

Helicobacter pylori

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H Eguchi, S F Moss

For life or death?

Helicobacter pylori infection leads in some individuals to the development of gastric cancer.¹ Because both *H pylori* infection and gastric cancer are relatively common worldwide, this association has spurred considerable interest into determining whether gastric cancer can be prevented by the eradication of *H pylori* and into investigating the mechanisms by which this extracellular bacterium and its associated inflammatory response promote carcinogenesis. In this regard, *H pylori* may provide important clues for the pathogenesis of other tumours associated with chronic inflammatory states.

Despite the fact that *H pylori* does not invade epithelial cells, it has important and profound effects on the gastric epithelial cell, which include provoking a state of chronic epithelial hyperproliferation that has long been recognised as a precursor of malignancy in the stomach as it has in many other cancer prone organs. How does *H pylori* induce proliferation? A considerable amount of evidence from cell culture and animal models implicates apoptosis as the primary response of gastric epithelial cells to *H pylori*, with epithelial hyperproliferation as a secondary, presumably compensatory, occurrence.² The attachment of *H pylori* is generally thought to be necessary for the induction of apoptosis in gastric cells, based on experiments in co-culture systems. However, the downstream pathways involved in the transduction of proapoptotic signals triggered by *H pylori* remain to be clarified.

In general, two major pathways of apoptosis have been implicated in *H pylori* induced apoptosis—the death receptor pathway and the death receptor independent “stress” or mitochondrial pathway. In support of the Fas–Fas ligand death receptor pathway, the use of antagonistic anti-Fas antibodies has been shown to block the apoptosis induced by *H pylori* in gastric³ and intestinal epithelial cells⁴ and in T cells.⁵ In addition, the lack of functional Fas ligand impairs the epithelial response to *Helicobacter felis* in a mouse model.⁶ In contrast, transfecting the vacA vacuolating cytotoxin of *H pylori* into HEP-2 cells resulted in vacA translocating to the mitochondria and releasing mitochondrial cytochrome c,⁷ indicating that the mitochondrial pathway may be involved in *H pylori* induced apoptosis. This

is consistent with observations that the expression of the proapoptotic Bcl-2 family members, Bak and Bax, confirmed releasers of mitochondria cytochrome c, is increased during the induction of apoptosis by *H pylori*.^{8,9} Recently, further strong support for the mitochondrial pathway being the dominant proapoptotic pathway activated by *H pylori* has been provided by a series of carefully conducted experiments using a defined wild-type *H pylori* strain and several isogenic mutants co-cultured with gastric cells. In these studies, Maeda *et al* have shown that during apoptosis induced by *H pylori* the death receptor pathway and its downstream effectors, including caspases 8, 3, and 7 were indeed activated, but inhibiting this pathway with antagonistic anti-Fas antibody did not influence apoptosis.¹⁰ Furthermore, *H pylori* was found to stimulate mitochondrial cytochrome c release accompanied by the translocation of Bax from cytosol to the mitochondrial membrane during *H pylori* induced apoptosis.

The second major finding in this paper by Maeda and colleagues was the intriguing observation that at the same time as inducing apoptosis, *H pylori* also had an anti-apoptotic effect, via the activation of the nuclear factor κ B (NF- κ B) transcription factor. This effect was revealed by transiently transfecting a kinase deficient I κ B construct to inhibit NF- κ B activation. In many respects, the demonstration of this antiapoptotic effect of *H pylori* was not surprising because it is well established that contact between *H pylori* and gastric cells results in the activation of NF- κ B.^{11,12} Although NF- κ B may behave as a positive regulator of apoptosis in some contexts, in most situations NF- κ B activation by—for example, tumour necrosis factor α , chemotherapeutic drugs, or ionising radiation—protects against apoptotic cell death.¹³ Candidate downstream molecules involved in NF- κ B mediated protection against *H pylori* stimulated apoptosis include the mitochondrial membrane stabilising proteins Bcl-xl and Blf-1, the caspase inhibitors cIAP1/cIAP2 and XIAP, the tumour necrosis factor receptor associated TRAF1 and TRAF2 molecules, and the cell cycle regulatory protein cyclin D1.¹³ However, quite a different conclusion regarding the role of NF- κ B in *H pylori* induced

