

## Original Article

# Increased lysozyme expression in gastric biopsies with intestinal metaplasia and pseudopyloric metaplasia

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**Abstract:** Lysozyme is an innate enzyme with potent non-immunological antibacterial properties in the upper intestinal tract. Lysozyme expression (ly-ex) was investigated in 80 consecutive sets of gastric biopsies having normal gastric mucosa (n=20), chronic gastritis (n=20), gastric intestinal metaplasia (IM, n=20), and pseudopyloric metaplasia (PpM, n=20). In biopsies with normal mucosa and with chronic gastritis, the foveolar epithelium and the mucus neck cells of the fundic mucosa as well as the antropyloric glands had moderate (++) to marked (+++) ly-ex whereas the fundic glands proper did not express lysozyme. In IM the goblet and the Paneth cells showed marked (+++) ly-ex. PpM, developing in patients with autoimmune (corpus) gastritis, had moderate ly-ex (++), thus contrasting with the negative ly-ex in the normal or inflamed fundic mucosa. The *Helicobacter pylori* did not proliferate in areas with IM or with PpM. The lysozyme production in IM and in PpM might be upregulated to eradicate ingested, proliferating bacteria in acid-deficient stomachs.

**Key words:** Lysozyme, gastric intestinal metaplasia, pseudopyloric metaplasia, chronic gastritis

## Introduction

During a deliberate search for medical antibiotics, Alexander Fleming [1] discovered one of the natural defence substances against infection, that he denominated lysozyme. Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, is a family of enzymes (EC 3.2.1.17) which damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins [2]. The lysozyme enzyme in human, is encoded by the LYZ gene [3].

Today lysozyme is regarded as the innate enzyme having potent non-immunological antibacterial properties in the upper intestinal tract [4]. Lysozyme immunoreactivity has been recorded in the granules of the Paneth cells of the small intestine [5], in the colorectal

mucosa with inflammatory bowel disease [6] (IBD) and in gastrointestinal tumors [7-10]. Saito et al. [11] and Santini et al [12] also studied the localization of lysozyme in the normal gastric mucosa. These authors [11,12] found that lysozyme was expressed in the cells of the neck region of the body-fundic mucosa and in the antropyloric glands, but not in the surface epithelial cells of both the body and the antropyloric mucosa.

While reporting gastric biopsies [13] we also noticed that lysozyme was expressed in the cells of the neck region of the body-fundic mucosa, in the antropyloric glands and contrary to Saito and Santini [11,12], in the surface-foveolar epithelium of the body-fundic mucosa. In addition, we also found lysozyme expression in the goblet cells of intestinal metaplasia and in pseudopyloric glands in autoimmune gastritis. The purpose of this communication is to report these observations in a cohort of gastric biopsies.

## Material and methods

From the data-base of our Department, a total of 80 consecutive sets of gastric biopsies diagnosed as normal gastric mucosa (n=20), chronic gastritis (n=20), gastric intestinal metaplasia (IM) (n=20) and pseudopyloric metaplasia (n=20)), were retrieved.

Following the recommendations from the updated Sydney System for the classification and grading of gastritis [14], gastric biopsies were taken from 5 different sites: two from the antrum, one from the incisura angularis and two from the corpus.

Sections were stained with hematoxylin-eosin (H&E) and with Giemsa stains, and immuno-histochemically with anti-human lysozyme antiserum (DAKO, Glistrup, Denmark), dilution 1:1600, using the standard protocol with DAB as a detector and appropriate positive control.

## Definitions

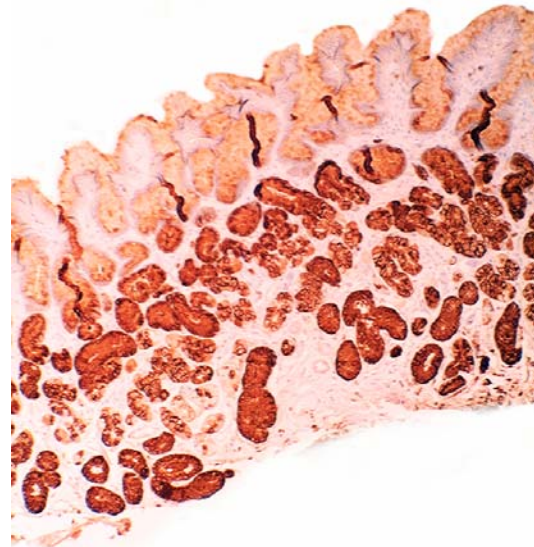
Two main forms of atrophic gastritis are recognized [13]:

