

Ultrastructural patterns of *Helicobacter pylori*

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Abstract

Ultrastructural morphology of the bacterial bodies was studied in 40 *Helicobacter pylori* positive cases. Two bacterial patterns were identified, which were associated with different modes of contact with the epithelial cells and possibly with different stages of the natural history of the infection.

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We have already described three ultrastructural patterns of contact between *Helicobacter pylori* (*H pylori*) and gastric epithelium¹: (1) *H pylori* in the mucus layer resting on the short microvilli of normal looking epithelial cells; (2) *H pylori* at intercellular junctions; (3) *H pylori* adherent at specific junction zones directly or with filamentous appendages anchoring the organisms to an epithelial surface, which has fewer microvilli and mucoid granules in the absence of a mucus layer. Our histological patterns² were very similar to the ultrastructural ones: a normal epithelium colonised by an interrupted file of organisms lying on the epithelial surface; a degenerated epithelium colonised by a reduced number of bacteria that seemed adherent to the epithelial surface or at intercellular junctions. During our ultrastructural examinations we noted that bacteria did not show a uniform morphological aspect. Many bacterial bodies presented with intracytoplasmic vacuole like structures and a separation between cytoplasmic content and parietal structures. These bacterial factors seemed related to the conditions of epithelial damage. To evaluate our first impressions we examined transmission electron microscopy findings in 40 *H pylori* positive cases out of 94 randomised cases.

Materials and methods

Three biopsies of the gastric antrum were taken in 94 random patients referred for routine gastroscopy. Biopsy specimens were fixed in 2.5% phosphate buffered glutaraldehyde, and then fixed for one hour in 2% osmium tetroxide in the same buffer. The tissues were dehydrated with alcohol and embedded in epoxy resin. Semi-thin sections were prepared by means of a Reichert OmU3 Ultramicrotome and stained with

toluidine blue: in our experience this technique provides particularly easy to read histological pictures.³ In each of the 40 *H pylori* positive cases an ultra-thin section of a selected area was contrasted with uranyl acetate lead citrate and studied with a Hitachi H-800 electron microscope. The Table shows the relation of the number of micro-organisms with both bacterial morphology and their modes of contact with gastric epithelium.

Results

A wide variation in the number of bacteria was seen in ultra-thin sections (between 17 and 96, mean 47.6). We recognised two ultrastructural bacterial patterns. Firstly, *H pylori* with homogeneously distributed chromatinic and cytoplasmic content within the bacterial body (Fig 1), which was associated with all the patterns of epithelial state and was seen in all the examined ultrastructural sections; this pattern, however, was prominent in the first pattern of contact with gastric epithelium presenting normal looking epithelial cells. Secondly, *H pylori* showing an evident separation between cytoplasmic and chromatinic content and parietal structures of the bacterial cells, and with the presence in many sections of a typical ring shape intrabacterial vacuolisation (Fig 2). We now know that these spherical electron opaque 'vacuoles' are indeed polyphosphate structures,^{4,5} functionally related to an energy and phosphorus reservoir, which are utilised in the absence of an exogenous source of energy. This second bacterial pattern was only seen associated with a degenerated epithelium presenting with few apical mucoid granules, fewer microvilli, cytoplasmic vacuolisations, and absence of mucus layer, and it was found in all the ultrastructural sections in which the second or third pattern of contact with gastric epithelium was present (sections from biopsy specimens of 31 patients).

Discussion

We proposed previously that the three patterns of physical relation between *H pylori* and gastric epithelium could represent stages in evolution of bacterial infection.¹ We now conclude that two ultrastructural bacterial patterns exist, and that these two patterns are linked to the modes of contact with gastric epithelium. In particular, the second *H pylori* pattern seems only related to the second and third patterns of contact, which are characterised by evident epithelial damage. We had supposed that the second bacterial pattern, with separation between cytoplasmic and chromatinic content and parietal structures and often of typical ring shape intrabacterial electron opaque vacuolisation, represented the morphological consequence of *H pylori* suffering

Number of bacterial bodies, counted in ultra-thin sections, in relation to both bacterial morphology and modes of contact with gastric epithelium

Patterns of bacterial morphology	Patterns of contact between <i>H pylori</i> and gastric epithelium	
	1	2 and 3
1	678 Bacterial bodies	207 Bacterial bodies
2	0 Bacterial bodies	1019 Bacterial bodies

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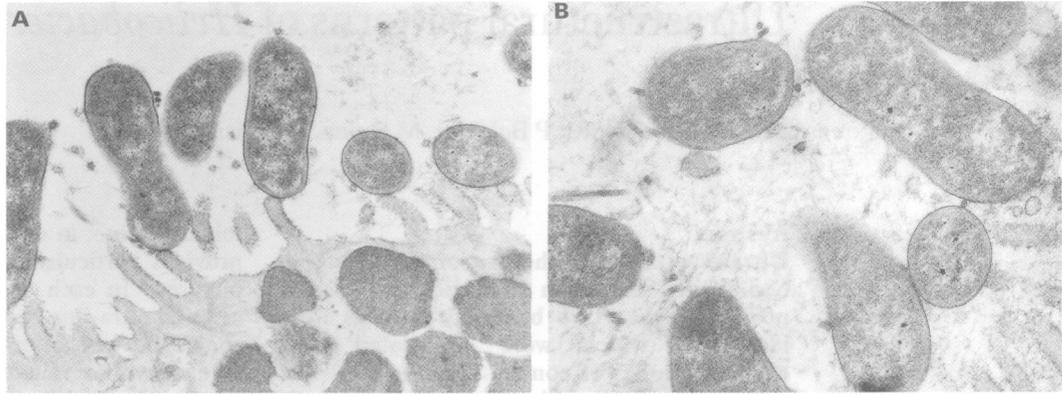


