

Paneth Cells and Goblet Cells Express the Neuroendocrine Peptide Synaptophysin.

I- Normal Duodenal Mucosa

CARLOS A. RUBIO

Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden

Abstract. Paneth cells are known to produce lysozyme, α -defensins, phospholipase, matrilysin and guanylin. This study reports, for the first time, that duodenal Paneth cells may also produce the neuroendocrine peptide synaptophysin. Normal duodenal biopsies from 37 patients were immunostained for synaptophysin and lysozyme. Synaptophysin was expressed in Paneth cells, in goblet cells (in the mucus and cytoplasm) and in the neurons/synapses in the mucosa and submucosa. Mucous glands in the submucosa (Brunner's glands) were synaptophysin negative. Lysozyme was expressed in Paneth cells, goblet cells (in the mucus but not in the cytoplasm) and in the mucous glands in the submucosa. Lysozyme was not expressed in the cytoplasm of goblet cells, nor in the neurons/synapses of the mucosa and submucosa. The differences in expression between synaptophysin and lysozyme seem to validate the immunospecificity of synaptophysin. The finding that synaptophysin is also produced by Paneth cells adds new information which might help to unravel the riddle of the ultimate biological significance of these puzzling cells.

The duodenal mucosa is histologically organized into crypts and villi. Cell renewal is zealously controlled by stem cells, confined to the basal aspect of the crypts (1). Stem cells generate several types of committed precursors that actively divide within the proliferative compartment, yielding differentiated mature cell families of enterocytes (absorptive cells) and secretory cells (goblet, enteroendocrine and Paneth cells) (2, 3). While

enterocytes, goblet cells and enteroendocrine cells migrate upwards, along the villus vertical axis, Paneth cells migrate in a retrograde fashion, downwards, towards the base of the crypts of Lieberkuhn (4).

The serozymogenic Paneth cells can be stained with many dyes, but hematoxylin and eosin (H&E) is the one most commonly used. With this dye Paneth cells are easily identified by their bright eosinophilic cytoplasmic granules when observed with transmitted light (5) and by their autofluorescent coarse cytoplasmic granules when using indirect light fluorescence (5). Paneth cells are also labeled by the antimicrobial enzyme lysozyme (6). Recently, while evaluating duodenal biopsies, Paneth cells were also found to be highlighted by immunostain for synaptophysin.

Synaptophysin is a major synaptic neuroendocrine protein encoded by the *SYP* gene. This protein has four transmembrane domains weighing 38 kDa. Synaptophysin is present in neuroendocrine cells and in virtually all neurons that participate in synaptic transmission in the central nervous system. It acts as a marker for neuroendocrine tumors, and because of its ubiquity, it is used to quantify synapses. The exact function of this protein is unknown, but it seems to interact with the essential synaptic vesicle protein synaptobrevin (7).

The purpose of this study was to report, for the first time, that Paneth cells express the neuroendocrine protein synaptophysin. Another important objective was to audit whether synaptophysin was expressed in all Paneth cells. For this purpose consecutive sections were stained with anti-lysozyme.

Materials and Methods

Normal duodenal biopsies from 37 patients complaining of vague symptoms in the upper digestive tract, 5 normal biopsies from the distal ileum and 4 normal biopsies from the cecum, obtained at colonoscopy in individuals complaining of vague symptoms in the lower digestive tract were investigated.

Correspondence to: C.A. Rubio, MD, Ph.D., Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, Stockholm, Sweden. Fax: +46 851774524, e-mail: Carlos.Rubio@ki.se