Environmental (formerly termed type B gastritis), starts at the incisura angularis [15] as glandular atrophy and intestinal metaplasia and spreads into the antrum and less frequently, into the corpus. It is often patchy (multifocal) and mainly associated with chronic *Helicobacter pylori* infection, and autoimmune (formerly termed type A gastritis), due to an autoimmune destruction of the specialized oxyntic glands of the body and fundus. It lacks the association with chronic *Helicobacter pylori* infection. Inflammatory infiltrates, glandular atrophy and intestinal metaplasia are restricted to the oxyntic mucosa and do not compromise the antrum. It is accompanied by a neuroendocrine (enterochromaffin-like-ECL-) hyperplasia in the corpus.

## Intestinal metaplasia (IM)

Metaplastic transformation of the foveolar and/or the glandular epithelium of the gastric mucosa by intestinal-like epithelium with goblet cells, enterocyte-like cells and Paneth cells. The last two cell phenotypes are missing in the so-called incomplete IM.

IM is found in patients with chronic gastritis.



**Figure 1.** Normal antrum showing slight lysozyme expression in the foveolar epithelium and moderate to marked expression in the pyloric glands proper (Lysozyme immunostain, left and H&E, right, x 4 objective).

Pseudopyloric metaplasia (PpM). Mucous-cell metaplastic transformation of the fundic-oxyntic mucosa. It is found in patients with autoimmune (corpus) gastritis.

Immunohistochemical evaluation: Areas containing a priori the most intense lysozyme stain were chosen to assess the numbers of lysozyme-positive gastric cells. Using a x40 magnification, cases were divided according to the degree of intensity of lysozyme staining as follows: negative (0), slight (+), moderate (++) or marked (+++). In biopsies having various degrees of staining intensity, the highest degree of intensity was applied to classify individual cases.

The Regional Ethical Committee approved the study.

## Results

The results are shown in **Table 1**.

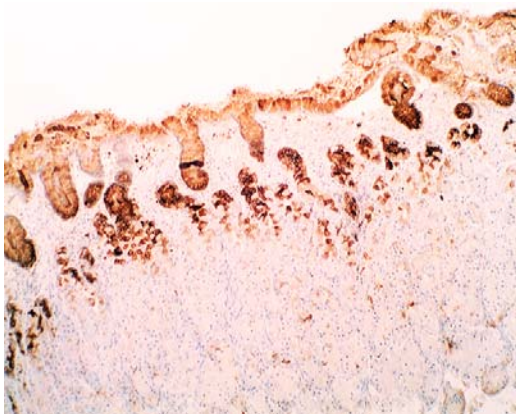
## Normal gastric mucosa

The antral mucosa in the 20 normal cases showed slight (+) lysozyme expression in the foveolar epithelium, and moderate (++) to marked (+++) expression in the pyloric glands

## Lysozyme expression in gastric biopsies with metaplasia

**Table 1.** Lysozyme expression in 80 gastric biopsies having either normal gastric mucosa (n=20), chronic gastritis (n=20), intestinal metaplasia (n=20), and pseudopyloric metaplasia (n=20)

Histology/ lysozyme-expression	Slight expression	Moderate expression	Marked expression	No expression
Normal antrum: foveolar epithelium	+			
Normal antrum: antropyloric glands		++	+++	
Normal corpus; foveolar epithelium	+	++		
Normal corpus; mucus neck cells			+++	
Normal corpus; fundic mucosa				0
Gastritis; foveolar epithelium (antrum)	+			
Gastritis; antropyloric glands		++		
Gastritis; foveolar epithelium (corpus)				0
Gastritis; mucus neck cells (corpus)		++		
Gastritis; fundic mucosa				0
Intestinal metaplasia; (goblet cells)			+++	
Intestinal metaplasia; (Paneth cells)			+++	
Pseudopyloric metaplasia: foveolar epithelium	+			
Pseudopyloric metaplasia: pseudopyloric glands		++		