apoptosis has been reached by Gupta *et al*, who reported that the upregulation of NF- κ B by *H pylori* could induce apoptosis and that the apoptosis can be suppressed by activation of the peroxisome proliferator activated receptor γ .¹⁴ An easy explanation for these discrepant results is not immediately obvious, but it may reflect differences in the experimental methods used because the precise co-culture conditions and approaches frequently vary widely between groups of investigators in this field, and some of the model systems may only poorly reflect the interactions that take place in the gastric mucosa.

The activation of apoptosis by *H pylori* is probably important in the stimulation of the compensatory epithelial hyperproliferation seen in chronic gastritis and in the aetiology of the tissue damage occurring in gastroduodenal ulceration as a result of *H pylori*. Is the activation of antiapoptotic pathways by *H pylori* also relevant to clinical conditions? Conceivably, the induction of antiapoptotic pathways by *H pylori* may provide explanations for several interesting phenomena. For example, Mongolian gerbils experimentally infected by *H pylori* exhibit increased cell turnover early after infection but later display evidence of an adaptive decrease in apoptotic and proliferative cell numbers.² Moreover, we have described how the repeated addition of *H pylori* to epithelial cells in vitro can induce or select for gastric epithelial cells that exhibit an apoptosis resistant phenotype, characteristic of the apoptosis resistant cells found all too frequently in human gastric cancer.¹⁵ Thus, in some situations the stimulation of survival pathways by *H pylori* may be more important than its ability to promote apoptosis in the gastric epithelium.

In part, the effect of *H pylori* on epithelial cell cycle events may relate to the virulence of the infecting strain. The ability of *H pylori* to activate NF- κ B is known to be dependent on genes within the cag pathogenicity island of *H pylori*. However, this relation does not exclude the possibility that cag negative strains can activate other epithelial survival pathways, such as those regulated by cyclooxygenase 2.

The demonstration that *H pylori* can promote both apoptosis and cell survival simultaneously is novel, but should not really surprise us. If there is one lesson we have learned from this organism, it is to expect the unexpected.

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Authors' affiliations

H Eguchi, S F Moss, Division of Gastroenterology, Department of Medicine, Rhode Island Hospital/Brown University, Providence, RI 02903, USA

Correspondence to: Dr S F Moss, Division of Gastroenterology, Department of Medicine, Rhode Island Hospital, 593 Eddy St, APC 445, Providence, RI 02903, USA; smoss1@pol.net

Correspondence to: Dr S F Moss, Division of Gastroenterology, Department of Medicine, Rhode Island Hospital, 593 Eddy St, APC 445, Providence, RI 02903, USA; smoss1@pol.net

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ECHO

Can electroporation make all the difference in gene therapy?



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Gene therapy has so far been hampered by the methods used to transfer DNA. But electroporation might overcome the problems associated with using a viral vector or simple DNA injection for the treatment of liver damage, suggests Japanese research.

The researchers transferred 50 µg rat hepatocyte growth factor in a modified pKSCX plasmid into the skeletal muscle of 8 week old female mice by electroporation, using a pulse generator. Four days later carbon tetrachloride was used to induce acute liver injury. ELISA was used to determine plasma HGF every other day for three weeks, starting four days after transfer. A fluorescent green plasmid was also transferred to check how effective electroporation had been.

Strong green fluorescence was evident in the muscle where the fluorescent protein had been transferred. HGF was up to four times as high as levels before transfer, peaking at six to nine days and then rapidly diminishing over the three weeks of the study. And hepatocyte apoptosis more than doubled after two days and ALT activity was significantly higher—and took longer to return to normal—in mice not given gene therapy.

The authors point out that, unlike simple DNA injection, electroporation works equally well in regenerating and in normal muscle tissues. And unlike viral vectors, it does not seem to activate an immune response or be mutagenic. The effects of electroporation can also be sustained using repeated transfer.

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