

ORIGINAL ARTICLE

# Intraepithelial Infiltration by Mast Cells in Human *Helicobacter pylori* Active Gastritis

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## ABSTRACT

Recent observations suggest an involvement of mast cells in *Helicobacter pylori* gastritis, but the mechanism of intraepithelial mast cell activation in *H. pylori*-infected patients remains to be clarified. Intraepithelial mast cells, identified by immunohistochemistry for CD117, were quantified in antral biopsies from 6 patients with *H. pylori* “active” chronic gastritis, 7 patients with *H. pylori* “nonactive” gastritis, and 9 controls. Antral biopsies from patients with *H. pylori* “active” gastritis showed higher intraepithelial mast cell counts than those from patients with *H. pylori* “nonactive” gastritis and from controls. Electron microscopy, selectively performed in 6 cases of *H. pylori* “active” gastritis, confirmed the presence of intraepithelial mast cells and allowed their subdivision into mature cells with intact electron-dense granules or degranulated cells. Other mast cells appeared to migrate through defects in the basement membrane into the epithelial layer. Mast cells in these areas often showed piecemeal degranulation or were characterized by large canaliculi, expanded Golgi areas, and a few granules, a process similar to the phase of recovery from anaphylactic degranulation of isolated human mast cells. The possible significance of these unusual ultrastructural findings is discussed.

**Keywords** electron microscopy, *H. pylori* gastritis, immunohistochemistry, intraepithelial mast cells

## INTRODUCTION

*Helicobacter pylori* colonizes the human stomach and establishes a long-term infection of the gastric mucosa [1]. The persistent colonization induces gastritis and is associated with the development of peptic ulcer disease, atrophic gastritis, and gastric cancer [2]. Neutrophils play an important role in *H. pylori* gastritis, of which they mark the “activity” and severity [3]. They commonly accumulate in the lamina propria beneath infected epithelium or around noncolonized renewal zone epithelium of gland necks [3]. In the early phase of *H. pylori* infection or during highly active chronic gastritis, neutrophils may also appear inside the epithelium and reach the lumen [3,4].

Mast cells are best known for their classic role in allergic hypersensitivity type I reaction, but they also play an important role in innate immune responses against bacteria by releasing cytokines and by recruitment of neutrophils [5]. Ultrastructural analysis of

degranulation mechanisms, which mast cells use to effect the release of cellular products, revealed two basic morphologic patterns that may represent separate points along a degranulation continuum which is important to the function of these cells [6]. These two basic patterns have been termed anaphylactic degranulation and piecemeal degranulation (reviewed by Dvorak [7]). Anaphylactic degranulation in mast cells is an explosive and rapid secretory event that is completed within minutes of stimulation [7]. This type of mast cell degranulation is characterized by secretion via degranulation channels or by direct fusion of granule containers with the cell membrane [7]. Piecemeal degranulation is defined as the presence of partially or completely empty granule chambers in the absence of intergranular fusion [7].

Infiltration of epithelia by mast cells occurs to a limited extent in normal mucosa, whereas it is increased in certain inflammatory states [8–14]. Recently, Hofman et al. [15] have confirmed that mast cells are also

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involved in chronic gastritis and increase as the disease worsens and progresses. They showed that the density of mast cells was higher in patients infected with *cagA*, *vacAs1/m1*, *babA2*, and triple-positive *H. pylori* strains than in patients infected with other *H. pylori* strains or in patients with NSAID-induced gastritis [15].

Although several lines of evidence show that mast cells participate in gastritis caused by *H. pylori* [8–15], the mechanism of intraepithelial mast cell activation in *H. pylori*-infected gastric mucosa remains to be clarified. Although electron microscopy is a useful tool for the investigation of chronic inflammation [16–18], there are a few ultrastructural studies on the morphology of intraepithelial mast cells in *H. pylori* gastritis [8]. Therefore, we used light and transmission electron microscopy coupled with immunohistochemistry to investigate intraepithelial mast cells in gastric biopsies with *H. pylori* infection.

