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Helicobacter pylori is invasive and it may be a facultative intracellular organism

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Summary

The pathogenicity of many bacteria colonizing the gastrointestinal tract often depends on their ability to gain access to cells that are normally non-phagocytic. *Helicobacter pylori* colonizes the stomach of over half the world population and is the main cause of peptic ulcer disease and gastric cancer. It is generally considered to be a non-invasive pathogen present only in the lumen of the stomach and attached to gastric epithelial cells although a number of *in vivo* and *in vitro* studies have demonstrated that *H. pylori* is in fact invasive. In addition, *H. pylori* can repopulate the extracellular environment after complete elimination of extracellular bacteria with gentamicin, suggesting it may be considered a facultative intracellular bacterium. This review examines the validity of these observations and describes the evidence suggesting that the intracellular presence of *H. pylori* plays a role in the induction of diseases, in immune evasion, and in life-long persistence of the bacterium in the stomach of a majority of humans.

Introduction

The gastric lumen has long been considered a sterile environment, although it is now established that the presence of many bacteria can be detected in the stomach. Since the start of the 20th century, many authors observed the presence of spiral organisms in the stomach and suggested that these bacteria were causing gastric diseases. Their observations were mostly ignored until 1985, when Warren and Marshall demonstrated convincingly that *Helicobacter pylori* causes gastritis (Marshall *et al*., 1985a), a discovery for which they received the 2005 Nobel Prize in Physiology or Medicine. In addition, the presence of *H. pylori* is strongly associated with peptic ulcer disease (Marshall *et al*., 1985b;Anonymous, 1994a) and gastric cancer (Anonymous, 1994b). Today, we know that *H. pylori* colonizes the stomach of over half of the word population and that it is consistently associated with chronic active gastritis, although only 15–20% of *H. pylori* positive subjects experience gastric diseases and some patients with peptic ulcer or gastric cancer appear to be *H. pylori* negative.

This apparent paradox suggests that the *H. pylori* presence in the stomach is not sufficient to cause gastric disease and that one or several additional conditions need to be fulfilled. For example, *H. pylori* has to carry specific virulence genes (e.g. the *cag* pathogenicity island and the *vacA* and *babA* genes) (Peek and Blaser, 2002) and the host may have to be predisposed to develop disorders during infection [e.g. IL-1β or NOD gene polymorphism in certain populations (El-Omar *et al*., 2000;Rosenstiel *et al*., 2006)]. In addition, undefined environmental factors that precipitate the occurrence of disease may have to be present, and it is believed that protective factors such as vitamins are protective. Finally, other factors may

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play a role in *H. pylori* pathogenicity, life-long persistence, and resistance to antibiotic treatment.

Because the invasiveness of many pathogens is believed to play a role in their virulence and pathogenicity, it is possible that invasiveness plays a role in *H. pylori* pathogenicity, as reviewed in 2003 (Petersen and Krogfelt, 2003). The present review updates the information available at the time and summarizes the *in vivo* and *in vitro* data demonstrating that *H. pylori* can invade the gastric mucosa and be present within gastric epithelial cells and immunocytes. The review also discusses whether *H. pylori* invasiveness plays a role in the persistence and pathogenicity of the bacterium.

Observations in *H. pylori*-infected humans and animals

Helicobacter pylori infection is consistently associated with an intense cellular inflammatory response that is initiated by the innate and adaptive immune systems and is characterized by an influx of neutrophils, mononuclear cells, and T-helper 1 cells. Although this response is typically aimed at clearing intracellular infections (Meylan *et al*., 2006), the bacterium is not generally considered to be an invasive bacterium and some of the early publications did not report the intracellular presence of the bacterium (Hazell *et al*., 1986;Hessey *et al*., 1990;Morris *et al*., 1990;Thomsen *et al*., 1990). However, many early ultrastructural studies in patients with gastritis and ulcers demonstrated that, in addition to intimately adhering to epithelial cell surface, '*Campylobacter*-like organisms' invaded the intercellular spaces and were present within some of the gastric and duodenal epithelial cells, parietal cells and immunocytes (Shousha *et al*., 1984;Buck *et al*., 1986;Chen *et al*., 1986;Tricottet *et al*., 1986;Bode *et al*., 1987;1988); the presence of the bacteria was associated with the formation of pedestals on the surface of gastric epithelial cells, disruption of the mucus granules and of the tight-junction complexes and degeneration of intraepithelial polymorphonuclear neutrophils (PMNs). Subsequent studies confirmed the invasive nature of *H. pylori* in patients with gastritis, peptic ulcer, precancerous lesions and gastric cancer (Kazi *et al*., 1990;Wyle *et al*., 1990;Noach *et al*., 1994; el Shoura, 1995;Genta *et al*., 1996;Papadogiannakis *et al*., 2000;Semino-Mora *et al*., 2003;Necchi *et al*., 2007). Similar observations were made in rhesus monkeys naturally infected by *H. pylori* (Dubois *et al*., 1994) and in experimental infection of mice (Oh *et al*., 2005). Finally, the use of confocal microscopy combined with immunohistochemistry (IHC) or fluorescence *in situ* hybridization (FISH) (Semino-Mora *et al*., 2003) and transmission electron microscopy (TEM) studies combined with immunogold cytochemistry (Necchi *et al*., 2007) suggested that intracellular bacteria were viable because intracellular *H. pylori* observed in patient tissues can express *H. pylori* mRNA and antigens, and that their ultrastructural appearance is well preserved.