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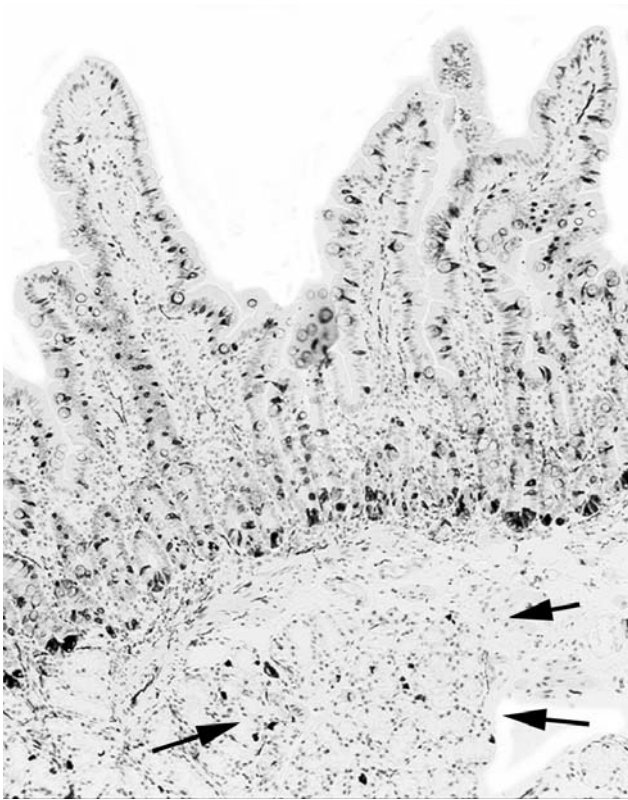


Figure 1. Normal duodenal mucosa showing Paneth cells at the base of the crypts and mucin and cytoplasm in goblet cells, expressing synaptophysin. Note lack of synaptophysin expression in Brunner's glands in the submucosa, at arrows (duodenal biopsy, synaptophysin immunostain, $\times 10$).

Sections were cut at 6 μm and stained with H&E, with anti-synaptophysin (Leica Microsystems, Wetzlar, Germany) as well as with anti-lysozyme (Leica). The preparations were scrutinized in transmitted light with a conventional microscope using a $\times 10$ objective.

Synaptophysin immunostain. Sections were stained with anti-human synaptophysin antiserum (Leica Microsystems). The antibody is ready-to-use, implying that the producer has optimized the dilution. The preparations were incubated for 30 min on a Leica Bond XT (Leica Microsystems).

Lysozyme immunostain. Sections were stained with anti-human lysozyme antiserum (Dako A 0099; Dako, Glostrup, Denmark), at dilution 1:1600, and incubation time 30 minutes on a Leica Bond XT (Leica Microsystems).

Previous studies with duodenal biopsies (5, 8) indicated that single Paneth cells had distinct boundaries, whereas those arranged in tight groups had poorly defined intercellular borders, a phenomenon that hampered *bona fide* counting of individual cells in tissue sections. Consequently, the results are restricted to qualitative descriptions of Paneth cells and other cell phenotypes stained by anti-synaptophysin.

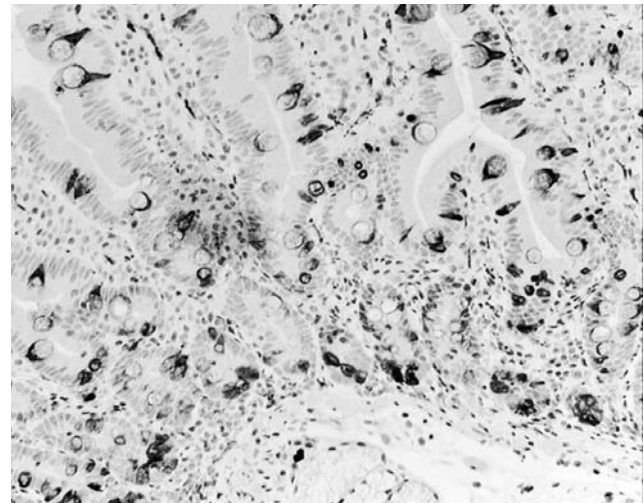


Figure 2. Detail of normal duodenal mucosa showing synaptophysin expression in goblet cells (duodenal biopsy, synaptophysin immunostain, $\times 20$).

Table I. The expression of synaptophysin and lysozyme in the normal duodenal mucosa.