## SUBJECTS AND METHODS

### Study Subjects and Sampling

Among the patients observed in the Department of Internal Medicine between March 2006 and February 2007 at the University Hospital of Messina, those having dyspeptic symptoms and clinical criteria for upper digestive endoscopy were consecutively invited to participate in this study. The present series includes 22 patients (13 males and 9 females; aged between 33 and 56 years). None of the subjects had taken any antibiotics, proton-pump inhibitors, H<sub>2</sub>-antagonists, nonsteroidal anti-inflammatory drugs, or corticosteroids in the 2 months preceding this study. Protocol included the collection of 6 biopsy specimens (4 for diagnosis and 2 for electron microscopy) from the gastric antrum and corpus of 22 patients. *H. pylori* was diagnosed on the basis of histology (modified Giemsa stain) and rapid urease test.

For light microscopy, 4 biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. We graded the histopathological findings of gastritis (glandular atrophy, intestinal metaplasia, mononuclear cells, neutrophils, *H. pylori* density) on a scale of 0 (normal), 1 (mild), 2 (moderate), and 3 (marked) using the visual analog scale of the Updated Sydney System [3]. All cases were histologically examined by two of the authors (RC and FF). If their opinions differed, the specimens were jointly reconsidered until a consensus was reached.

### Mast Cell Histochemistry

Paraffin-embedded sections were stained with toluidine blue, which stains all mature mast cells by binding to serglycin proteoglycans in their secretory granules [19,20].

### Immunohistochemistry

For immunohistochemistry, paraffin-embedded 5- $\mu$ m sections were incubated with *c-kit* anti-human rabbit polyclonal antibody (CD117, Dako, Copenhagen, Denmark) at a dilution of 1:50. As a negative control the primary antibody was omitted and replaced with phosphate-buffered saline. A standard avidin–biotin complex (ABC Elite kit, Vector, Burlingame, CA, USA) technique was employed for the immunostain. The slides were counterstained with hematoxylin. Human gastrointestinal stromal tumor tissue was used as a positive control for CD117 expression.

### Quantization of Intraepithelial Mast Cells

Intraepithelial mast cell density was determined in antral biopsies for each case by manually counting the number of CD117-immunoreactive cells in 1000 epithelial cells with a  $\times 40$  objective. For all measurements, only cells with an identifiable portion of the nucleus were included. For each case, three different counts were performed and the highest score was chosen as a corresponding index.

### Electron Microscopy

For electron microscopy, two biopsies from the gastric antrum and corpus were immediately fixed in 3% phosphate-buffered glutaraldehyde, pH 7.4, and post-fixed in 1% osmium tetroxide. Semithin araldite-embedded sections were stained with toluidine blue for selection of fields. For electron microscopy, ultrathin sections were double-stained with uranyl acetate and lead citrate; they were then examined in a Zeiss EM 902 electron microscope. A total of 140 intraepithelial mast cells were identified and photographed at 6000 $\times$  magnification.

### Ethics

All patients signed an informed consent form, and the protocol was approved by the ethics committee of the University Hospital of Messina (Messina, Italy).

### Statistical Analysis

Intraepithelial mast cell counts, expressed as median and ranges, were not normally distributed and were thus compared between the groups of study subjects using the Kruskal-Wallis test (STATA 9). A nonparametric Mann-Whitney test was then applied as the initial Kruskal-Wallis test was significant. Differences with *p* values lower than .05 were considered significant.

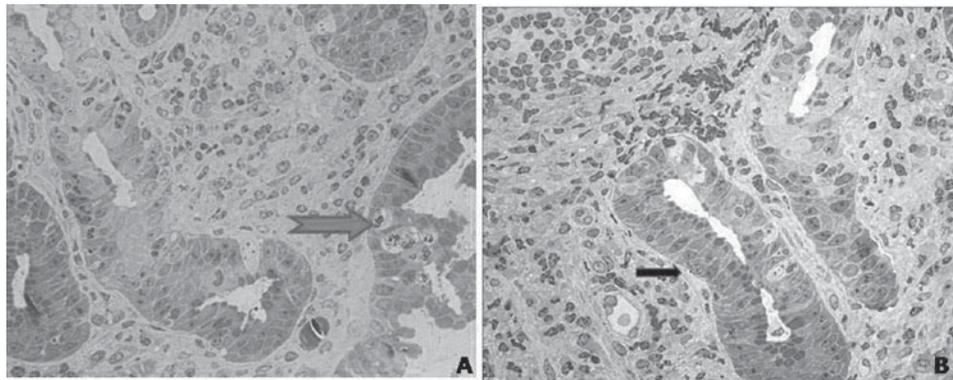


FIGURE 1 (A) *H. pylori* chronic “active” gastritis. Note the presence of intraepithelial neutrophils and *H. pylori* colonization of foveolar epithelium (arrow). Semithin section; toluidine blue  $\times 400$ . (B) Intraepithelial mast cells show loss of metachromasia over portions of the cytoplasmic area (arrow). A portion of lymphoid follicle is noticed in the upper left corner. Semithin section; toluidine blue,  $\times 400$ .