The lack of recognition that *H. pylori* can be intracellular may be due to the fact that electron microscopy is less adapted to screening large number of samples, is difficult to apply to archived material, and also requires excellent preservation of the samples. In contrast, the use of high-power histochemistry, IHC and FISH is efficient in screening large number of biopsies (including archived tissues) and in characterizing small organisms (Makristathis *et al*., 2004). When such methods are applied, *H. pylori* is detectable in the lamina propria and within gastric epithelial cells and immunocytes of patients with gastric diseases (Andersen and Holck, 1990;Genta *et al*., 1996;Engstrand *et al*., 1997;Semino-Mora *et al*., 2003;Necchi *et al*., 2007).

High-magnification observation of Genta-stained biopsies readily visualizes silver-stained spiral organisms not only in the lumen of the stomach, but also within normal and metaplastic gastric epithelial cells, in the lamina propria, and in post-venous capillaries where they appear to adhere to red blood cells (Fig. 1). In addition, the size and appearance of the silver-stained

organisms in the lamina propria are strikingly similar to those of intraluminal *H. pylori*. However, silver may stain the host's tissue membranes and is not specific for *H. pylori* and many pathologists discount such observations. In contrast, confocal microscopy observations of IHC and FISH preparations and ultrastructural studies with immunogold revealed the specific identity of the organisms and their intracellular localization (Semino-Mora *et al*., 2003;Necchi *et al*., 2007) and also confirmed *H. pylori* presence in gastric lamina propria capillaries (Aspholm *et al*., 2006;Necchi *et al*., 2007).

In vitro observations in cell cultures of epithelial cells and immunocytes

Helicobacter pylori were also observed inside epithelial cell lines infected with the bacterium (Evans *et al*., 1992;Segal *et al*., 1996;Lofman *et al*., 1997;Su *et al*., 1999;Amieva *et al*., 2002;Bjorkholm *et al*., 2000;Kwok *et al*., 2002). The fraction of cell-associated *H. pylori* entering cell lines was insignificant in HeLa cells (Rautelin *et al*., 1995) and was 0.1% in human embryonic kidney HEK293 (Viala *et al*., 2004) but was in the 1% range in epithelial cell lines such as gastric cancer AGS cells (Birkness *et al*., 1996;Amieva *et al*., 2002). Persistent intraepithelial *H. pylori* were located within large vacuoles (Amieva *et al*., 2002) and these bacteria were viable as demonstrated by the acridine orange method (Wilkinson *et al*., 1998), perhaps because intravacuolar *H. pylori* are protected against bactericidal components of the lysosomal pathway. In addition, approximately 1% of *H. pylori* infecting an AGS cell monolayer survived for up to 3 days of continuous gentamicin treatment (an antibiotic that is considered not to enter eukaryotic cells when in concentrations of 200 μg ml^{-1}), and this tolerance was not due to selection for gentamicin resistance mutations. Furthermore, live internalized *H. pylori* exhibit stop and go directional movements within the confines of the vacuole for up to 3 h, and they can repopulate the extracellular environment upon removal of gentamicin (Amieva *et al*., 2002). Finally, *H. pylori* was also able to pass through a bilayer of AGS cells and an endothelial layer (Birkness *et al*., 1996). This latter observation provides further experimental support for the presence of the bacterium within capillaries of the lamina propria (Aspholm *et al*., 2006;Necchi *et al*., 2007), but the clinical significance of the observation is unknown, and *H. pylori* bacteraemia has been reported in only one case (Ndawula *et al*., 1994).

At this time, however, there is no direct proof that *H. pylori* can replicate intracellularly because no time-lapse study has shown *H. pylori* dividing within cells and colony-forming units (cfu) counts did not increase within AGS cells treated with gentamicin (Amieva *et al*., 2002). This latter observation indicates that these specific *in vitro* experimental conditions do not provide a self-sustaining environment but does not completely exclude intracellular replication of *H. pylori*. Indeed, intracellular cfu counts represent a complex balance between entry, intracellular growth, intracellular death, and exit from cells, and this delicate balance may be disrupted by extracellular gentamicin. *In vivo*, it is possible that *H. pylori* temporarily hides intracellularly and then egresses once the danger is passed, similar to what may happen with *Campylobacter jejuni*, another facultative intracellular organism.

The complex relationship between *H. pylori* and immunocytes that are tasked with their elimination may help explain why the infection usually persists for the life of the individual. *In vitro*, phagocytosed *H. pylori* remained ultrastructurally unimpaired when incubated with polymorphonuclear leucocytes and monocytes in the absence of complement or serum, whereas the addition of complement or serum to the incubation medium led to the destruction of the bacteria (Andersen *et al*., 1993;Kist *et al*., 1993). Similarly, addition of immune serum plus an excess of monocytes reduced the number of *H. pylori*, whereas an excess of PMNs resulted in complete killing of *H. pylori* (Andersen *et al*., 1993). However, some of the phagocytosed bacteria were unaffected after incubation with monocytes and serum, and often were surrounded by large aggregates of platelets (Andersen *et al*., 1993). This latter observation

may be relevant to the normalization of platelet count observed in nearly half of patients with immune thrombocytopenic purpura after *H. pylori* eradication (Kuwana and Ikeda, 2006).

