CagA: a role at last


Abstract

*Helicobacter pylori*, present in half of the world’s population, is a very successful pathogen. It can survive for decades in the human stomach with few obvious consequences to the host. However, it is also the cause of gastric diseases ranging from gastritis to ulcers to gastric cancer and has been classified a type 1 carcinogen by the World Health Organization. We have previously shown that phosphorylation of a 145-kDa protein and activation of signal transduction pathways are associated with the attachment of *H. pylori* to gastric cells. Here we identify the 145-kDa protein as the *H. pylori* CagA protein. We also show that CagA is necessary to induce a growth-factor-like phenotype (hummingbird) in host gastric cells similar to that induced by hepatocyte growth factor (HGF). Additionally, we identify a second cellular phenotype induced after attachment by *H. pylori*, which we call SFA (stress fiber associated). SFA is CagA independent and is produced by type I and type II *H. pylori*.

Comment

Many strains of *Helicobacter pylori* produce an immunogenic high molecular weight protein called cytotoxin associated gene product A (CagA) and infection with such strains can be detected by a simple serological test for anti-CagA antibodies. Numerous studies over the past nine years have shown that CagA positive infections are more likely than CagA negative infections to result in gastrointestinal ulceration or gastric carcinoma. Despite this, no direct role for CagA in pathogenesis or function has been found. Now, four independent groups have shown that CagA is delivered by *H. pylori* into gastric epithelial cells where it is phosphorylated, and triggers profound changes in the host cytoskeleton. This should help explain the enhanced virulence of CagA positive *H pylori* strains.

Previously, *H. pylori* has been shown to induce changes in gastric epithelial cells during attachment. The microvilli disappear at the site of attachment, the cytoskeleton is rearranged beneath the bacterium, and a cup shaped pedestal forms beneath the bacterium. At the same time, intracellular signalling changes can be detected inside the epithelial cell with phosphorylation of proteins on tyrosine residues—a classic cellular signalling pathway. The major phosphorylated protein has now been shown not to be a host cell protein, but *H pylori* CagA. The four groups involved have shown this conclusively using a variety of methods: the phosphorylated protein varies in size according to the size of CagA in the *H pylori* strain and is not induced by CagA negative mutants; it is 35S labelled if the bacterium is so labelled before allowing it to interact with cultured epithelial cells; it reacts with anti-CagA and antiphosphotyrosine antibodies; and if antiphosphotyrosine antibodies are used for purification, direct sequencing shows the protein to be CagA. Furthermore, indirect immunofluorescence and confocal microscopy elegantly show colocalisation of CagA and phosphotyrosine next to the attachment site of the bacterium.

Immunofluorescence studies performed by Segal et al have allowed CagA, the *H pylori* bacterium, and epithelial intracellular structures to be visualised. The images produced show that CagA is inserted into cells and then forms a cylinder next to or surrounding the bacterium. This is associated with active cytoskeletal rearrangement next to the cylinder. Under some conditions, the whole cell is stimulated to spread and elongate in a fashion identical to that produced by a human growth factor called HGF (hepatocyte growth factor) and this change is dependent on tyrosine phosphorylation of CagA. Other cellular changes include dephosphorylation of some normally phosphorylated proteins, showing that signalling changes can be widespread. Further evidence that CagA may suppress some cellular responses comes from the finding that CagA negative mutants cause more marked induction of a second cellular change associated with extensive stress fibre formation than their CagA positive parents.

Recent studies also provide evidence concerning how CagA is delivered into epithelial cells. The cagA gene is known to be at one end of the cag pathogenicity island (PaI), a segment of DNA containing 31 putative genes and thought to have been acquired by *H pylori* relatively recently in its evolution. The cag PaI genes are thought, by comparison with similar genes in other bacteria, to encode a complex syringe-like structure called a type IV secretory apparatus. Free CagA cannot enter epithelial cells and inactivating various cag PaI genes abolishes CagA delivery and phosphorylation. This is consistent with CagA delivery by a cag encoded syringe into the host epithelial cell.

The knowledge that CagA is delivered into epithelial cells where it hijacks cell signalling will provide new impetus for research into *H pylori* virulence. Comparisons with similar systems in other bacteria should help rapid progression in this field. The most striking functional similarity is with enteropathogenic *Escherichia coli* (EPEC) which also expresses a molecular syringe (although this is of a structurally and phylogenetically different type called a type III system) through which it injects a protein called translocated intimin receptor (Tir). In common with CagA, Tir is phosphorylated in the host cell and induces cytoskeletal rearrangement with pedestal formation.
expressed on the cell surface after phosphorylation and acts as a receptor for bacterial adherence. A number of other bacteria have type III or type IV secretory systems, and in many cases the secreted proteins are well characterised. Although their functions are diverse, many induce cytoskeletal changes and may provide clues as to the mechanism of action of CagA.

For the gastroenterologist, the most immediate question is the relation between the enhanced pathogenicity of CagA positive strains, intracellular phosphorylation, and disease. *H pylori* strains which express CagA are more often associated with peptic ulceration and gastric adenocarcinoma than are CagA negative strains.8 9 This is thought to be because they induce more intense gastric inflammation.9 CagA positive strains induce epithelial cells to produce large amounts of proinflammatory cytokines, such as interleukin 8, yet it is known that this property is also seen in CagA negative mutants.6 7 11 However, mutants in which other *cag* PAI genes have been inactivated (presumably interfering with syringe assembly) have a diminished ability to induce interleukin 8.6 7 11 Thus it appears that either another translocated protein induces this proinflammatory effect or that it is induced by the syringe structure itself. Furthermore, neither of the strains for which the complete genome has been sequenced induce phosphorylated CagA in host cells, despite the fact that one of these (J99) was isolated from a patient with duodenal ulceration. Interestingly, the predicted J99 CagA protein lacks any tyrosine phosphorylation motifs.7 The other strain, 26695, is also interesting as it has a seemingly complete *cag* PAI and a CagA protein with tyrosine phosphorylation motifs, but its *cag* encoded injection machinery is non-functional.4 Thus the only method of determining whether a strain can induce CagA host phosphorylation may be to test this directly.

The future for research into the importance of CagA in pathogenesis seems bright. There is clearly a place for mechanistic in vitro studies, and these should help identify the precise actions of CagA on the epithelial cell and how these could allow persistence of infection or cause disease. The effect of defined strains in animal models should be assessed, and strains from patients with carefully defined gastroduodenal pathology should be studied. In particular, it will be important to compare strains which are able to inject CagA into epithelial cells and induce its tyrosine phosphorylation with other CagA positive strains which are unable to do this.

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