Histologic structure	Synaptophysin	Lysozyme
Paneth cells	+++	+++
Goblet cells (Mucin)	+ / ++	+ / ++
Goblet cells (Cytoplasm)	+++	0
Brunner's glands (Mucin)	0	+++
Neurons/synapses (mucosa)	+++	0
Neurons/synapses (submucosa)	+++	0

0: No stain; +: slightly stained; ++: moderately stained; +++: markedly stained

Results

Synaptophysin immunostained biopsies. Results in Table I show that synaptophysin was expressed in Paneth cells and in goblet cells both in the mucin contained in the cytoplasm and in the cell membrane surrounding the mucin (Figures 1 and 2). In addition, synaptophysin was expressed in the neurons/synapses present in the mucosa and submucosa (Figure 3). On the other hand, mucin-producing glands in the submucosa (Brunner's glands) were non-reactive for synaptophysin (Figure 1, arrows). Sections were also examined under high-power microscopy ($\times 40$) without the microscope's front lenses. This technique permitted Paneth cell granules to be observed. Results showed that none of the unstained cells for synaptophysin had granules, indicating that all Paneth cells were highlighted by this immunostain.

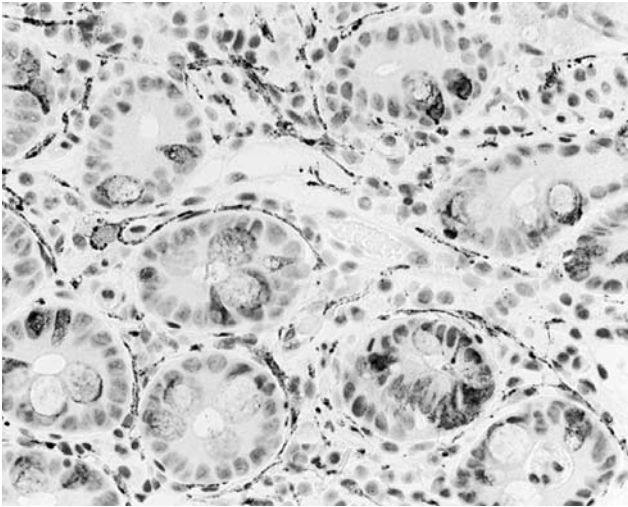


Figure 3. Normal duodenal mucosa showing synaptophysin expression in neurons/synapses in the lamina propria mucosa (duodenal biopsy, synaptophysin immunostain, $\times 40$).

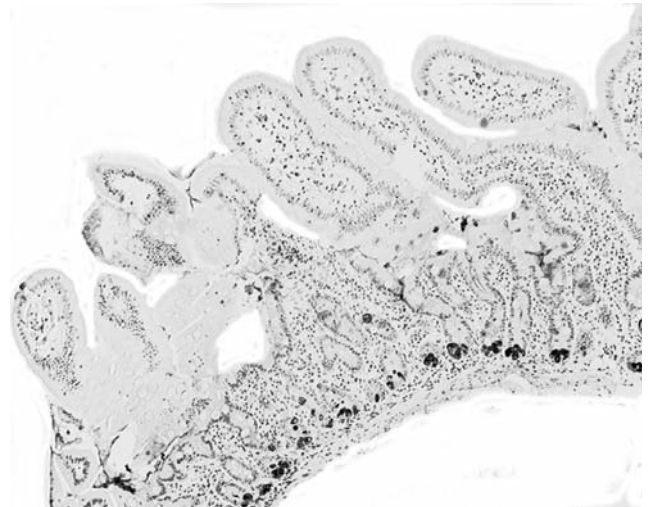


Figure 4. Normal duodenal mucosa showing Paneth cells at the base of the crypts and mucin expressing lysozyme in some in goblet cells (duodenal biopsy, lysozyme immunostain, $\times 10$).

Lysozyme immunostained biopsies. Table I shows that lysozyme was expressed in Paneth cells, in the mucin contained in the cytoplasm of some goblet cells (Figure 4) and in the mucous glands in the submucosa (Brunner's glands) (Figure 5). In contrast, lysozyme was not expressed in the cytoplasm surrounding the cytoplasmic mucin, nor in neurons/synapses of the mucosa or submucosa.