Helicobacter pylori-induced non-opsonic activation of human neutrophils occurs by lectinophagocytosis, i.e. adherence of the sialic acid binding adhesin SabA to neutrophils surface gangliosides (sialylated glycolipids) that activate phagocytosis (Unemo *et al*., 2005). SabA was recently identified as the long-sought sialic acid-dependent *H. pylori* haemagglutinin, which aggregates bacterial cells with erythrocytes *in vitro*. In addition, *H. pylori* is attached to erythrocytes within small vessels in the gastric mucosa, suggesting that haemagglutination may be of direct biological relevance and mechanistically active *in vivo* (Aspholm *et al*., 2006). In the gastric mucosa, *H. pylori* is often in excess compared with the phagocytes. If the *in vitro* results reflect the *in vivo* situation, the phagocytes may be ineffective in *H. pylori* killing, which may play a role in the persistence of *H. pylori* in the gastric epithelium.

Similar events have been observed in macrophages. Immunofluorescence and electron microscopy demonstrated that virulent *H. pylori* containing the *cag* pathogenicity island were rapidly internalized into actin-rich phagosomes (Allen *et al*., 2000). Homotypic phagosome fusion then led to formation of ‘megasomes’ containing multiple *H. pylori* that are viable for ≥ 24 h (Allen *et al*., 2000). In contrast, *H. pylori* strains that did not express CagA or VacA were readily phagocytosed and killed, and no megasomes were observed (Allen *et al*., 2000). Megasome formation and *H. pylori* survival were shown to depend on urease and urease-derived ammonia, and acidification of phagosomes (Schwartz and Allen, 2006). The intracellular presence of *H. pylori* within immunocytes was associated with production of apoptosis in T- and B-cell lines (Singh *et al*., 2006) and *in vivo* (Semino-Mora *et al*., 2005), which may play a role in the persistence of the infection (Singh *et al*., 2006).

Mechanism and time-course of *H. pylori* invasiveness

Before entry into a cell can occur, *H. pylori* needs to establish adherence with the epithelial cells, and the two best-described *H. pylori* adhesins BabA and SabA may be involved. Of particular relevance are the facts that the SabA-mediated binding to gangliosides stimulates G-proteins, activates phagocytosis and oxidative burst reactions of neutrophils and that the associated phosphorylation signalling triggers remodelling of the actin skeleton. The CagA-mediated reorganization of the host cell membrane and signalling probably forms the essential prerequisites for bacterial adhesins to accumulate in the bacterial–host cell interface. The multivalent interactions recruits additional glycolipids to the interface and local membrane density increases, which can lead to raft formation. Such reorganization of the cell membrane with local accumulation of rafts rich in cholesterol moieties may be used by *H. pylori* because, like some *Mycoplasma* species, it acquires cholesterol from the host cells. The high density of cholesterol and the resulting reorganization of local membrane melting point might develop to a critical combination that leads to intimate membrane association and phagocytosis (Wunder *et al*., 2006). Thus, lectinophagocytosis might allow ‘hitchhiking’ of *H. pylori* into the host cells during periods of activated and intense host responses, and may aid its immune evasion.

Time-lapsed scanning electron microscopy (SEM) and TEM documented the time-course of initial adhesion and subsequent entry of *H. pylori* into HEp2 cells (Fig. 2) (Lofman *et al*., 1997). Entry appears to involve a different mechanism in AGS cells, with intimate adhesion of *H. pylori* to pseudopods emerging from AGS cells, leading to the progressive engulfment of *H. pylori* in a zipper-like fashion (Fig. 3) (Kwok *et al*., 2002).

Molecular studies showed that entry into epithelial cells was achieved by receptor-mediated endocytosis (Evans *et al*., 1992) and through an active process of bacterial motility and penetration of the cell membranes (Bjorkholm *et al*., 2000). The bacteria were then bound in

tight vacuoles in close association with condensed filamentous actin and tyrosine phosphorylation signals. These events were inhibited dose-dependently by TNF-α and inhibitors of phosphatidylinositol 3-kinase and of protein kinase C, and enhanced by an inhibitor of tyrosine phosphatases and ATPases (Kwok *et al*., 2002).

Differential interference contrast video and immunofluorescence microscopy (Fig. 4) illustrated that *H. pylori* can enter large cytoplasmic vacuoles of multiple epithelial cell lines including gastric and colon adenocarcinoma and kidney cell line (Amieva *et al*., 2002). Cytochalasin D treatment did not affect vacuole formation but suppressed entry into the cells, suggesting that the actin cytoskeleton plays a role in cell entry (Amieva *et al*., 2002). Once inside the cells, *H. pylori* remained motile within vacuoles for several hours, as demonstrated by time-lapse microscopy. Pulsed treatment with gentamicin demonstrated that the half-life of intravacuolar bacteria was approximately 24 h, and that some intravacuolar bacteria remained viable, were released and did repopulate the extracellular environment after gentamicin was removed.