**Figure 2.** Normal corpus with slight lysozyme expression in the foveolar epithelium and marked expression in the mucus neck glands. The oxyntic mucosa does not express lysozyme (Lysozyme immunostain, left and H&E, right, x 4 objective).

proper (**Figure 1**). The fundic mucosa in the 20

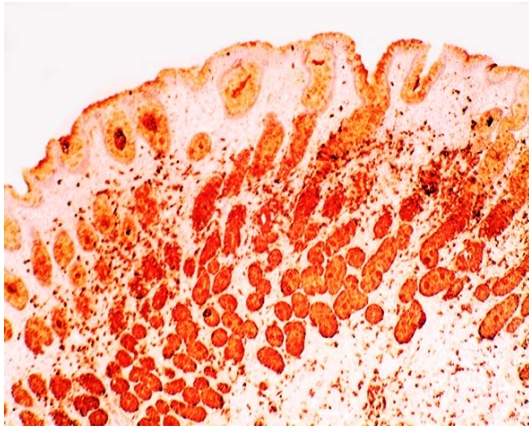
normal cases showed slight (+) to moderate (++) lysozyme expression in the foveolar epithelium and marked (+++) expression in the mucus neck cells (**Figure 2**). Lysozyme was not expressed (0) in the fundic glands proper (**Figure 2**).

### *Chronic gastritis*

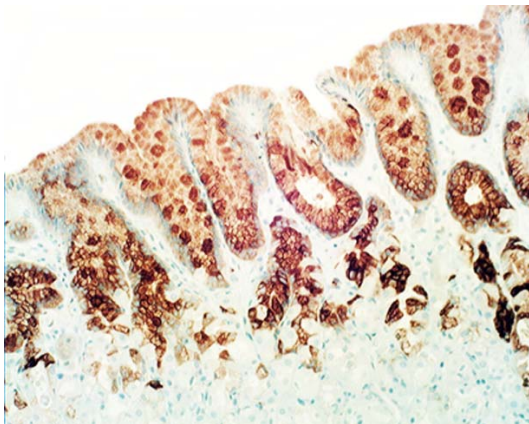
The degree of lysozyme expression was similar to that of the normal gastric mucosa in the 20 cases with chronic gastritis, except in the lower portion of the foveolar epithelium of the antrum, where the lysozyme expression was marked (+++) (**Figure 3**).

### *Intestinal metaplasia*

Marked (+++) lysozyme expression was found in  $\geq 90\%$  of the goblet cells in 11 of the 20 cases with IM (**Figure 4**) and in  $\leq 10\%$  of the goblet cells in the remaining 9 cases. Paneth (metaplastic) cells occurred in 12 of the 20



**Figure 3.** Chronic gastritis (antrum) showing similar lysozyme expression as in Figure 1. In the lower portion of the foveolar epithelium, however, the lysozyme expression was more accentuated (Lysozyme immunostain, left and H&E, right, x 2 objective).



**Figure 4.** Intestinal metaplasia (corpus). Note marked lysozyme expression in goblet cells and in mucus neck cells (Lysozyme immunostain, left and H&E, right, x 10 objective).

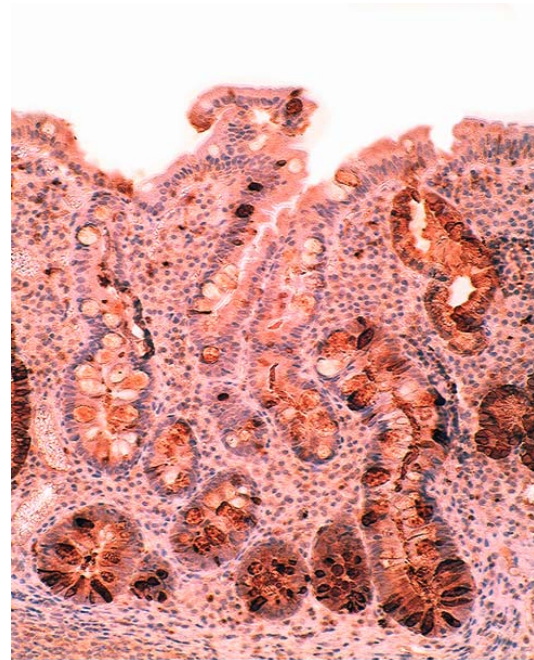
cases with IM. All Paneth cells in these 12 cases showed marked (+++) lysozyme expression (**Figure 5**).