## RESULTS

### *H. pylori* colonization and histopathological diagnosis

*H. pylori* was identified in 13 out of 22 cases. Six of the positive cases revealed “active” gastritis where neutrophils were consistently observed also inside the *H. pylori*-colonized foveolar epithelium (Figure 1). According to Updated Sydney System [3], these cases were graded with regard to glandular atrophy (0), intestinal metaplasia (0 or 1), mononuclear cells (1 or 2), neutrophils (2–3), and *H. pylori* density (1–3). Seven of the positive cases revealed “nonactive” gastritis, characterized by chronic mononuclear cell infiltration consisting of various amounts of lymphocytes, plasma cells, mast cells, and macrophages in the lamina propria without the presence of neutrophils. These cases were graded with regard to glandular atrophy (0–1), intestinal metaplasia (0 or 1), mononuclear cells (1 or 2), neutrophils (0), and *H. pylori* density (1 or 2). Of the *H. pylori*-negative cases, 6 had an essentially normal mucosa while 2 showed mild lamina propria infiltration of round mononucleated cells. These cases were considered as control cases and were graded with regard to glandular atrophy (0), intestinal metaplasia (0), mononuclear cells (0 or 1), neutrophils (0) and *H. pylori* density (0).

Antral biopsies from patients with *H. pylori* chronic “active” gastritis showed higher intraepithelial mast cell counts than those from patients with *H. pylori* chronic “nonactive” gastritis and from controls ( $p < .01$ ). In 2 patients with *H. pylori* chronic “active” gastritis, we found focal areas of intestinal metaplasia. Although *H. pylori* was absent in these areas, an increased number of intraepithelial mast cells was noticed (Figure 2). In addition to increase of intraepithelial mast cells, CD117 immunohistochemistry revealed also the appearance of spindle-shaped mast cells within the gastric epithelium in *H. pylori* chronic “active” gastritis (Figure 2).

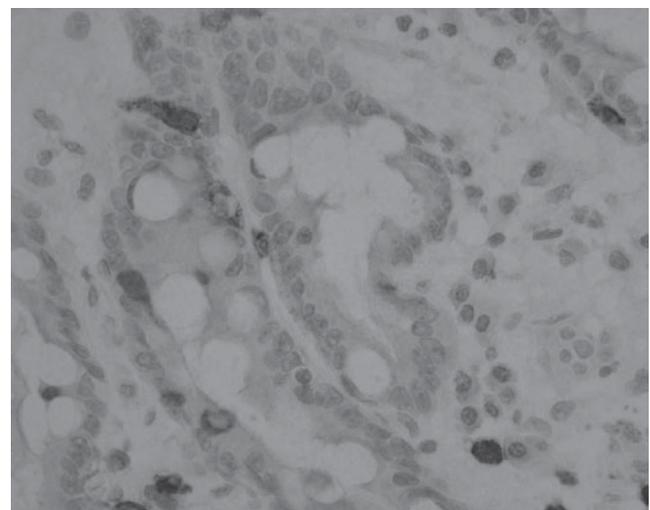


FIGURE 2 Focal areas of intestinal metaplasia in *H. pylori* chronic “active” gastritis. Numerous mast cells infiltrate into the metaplastic epithelium. Note the transepithelial migration of some spindle-shaped mast cells (anti-CD117 antibody,  $\times 200$ ).