Vacuoles containing *H. pylori* have the same morphology as late endosomal multivesicular bodies induced by VacA (Amieva *et al*., 2002). They are formed by the fusion of late endocytic organelles in a VacA-dependent process involving retention of the small GTPase Rab7 and interactions with its downstream effector, the Rab-interacting lysosomal protein (Terebiznik *et al*., 2006). However, the role of *H. pylori* virulence factors in cell invasion *in vitro* is controversial at this time, probably due to differences in experimental design. On one hand, invasion was more prevalent with fresh clinical isolates (Segal *et al*., 1996) and with strains carrying the *cag* pathogenicity island (Allen *et al*., 2000), and a larger proportion of cagA- and VacA-positive strains attached to AGS cell membrane and also invaded the cells (Petersen *et al*., 2000). Furthermore, *H. pylori* resided in vacuoles formed through a VacA-dependent process that is responsible for the generation of the large vacuoles containing *H. pylori*, although VacA did not influence *H. pylori* capacity to invade AGS cells (Terebiznik *et al*., 2006). On the other hand, entry and intracellular survival were similar with isogenic *vac*A mutants and with the wild-type strain, suggesting that neither CagA nor VacA was required for these events to occur (Amieva *et al*., 2002). Therefore, it is likely that other, yet undefined, *H. pylori* virulence factors may be involved in cell invasion.

One of those could be an invasin (InvA) protein, similar to the one produced by enteropathogenic *Yersinia* and that binds to intestinal M cells integrin receptors and allows entry into these cells (Isberg *et al*., 2000). In the case of *H. pylori*, the J99 strain has a homologous invasin protein InvA and an *invA* gene, while similar sequences have been named NudA and *nudA* in the 26695 strain and also are present in the recently published HPAG1 complete genomes (access numbers CP000241 and NC_008086) although no name has been given (Oh *et al*., 2006).

The InvA protein was called NudA because it is a Nudix hydrolase. It belongs to the nucleoside polyphosphate hydrolase subgroup that hydrolyses diadenosine tetraphosphate with resulting asymmetrical cleavage of the molecule into ATP and AMP (Lundin *et al*., 2003). The biological importance of *H. pylori* NudA enzyme is illustrated by several observations. First, an insertion mutant has a 2.7-fold decrease in survival as compared with the wild type after hydrogen peroxide exposure but there was no difference in survival after heat shock or in spontaneous mutation frequency. In addition, NudA is constitutively expressed in *H. pylori* at different growth stages and during stress, thus indicating that this protein has a housekeeping function, and all the *H. pylori* strains tested to date harbour the *nudA* gene and show protein expression (Lundin *et al*., 2003). A gentamicin protection assay failed to demonstrate that a quantifiable difference in invasion frequency existed between the *nudA* mutant and the wild-type strain (Lundin *et al*., 2003). More recently, however, cell entry of an *invA-cam* mutant was found to

be reduced by 50% relative to the wild-type *H. pylori* and TEM indicated a sevenfold reduction of intracellular *H. pylori*. Finally, the number of membrane-bound bacteria was 25-fold greater with the *invA-cam* mutants than with the wild-type strain (Liu *et al*., 2007). Thus, deletion of *invA* appears to limit *H. pylori* to the AGS cell surface, where, even if partially protected by pseudopodial embrace, it may remain more vulnerable to host defence or therapeutic intervention, or less prone to trigger normal immune or carcinogenic response pathways.

Relevance of *H. pylori* invasiveness

Because pathogenicity of many invasive bacteria depends on their invasiveness, the *in vitro* observation that invasion frequency of viable *H. pylori* is similar to that of *Yersinia* and greater than that of *Shigella* (Wilkinson *et al*., 1998) supports the hypothesis that *H. pylori* invasiveness does play a role in its pathogenicity. Several clinical observations also are in favour of the hypothesis. First, the association of intramucosal *H. pylori* with histopathologic features was demonstrated in 104 *H. pylori*-positive human subjects and in the stomach of 17 mice persistently infected after inoculation with SS1 *H. pylori* strain (Min *et al*., 2003). By IHC, intraepithelial *H. pylori* were observed in 26% of the human biopsies and in 65% of the murine stomachs, and lamina propria *H. pylori* were present in 48% of the biopsies. Neutrophil-associated immunopositivity for *H. pylori* was observed in 24% of the biopsies with chronic active gastritis, and lamina propria and neutrophil-associated immunopositivity correlated significantly with the grade of acute inflammatory reaction gastritis. The lamina propria and/or neutrophil-associated *H. pylori* may play a more important role than intraepithelial *H. pylori* in the induction of acute inflammatory reactions (Min *et al*., 2003) and perhaps also in *H. pylori* persistence. In another study, gastric ulcers were associated with predominantly intracellular and intercellular colonization in 69% of the cases and with predominantly free-in-mucus *H. pylori* in only 39% of patients ($P < 0.01$) (Chan *et al*., 1992). Intracellular *H. pylori* were also found in the duodenal mucosa of some patients with duodenal ulcer (Bode *et al*., 1987). Finally, penetration of a large number of *H. pylori* into gastric epithelial cells was associated with cell damage and cell disintegration both *in vivo* (el Shoura, 1995) and *in vitro* (Wilkinson *et al*., 1998).