## Pseudopyloric metaplasia

The PpM glands in all 20 cases of autoimmune gastritis showed a moderate (++) lysozyme expression (**Figure 6**), thus contrasting with the negative lysozyme expression (0) of the fundic mucosa under normal conditions or with chronic inflammation (see **Figure 2**).

## Discussion

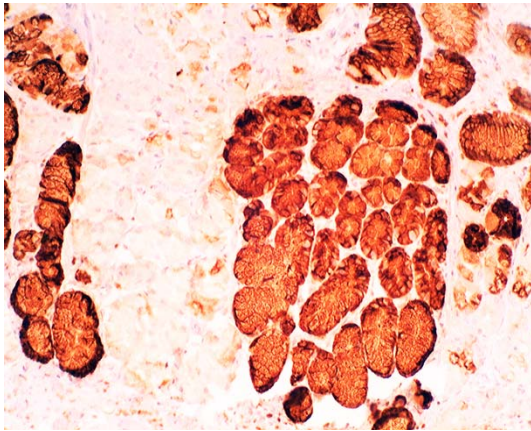
Moderate lysozyme expression (++) was demonstrated in the foveolar epithelium and the mucus neck cells in the fundic mucosa and in the antro-pyloric glands proper, both in the normal mucosa and in the mucosa with chronic inflammation. These results differ somewhat from those of Saito and Santini [11,12] as these authors found no lysozyme expression in the surface epithelial cells. It should be stressed, however, that the lysozyme antibody used by Saito et al. [11] more than 21 years ago and by Santini et al.



**Figure 5.** Intestinal metaplasia (antrum), demonstrating marked lysozyme expression in Paneth cells at the bottom of the crypts (Lysozyme immunostain, left and H&E, right, x 10 objective).

[12] more than 17 years ago might be somewhat different from the one used in the present investigation. In this respect, it should be mentioned that lysozyme is only a generic name, and that under this term at least 80 different compounds are being listed [8].

Marked lysozyme overexpression (+++) was found in the goblet cells and in the Paneth cells of IM. The increased lysozyme expression in the goblet cells of gastric IM appears to be a phenomenon unrelated to the presence of Paneth cell as lysozyme was similarly overexpressed in cases with complete IM (i.e.



**Figure 6.** Pseudopyloric glands (corpus) showing moderate lysozyme expression (Lysozyme immunostain, left and H&E, right, x 20 objective).

having Paneth cells) and with incomplete IM (i.e. without Paneth cells). As pseudopyloric glands are generated by the hyperplastic-metaplastic transformation of the mucous neck cells [13, 14] it is not surprising that PpM cells had retained the characteristics of the mucus neck cells, namely to express lysozyme.

Years ago, Giannella et al. [16] studied in vitro the bactericidal activity of the normal and achlorhydric gastric juice obtained from various patients. These authors found that 99.9% of the bacteria were killed within 30 minutes in vitro, when the pH was less than 4.0. According to Giannella et al. [16], the "gastric bactericidal barrier" is primarily pH-hydrochloric acid dependent, with other constituents of gastric juice contributing little, if any, to the destruction of microorganism. Consequently, in acid-deficient stomachs, a mechanism, other than the gastric acidity should contra rests the ingested, proliferating bacteria. That mechanism might be the neo-production of the antibacterial lysozyme in the goblet cells of IM and in the pseudopyloric glands in PpM. This possibility seems to be validated by the fact that the *Helicobacter pylori* was invariably absent in areas with IM and PpM.

In conclusion, the results of the present survey demonstrated an upregulation of lysozyme expression in IM and in PpM, an apparently natural mechanism of adaptation, aimed to eradicate proliferating bacteria in acid-deficient stomachs.

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