Intraepithelial mast cells were selectively studied by electron microscopy in the 6 cases of *H. pylori* chronic “active” gastritis. The mast cells were truly within the epithelial layer, i.e., on the luminal side of the basement membrane (Figure 3A). Intraepithelial mast cells include mature cells with full complement of granules displaying homogeneous, scroll, particle, and mixed pattern (Figure 3A) or degranulated cells. Piecemeal-type degranulation was the most common morphologic change in these intraepithelial mast cells. Some mast cells appeared to be passing through defects in the continuity of the epithelial basement membrane (Figure 3B) or were semicircumferentially surrounded by epithelial basement membrane (Figure 4). These cells assumed the polarized shape typical of motile cells, i.e., a distinct tail or uropod at the trailing end (Figure 3B). We also could observe that migrating mast cells find their way to gastric epithelium by crawling along subepithelial fibroblasts

(Figure 3B). These migrating mast cells showed piecemeal degranulation (Figure 3B) or were characterized by large canaliculi with internalized surface processes, expanded Golgi areas, a few granules, and numerous mitochondria (Figures 4–6). On no occasion was there release of granule content, with formation of channels that are continuous with the extracellular environment. Hypogranular mast cells showed no evidence of cytoplasmic or nuclear degeneration by ultrastructure.

## DISCUSSION

The present study agrees well with previous observations that mast cell density in the epithelium of patients with *H. pylori* chronic “active” gastritis is significantly higher than in patients with *H. pylori* chronic “nonactive” gastritis and control subjects. Quantitative analysis of mast cells was based on CD117-positive immunohistochemistry, which is not altered by massive degranulation and can be regarded as specific for mast cells in the gastrointestinal mucosa [14,21].

Electron microscopy, selectively performed in 6 cases of *H. pylori* “active” gastritis, confirmed the presence of intraepithelial mast cells and allowed their subdivision into mature cells with intact electron-dense granules or degranulated cells showing empty, slightly enlarged, nonfused granule containers, a process similar to piecemeal degranulation [6].

Our electron microscopic study revealed that other mast cells appeared to migrate through defects in the basement membrane into the epithelial layer. Migrating mast cells assumed a polarized shape, such as a distinct tail or uropod at the trailing end. These mast cells often

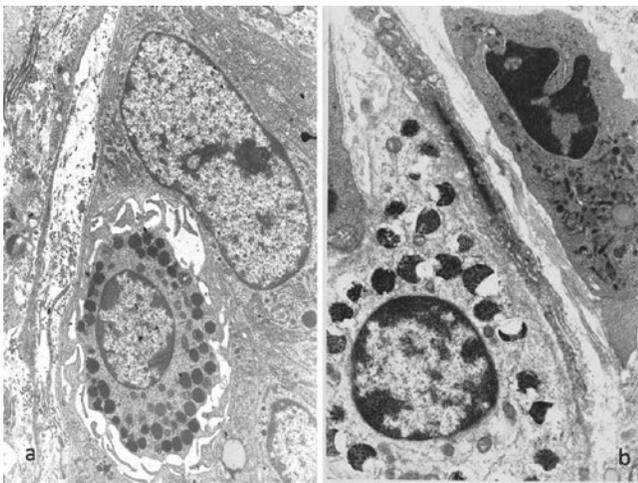


FIGURE 3 (A) Ultrastructural appearance of mature intraepithelial mast cell with irregular surface contour and numerous various shaped electron-dense secretory granules.  $\times 6000$  (B) Migrating mast cell shows a distinct tail or uropod at the trailing end that seems to crawl along a subepithelial fibroblast. It presented partially empty granule chambers, typical of piecemeal degranulation. A subepithelial neutrophil is also seen.  $\times 6000$

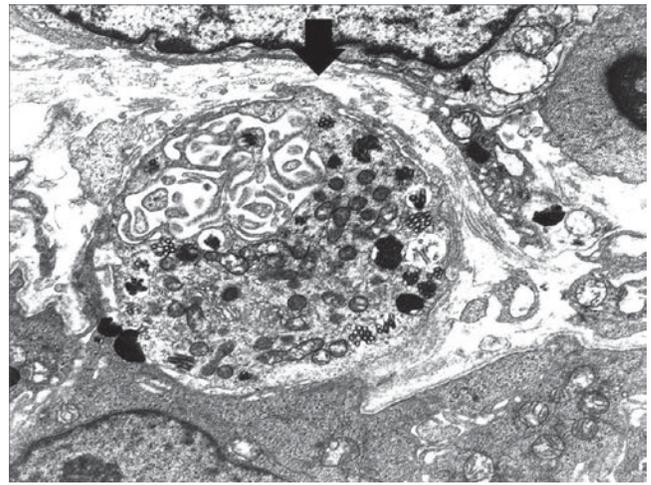


FIGURE 4 Epithelial basement membrane surrounds semi-circumferentially a mast cell showing large canaliculi, smooth surface, a few granules and many mitochondria. This mast cell appears to migrate through a defect in the basement membrane (arrow).  $\times 6000$