In addition, apoptosis of T lymphocytes can be induced through interactions between *Yersinia pseudotuberculosis* InvA protein and host transmembrane receptors such as β1-integrin (Arencibia *et al*., 2002). Similarly, interactions between *H. pylori invA* gene and host cells β1-integrin also may lead to apoptosis, as suggested by a study of gastric biopsies from patients with gastric precancerous and cancerous lesions (Semino-Mora *et al*., 2005). First, combined ISH (*invA*) and IHC (β1-integrin) showed that *invA* was expressed only by epithelial surface-bound and intracellular *H. pylori*, whereas *16S rRNA* and *cagA* were expressed in all locations. β1-integrin was present in the cytoplasm (cytosol) and in the cellular membrane of mucus-secreting, immune, precancerous goblet, and pleomorphic neoplastic cells. Apoptosis and expression of *invA* and β1-integrin were all increased in intestinal metaplasia and in gastric cancer compared to gastritis biopsies, and these parameters were significantly correlated with each other (Semino-Mora *et al*., 2005). These observations suggest that the increased apoptosis in precancerous and cancerous lesions may be mediated by increased expression of *H. pylori invA* and of the host's β1-integrin, and that this effect may play a role in persistence of the bacterium and perhaps in its carcinogenic effect.

The presence of *H. pylori* within epithelial cells may also play a role in the life-long persistence of the infection, as shown by recent experiments demonstrating the presence of intracellular *H. pylori* within progenitor neck cells in murine (Oh *et al*., 2005) and primate models (Semino-Mora *et al*., 2007). In a gnotobiotic transgenic mouse model of human chronic atrophic gastritis, a combination of TEM and multilabel immunohistochemi-cal methods demonstrated that intracellular *H. pylori* collections were present in dividing and non-dividing gastric epithelial

progenitors cells (Oh *et al*., 2005). The number of intracellular *H. pylori* ranged from a few solitary bacteria to consolidated populations and to microorganisms traversing breaches in the plasma cell membranes. Importantly, 1–5% of epithelial cells surrounded by *H. pylori* contain an intracellular population of bacteria. It is possible that these cell-associated clusters of bacteria are formed by intracellular replication, but the authors did not comment on this possibility. In monkeys persistently infected with *H. pylori*, a FISH study recently demonstrated the presence of intracellular *H. pylori* within SOX-2 positive neck cells (Semino-Mora *et al*., 2007). Therefore, it is possible that *H. pylori* is sheltered from the host immune system within a cell population with a long life span. This stationary haven may be used for the slow-dividing *H. pylori* to multiply and be present in some of the epithelial cells that migrate towards the surface of the stomach and have a 2–4 days' life span. These observations also suggest that other invasive pathogens may use a similar strategy to persist in the human gastrointestinal tract.

Finally, intracellular *H. pylori* are more resistant to antibiotic treatment and only high concentrations of antibiotics with intracellular activity can eliminate the bacterium from HEp2 cells. *In vivo*, IHC demonstrated that infection was not cured by antibiotics in patients with persistent intracellular *H. pylori* infection, even if elimination of extracellular bacteria was complete at 14 days after treatment (Engstrand *et al*., 1997). Thus, antibiotics with intracellular activity (e.g. macrolides) should be used preferentially for the treatment of *H. pylori* infection (Hulten *et al*., 1996;Pechere, 2001).

Conclusions

A quarter century after the discovery of *H. pylori* and the demonstration that the bacterium causes gastric adeno-carcinoma, MALT lymphoma, and peptic ulcers, convincing *in vivo* evidence demonstrates that the bacterium can enter and express mRNA and antigens within the gastric lamina propria. In addition, *in vitro* studies have shown that viable *H. pylori* survive within epithelial cells and immunocytes, exhibit directional movements within intra-cellular vacuoles, and repopulate the extracellular space after the extracellular bacterial population has been killed by gentamicin for up to 3 days. However, direct time-lapse observations have not provided direct proof of intracellular replication of *H. pylori* by visualization of bacteria dividing within epithelial cells, and no increase in intracellular counts have been observed. In addition, no *H. pylori* factor is known to be essential for invasion or intracellular survival. Finally, the fraction of intracellular versus intraluminal *H. pylori* was reported to be approximately 1% in gastrointestinal epithelial cells, and insignificant in HeLa cells, and it is tempting to consider that the invasiveness of the bacterium is not clinically important.

Nonetheless, a small number of bacteria located within the cytoplasm of a few rapidly dividing and strategically positioned cells may have the potential to modify a number of cytoplasmic and nuclear processes and may cause CpG methylation and DNA mutations, especially because virulence genes are known to be expressed within epithelial cells (Semino-Mora *et al*., 2003;Necchi *et al*., 2007). Thus, as many cancers are believed to have evolved from one single cell, a few intracellular *H. pylori* may trigger immune, carcinogenic, or other developmental response pathways, especially when they are present in epithelial progenitor cells that divide rapidly (Oh *et al*., 2005;Semino-Mora *et al*., 2007). This process is intuitively attractive to explain neoplastic transformation of epithelial cells leading to adenocarcinoma and of immunocytes leading to MALT lymphoma. Furthermore, complex interactions between *H. pylori* and immunocytes may moderate the host's immunity and play an important role in the persistence of the bacterium.

Despite the progress already accomplished, it is clear that additional *in vitro* and animal studies will have to be performed and the observations will have to be integrated with new clinical

observations before we can ascertain that *H. pylori* invasiveness plays a role in the bacterium pathogenicity, persistence, and resistance to antibiotics.