Figure 1: Transmission electron microscopy of a gastric biopsy specimen showing some bacteria with homogeneously distributed cytoplasmic content resting on the short and irregular microvilli of a normal looking epithelial cell. (A) original magnification $\times 30\,000$; (B) original magnification $\times 65\,000$.

linked to nutritional conditions or to an acid or cytotoxic attack in the absence of a mucus layer, or all three. Recently Bode *et al.*,⁴⁵ however, showed that the ring shape intrabacterial 'vacuoles' seen by us are indeed polyphosphate structures with a size ranging from 0.05 to more than 0.2 μm . Polyphosphate granules were identified by using the Neisser reagent in light microscopy and by electron spectroscopy with electron energy loss spectrometry in electron microscopy.

Polyphosphates have been identified in *H pylori* both in vivo and in strains cultured in liquid medium (personal unpublished findings).⁴ Because the nutritional conditions for growth are probably more difficult in liquid than in solid media and particularly the necessary microaerophil environment is more difficult to obtain in liquid medium,⁶ the presence of polyphosphate structures in liquid rather than in solid medium seems to confirm their supposed role in energetic metabolism of the microorganisms. Bode *et al* describe three different types of polyphosphate granules, but the large vacuole like granules seen by us and closely associated with specific epithelial conditions are functionally related with energy and phosphorus supply. This reservoir may be used by the bacterial cell to inhibit the utilisation of important cellular constituents such as RNA and structural proteins.⁷⁻⁹ The separation between

cytoplasmic content and parietal structures may represent the metabolic end point in which structural components must also be utilised. As similar structures have also been found in *H mustelae*, *H nemestrinae*, *Gastrospirillum hominis*, and *Gastrospirillum suis*,¹⁰⁻¹³ they may be essential and characteristic for the genus *Helicobacter*; they accumulate under certain growth conditions.

We now conclude that the presence of a separation between cytoplasmic and parietal structures and the evidence of large polyphosphate structures may represent the morphological consequence of nutritional conditions that are more and more difficult in the natural history of *H pylori* infection. We previously suggested a possible model for the natural history of *H pylori* infection¹: firstly *H pylori* colonise the mucous layer of a normal looking gastric type epithelium and then, probably during many years, the bacterium induces degradation of gastric mucoid granules and microvillar and cellular damage. Our results confirm this suggestion. The second ultrastructural pattern of *H pylori*, which is only associated with a damaged epithelium, seems related to the absence of a sufficient exogenous source of energy when a longstanding bacterial infection has depleted the local resources.

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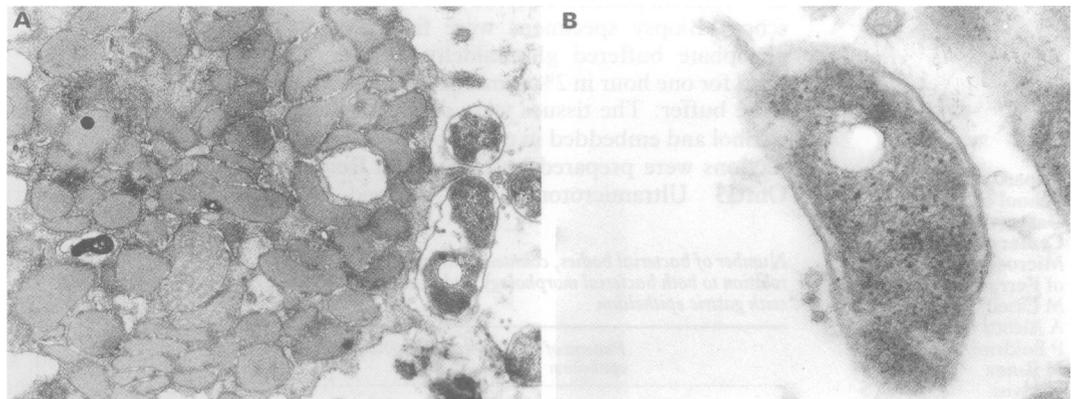


Figure 2: Transmission electron microscopy of a gastric biopsy specimen showing bacteria with an evident separation between cytoplasmic content and parietal structures joined to a damaged epithelium; ring shape electron opaque intrabacterial 'vacuolizations' (polyphosphate structures) are seen in longitudinally cut bacteria. (A) original magnification $\times 30\,000$; (B) original magnification $\times 100\,000$.

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