detect and react to DNA damage will be impaired and apoptosis may be either activated inappropriately or inhibited. A second pathway to apoptosis involves the external stimulus of growth factors and the extracellular matrix. Most growth factors in the stomach are thought to stimulate proliferation and/or inhibit apoptosis, such as hepatocyte growth factor or transforming growth factor-alpha, but it is noteworthy that transforming growth factor-beta has a predominantly negative regulatory effect on cell growth, through the induction of gastric epithelial cell apoptosis [26]. Thus, a lack of transforming growth factor-beta or an inability to respond to this peptide due to a mutation in the type II receptor, as occur in gastric carcinogenesis [27], would also result in decreased gastric epithelial cell apoptosis. Finally, an increasingly complex pathway to activate apoptosis occurs through the family of tumor necrosis factor receptors, stimulated by binding tumor necrosis factor or the Fas ligand and resulting in the activation of intracellular kinase cascades and ultimately transcriptional regulation [28]. Since tumor necrosis factor-alpha is one of the pro-inflammatory cytokines increased in the mucosa of *H. pylori* infected gastritis, this may well be a key promoter of apoptosis in the gastric epithelium. Once apoptotic pathways are activated, there is a common late downstream regulatory step that determines whether apoptosis will ultimately occur. This step is dependent upon regulation by the Bcl-2 family of proteins, some of which may be altered in *H. pylori* gastritis [29] and in
the later stages of gastric cancer [30]. We have little idea of the relative importance of each of these pathways and their regulatory molecules in the normal stomach. However, the Fas pathway has been implicated in a mouse model of autoimmune gastritis [31], and Mannick and colleagues have recently suggested that nitric oxide synthesis in the mucosa of H. pylori-infected gastritis may promote apoptosis through inducing DNA damage [32].

**SUMMARY AND CONCLUSION**

Chronic H. pylori infection may increase gastric epithelial cell proliferation by causing a higher than normal rate of apoptosis. How H. pylori induces apoptosis is not clear. After an initial phase of increased cellular turnover manifested by increased “physiological” apoptosis and increased cell proliferation, a shift in the balance between apoptosis and proliferation may result in dysregulated tissue growth. What precisely is responsible for this dysregulation is unclear. Candidates include the cagA phenotype of the bacteria, the acquisition of a mutation of p53 or the transforming growth factor-beta type II receptor, or other factors capable of regulating proliferation, apoptosis and the cell cycle. Although our knowledge of the relationship between proliferation and apoptosis in the stomach is still at a very early stage, it is possible to construct a model to test some hypotheses (Figure 6). In this model, the balance or imbalance between apoptosis and proliferation is assumed to determine the outcome of infection. Whether chronic gastritis progresses to ulcers and atrophy, or to cancer, may depend upon bacterial factors (such as the cagA phenotype) and perhaps more importantly, epithelial cell determinants (for example, p53 status, the transforming growth factor-beta type II receptor and Bcl-2 family expression). It is likely that in the future other epithelial cell regulatory molecules, oncogenes or tumor suppressor genes, which determine the outcome of infection with H. pylori, will be identified. Finally, it should be emphasized again that it is the relationship between apoptosis and proliferation that is important rather than apoptosis per se. Apoptosis is a healthy phenomenon when activated at the end of the cell life span, or when appropriately activated to eliminate cells carrying mutations. It is only when inappropriately activated or inhibited that abnormalities of apoptosis may contribute to disease. Understanding the normal control of apoptosis in gastric epithelial cells will help determine whether an interaction of H. pylori with the epithelium will result in deleterious consequences.
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