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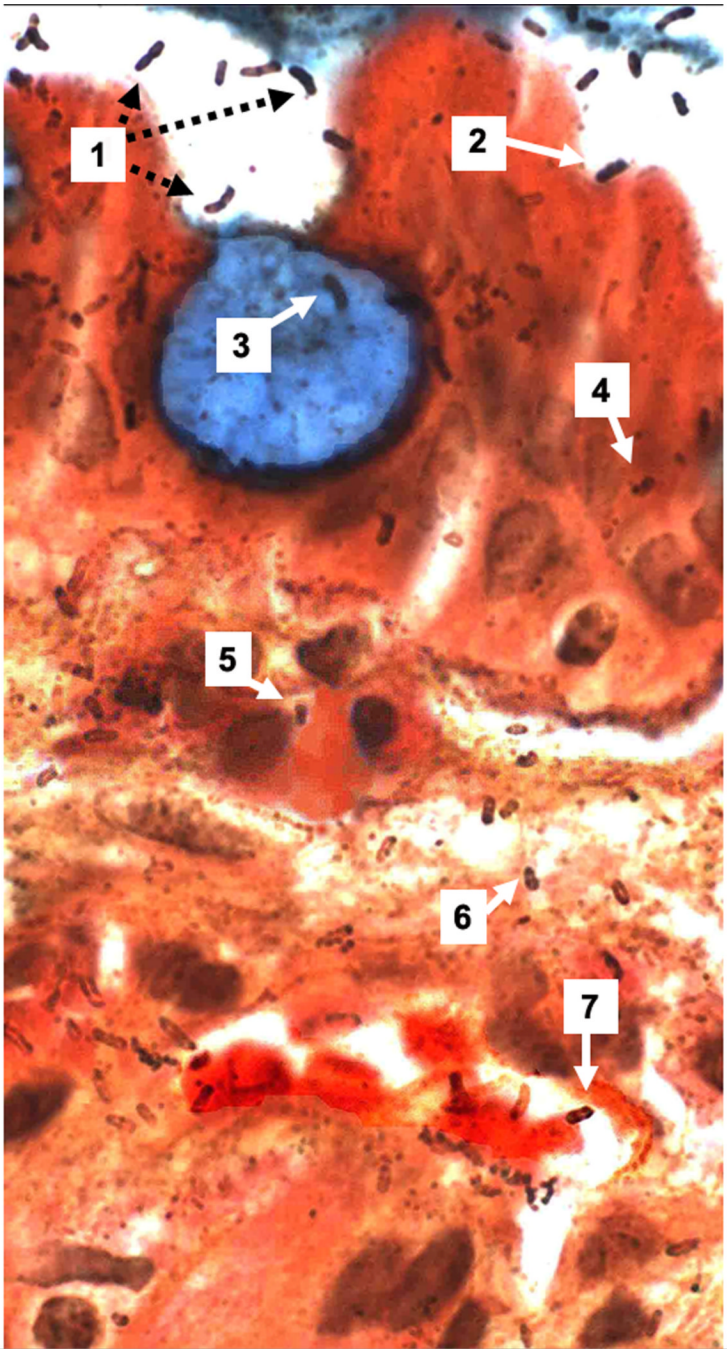

Fig 1.

Gastric biopsy of a patient with intestinal metaplasia (Genta stain, original magnification ×1000). A blue-stained goblet cell is surrounded by more or less normal epithelial cells. Intraluminal *H. pylori* are either free-floating (1) or adherent to epithelial cells (2), but not to the goblet cell. Rod-, comma- and spiral-shaped *H. pylori* are visible in the goblet cell (3), in epithelial cells (4), in inflammatory cells (5), in the lamina propria (6), and in a post-capillary venule, where they are closely associated with erythrocytes (7). Interestingly, the silver straining of intraluminal *H. pylori* appears to be stronger than for intracellular and interstitial bacteria.

