**Helicobacter pylori:** Bacterial Factors and Interaction with the Epithelial Cells

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*Helicobacter pylori* has been recognized as a major cause of most of the diseases of the stomach. These diseases are preceded by lesions of gastritis induced by *H. pylori*. This long-standing infection gives us a very good model of interaction between a bacterium and its host. We will review the direct and indirect effects of *H. pylori*.

**DIRECT ACTION**

*Helicobacter pylori* cytotoxin

It has been known since 1988 that *Helicobacter pylori* can produce a vacuolating cytotoxin (VacA) [1]. This molecule has been purified and characterized by Cover and Blaser. The cytotoxin is a 140 kD molecule secreted as a 91 kD protein that splits into two sub-units of 58 and 37 kD [2]. The gene vacA has been cloned, and an isogenic mutant strain that does not produce the cytotoxin has been developed. The gene is present in all *H. pylori* strains but is not always expressed. Recently, Atherton et al. identified signal sequences that correspond to strains producing high levels of cytotoxin (s1a), low levels (s1b) or no cytotoxin (s2) [3].

The cytotoxin is responsible for epithelial damage, erosion and perhaps ulceration but not inflammation [4]. Strains producing the cytotoxin have been found associated with ulcer diseases (60 percent versus 30 percent for gastritis only) [5].

The cag region

Early work showed that antibody response to a particular protein of *H. pylori* was associated with duodenal ulcer disease [6]. This protein was originally thought to be the cytotoxin, but the confusion was ended when the toxin was purified. The protein was named cagA since it was nevertheless very often associated with the expression of the cytotoxin. The gene encoding for cagA (3650 bp) is not present in all strains of *H. pylori* [7]. It can be detected by hybridization or by a combination of PCR using different primers since there is some variability within its structure.

The protein has a MW of 120 kD and is highly immunogenic. We do not know its role in pathogenesis, but it has been proposed as a marker of pathogenicity. Only strains of *H. pylori* that are cagA-positive are able to induce the production of IL-8 by epithelial cells [8]. After knocking out the cagA gene, IL-8 induction is still present indicating that cagA *per se* is not responsible for this property. By studying genes upstream of cagA, Blaser’s group found two other genes, cagB and cagC, which seem to be responsible for IL-8 induction [9]. These genes have been subsequently named picA and picB (for “Permit the Induction of Cytokines”).

Further work has been performed by Rappuoli and his group in Italy [10] and Berg and his group in Missouri [11]. They noted that, indeed, a cluster of 20-30 genes forms a

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cag region, which is not present in all *H. pylori* strains. Furthermore, this cag region may exist in two parts in different areas of the *H. pylori* genoma within some strains. The dissection of this cag region has not yet been fully performed, but it seems that it encodes for different genes involved in the transport of proteins, some being close to known genes from other pathogenic bacteria. For this reason, the cag region can be categorized as a so-called "pathogenicity island."

The cag region seems to be responsible for the significant inflammation present in the gastric mucosa when the gene is present, in comparison to the cases when it is absent. The induction of IL-8 from the gastric epithelial cell may be a critical factor.

In conclusion, while we do not yet have an idea of the mechanism, the detection of the cagA protein or the cagA gene in a *H. pylori* strain can be considered as the best marker of pathogenicity that we currently have.

**Consequence of adherence of *H. pylori* to epithelial cells**

The proportion of *H. pylori* organisms adhering to the epithelial cells is a matter of controversy. While some have claimed that 20 percent adhere, others have proposed that only two percent are in close contact with the cells at any instant. When a bacterium adheres to a cell, it allows the delivery of the toxic compounds in the vicinity of the cell and therefore increases the deleterious effects [12]. Adherence is probably important also in fighting peristalsis, especially in the duodenum when the bacteria colonize antral metaplastic areas.

There is also a direct consequence of adherence on the cell: a modification of the actin cytoskeleton. It has been shown that actin condensation is present at the site of adherence. In some cases, there is also an attaching-effacing effect of the microvilli and formation of pedestals, as is the case with enteroadherent *E. coli*. The exact mechanism is not known but may involve signaling pathways such as tyrosine phosphorylation [13]. One consequence may also be a decrease in mucin exocytosis and induction of IL-8 as previously mentioned. We do not know how this may be deleterious for the cell, but it could be a way of initiating apoptosis.

**INDIRECT ACTION**

Some enzymes produced by *H. pylori* have been proposed as being indirectly responsible for mucosal damage. These enzymes include urease, phospholipases and alcohol dehydrogenase.

Urease is undoubtedly important in bacterial colonization by generating ammonia [14]. The role of ammonia in the occurrence of the mucosal damage is more controversial. Ammonia is cytotoxic. It has a lysosomotropic effect that can be identified. However, this effect seems to occur only with the NH$_3$ molecule at alkaline pH. At pH 7, most of the compound is found in the form of an NH$_4^+$ ion, which is not cytotoxic. Nevertheless, as mentioned before, the direct delivery of the compound to the cell may contradict this fact.

Phospholipases A and C are produced by *H. pylori* at low concentrations and may induce the production of lysolecithin, a toxic product, as well as act directly on the cell membrane.

When there is an excess of ethanol, alcohol dehydrogenase may produce the harmful compound acetaldehyde, which may be a factor of atrophy. The exact role of these compounds (ammonia, lysolecithin and acetaldehyde) is not yet known, but they may be responsible for the background of lesions and inflammation present even when *H. pylori* strains do not produce cytotoxin and do not harbor the cag region.

Recently, another enzyme was found in *H. pylori*, the N-α-methyl-histamine-transferase, which generates N-α-methyl-histamine, a compound that is an agonist of the H$_3$ receptors of histamine [15]. This compound does not act on epithelial cells but could, for
example, act on D cells in order to decrease the release of somatostatin. The enzyme has not yet been purified, nor has the gene been cloned, but if it is confirmed it would give a nice explanation of the effects of *H. pylori* on acid secretion.

But the major indirect damage caused by *H. pylori* is probably the inflammatory response. There are a number of cytokines released in the depth of the mucosa, some being harmful to the tissue. Some strains seem, nevertheless, to induce a more intense and quick oxidative burst than others. The LPS from *H. pylori* has low biological activity but may induce an autoimmune response since some sugar terminal regions mimic Lewis group antigen x and y [16], and this will have to be considered in the future in *H. pylori*-associated diseases.

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