Discussion

The significance of Paneth cells has generated a large amount of scientific interest in the literature (9-15). It is well documented that these cells secrete lysozyme and α -defensins (cryptdins) (10-12), key anti-microbial peptides that keep the duodenum free of pathogenic bacteria; phospholipase A2, an enzyme that releases fatty-acids; matrilysin which regulates the activity of defensins; as well as guanylin, a 15 amino-acid polypeptide, present in goblet cells in the distal small intestine and colon.

In this work it was demonstrated for the first time that Paneth cells generate, in addition to the aforementioned natural products, the neuroendocrine peptide synaptophysin. Synaptophysin was found not only in Paneth cells of the normal duodenum but also in Paneth cells of the normal distal ileum and normal cecum. The secretion of synaptophysin seems to be controlled by the parasympathetic nervous system (B. Mirzai, personal communication). This regulation might explain why Paneth cells stain positively for synaptophysin.

It was also demonstrated for the first time that duodenal goblet cells express synaptophysin, not only in the retained mucus, but also in the cytoplasm surrounding the mucus. The

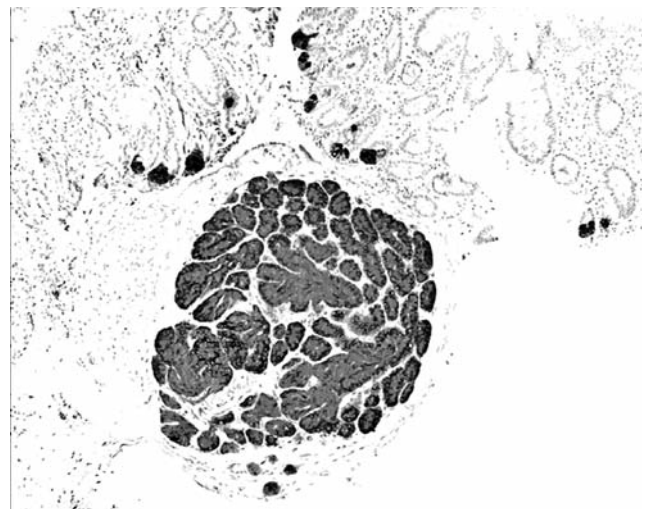


Figure 5. Normal duodenal mucosa showing submucosal Brunner's glands expressing lysozyme (duodenal biopsy, lysozyme immunostain, $\times 10$).

possibility that the sharp synaptophysin-positive cytoplasmic border might include the cell membrane, could not be totally excluded. If this is the case, one possibility is that the cell membrane of duodenal goblet cells might have specific receptors that bind, in a paracrine fashion, to the synaptophysin produced by Paneth cells. The fact that synaptophysin's expression in goblet cells may be a non-specific phenomenon appears less likely since synaptophysin was expressed in mucosal and submucosal neurons/synapses in the same sections, an internal control that confirms the utility of this antibody in highlighting structures known to

expresses the neuropeptide. The lack of the neuroendocrine peptide synaptophysin in goblet cells of the normal ileum and cecum suggests that the neuroendocrine role of synaptophysin may be essential in the duodenum.

It should be mentioned that some prostate adenocarcinomas display Paneth-like cells, so-called because they express synaptophysin but are negative for lysozyme immunostain (16).

In summary, Paneth cells and the mucus of goblet cells in duodenal biopsies exhibited a similar synaptophysin and lysozyme expression. In contrast, the cytoplasm and probably the cell membrane in goblet cells expressed synaptophysin, but no lysozyme. While synaptophysin was expressed in the neurons/synapses present in the mucosa and submucosa, these structures remained unstained with lysozyme. Moreover, the mucus glands in submucosa expressed lysozyme, but were synaptophysin unreactive. The differences in expression between synaptophysin and lysozyme seem to validate the immunospecificity of synaptophysin.

Despite Paneth cells first being described by the German anatomist and anthropologist Gustav Schwalbe in 1872 (17), these cells have remained an enigma (18). In later years, however, part of this mystery has been disclosed (9-15). The finding that synaptophysin is also produced by Paneth cells add new information that might help to unravel the riddle of the ultimate biological significance of these puzzling cells.

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