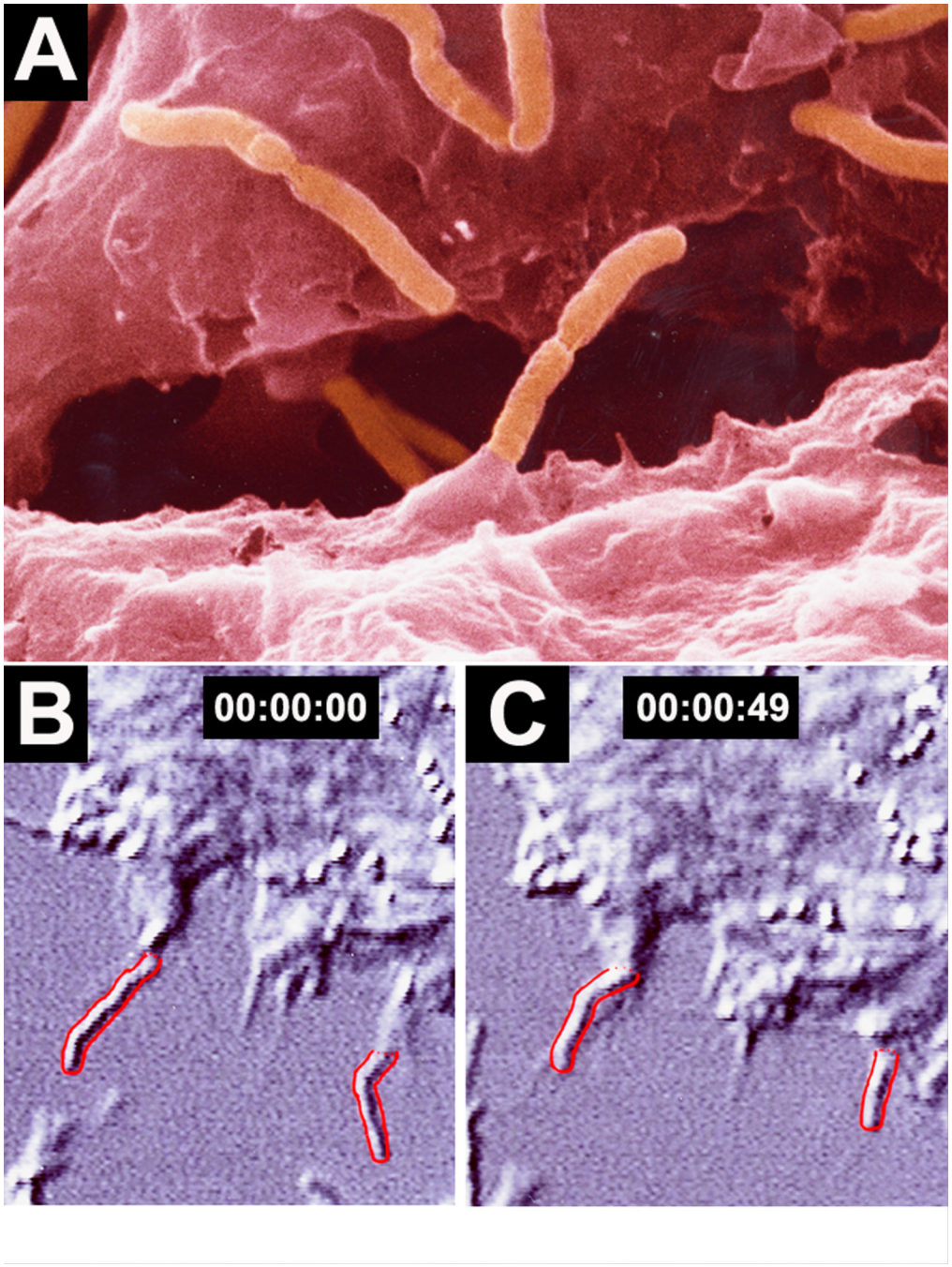

Fig 2.

A. SEM picture of *H. pylori* entry in an HEp2 cell (with permission from M. Block, after Engstrand and Graham, 1997). B and C. Time-lapse observation of two *H. pylori* outlined in red showing the progressive entry into an HEp2 cell over a 49 s time. Adapted by permission from Macmillan Publishers Ltd. *Nature Medicine* **3**: 930–931, copyright 1997.

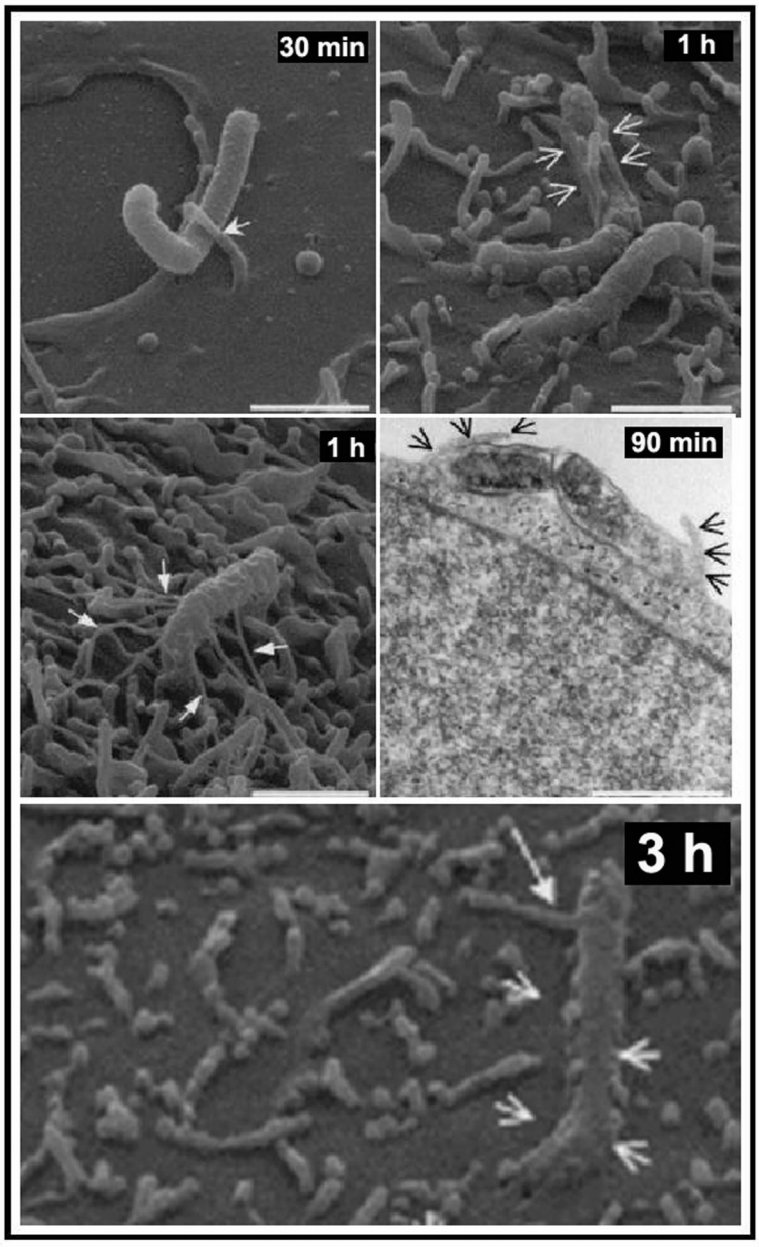

Fig 3.