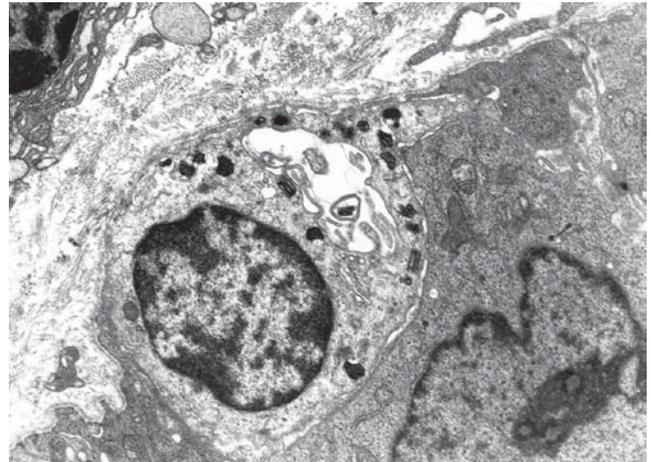


FIGURE 5 Migrating mast cell undergoing recovery from anaphylactic degranulation evidenced by large canaliculi with internalized surface processes. Note the association with a distinct tail or uropod on luminal side of epithelial basement membrane.  $\times 6000$

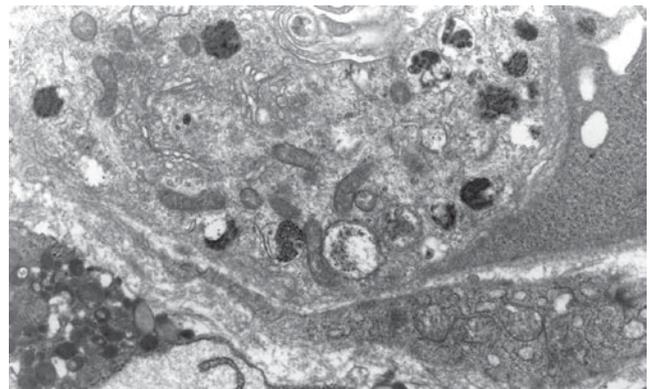


FIGURE 6 Intraepithelial mast cell containing large canaliculi and expanded Golgi area.  $\times 6000$

showed piecemeal degranulation or were characterized by many mitochondria, a few cytoplasmic granules, smooth contour, expanded Golgi areas, and intracellular canaliculi that occupied a large portion of cytoplasm. These are structures that occur infrequently in situ mast cell populations [22] and, to our knowledge, are not previously described in *H. pylori* chronic active gastritis. They increase dramatically in the recovery phase from anaphylactic degranulation [22]. There was no evidence of cytoplasmic or nuclear degeneration in intraepithelial mast cells, suggesting that their state of degranulation was the result of previous granule discharge and not due to degenerative or artifactual alterations. Thus, electron microscopic observations suggest the presence of migrating interepithelial mast cells undergoing piecemeal degranulation or recovery from anaphylactic degranulation. Additional studies are clearly needed to expand and confirm these preliminary ultrastructural observations.

There are a few studies on intraepithelial migration of mast cell in gastrointestinal mucosa. Recently, it has been suggested that the pericryptal zone appears as a critical area conveying myriad of modulatory signals to intestinal epithelial and villous stroma [23]. Our ultrastructural data reveal a close spatial relationship between activated mast cells, epithelial cells, and/or subepithelial fibroblasts. Mast cells produce and release trypsin and chymase that affect subepithelial fibroblasts and epithelial cell functions [12]. Therefore, our ultrastructural findings of mast cell degranulation may be considered as morphologic expression of cross-talk between mast cells, subepithelial fibroblasts, and/or epithelial cells during *H. pylori* chronic "active" gastritis.

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**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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