SEM and TEM analyses of *H. pylori* 26695 adherence to, and entry into, AGS cells. AGS cells infected with *H. pylori* 26695 at a multiplicity of infection of 100 for 12 h at 37°C. Samples obtained at various times were analysed by SEM and TEM. *H. pylori* adhered to AGS cells by intimate contact with the host cell microvilli (30 min, arrows). Features of zipper-like engulfment are observed at 1 h, 90 min and 3 h post infection. Bars, 1.5 μm. With permission from Kwok *et al*. (2002).

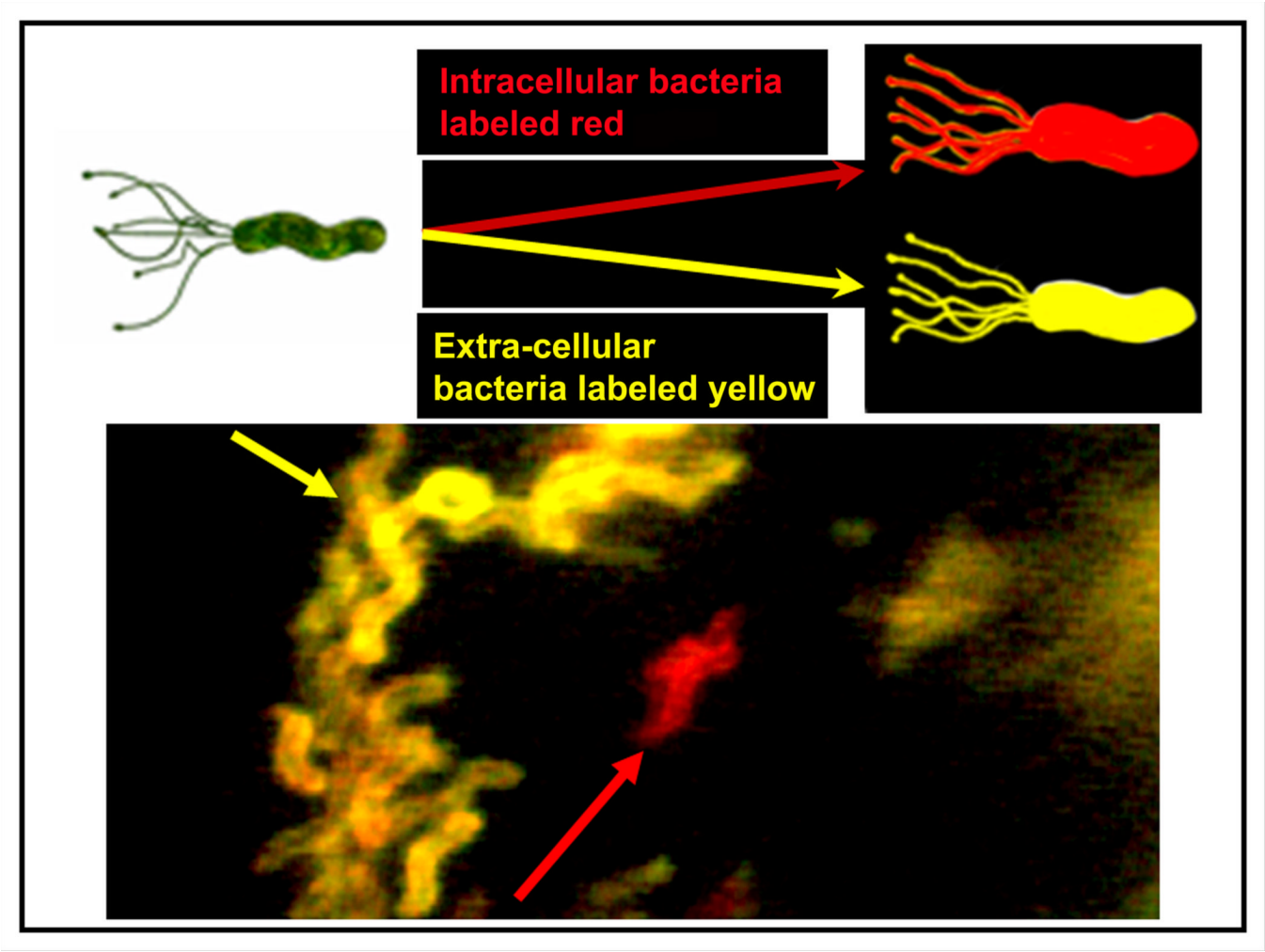

Fig 4.
Illustration of differential immunostaining of AGS cells cocultured with *H. pylori*. As illustrated by the yellow arrow, most *H. pylori* are stained yellow and are located outside the cells. One of the *H. pylori* is intracellular (red arrow) and is stained red (after Amieva *et al*., 2002 with permission).