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Immunocytolocalization of human gastric lipase in chief cells of the fundic mucosa

H. Moreau[1], A. Bernadac[1], Y. Gargouri[1], F. Benkouka[3], R. Laugier[2], and R. Verger[1] *

[1] CNRS – Centre de Biochimie et de Biologie Moléculaire, 31, chemin Joseph Aiguier, F-13402 Marseille Cedex, France
[2] INSERM – U315, 46, Bd de la Gaye, F-13009 Marseille, France
[3] INSERM – U10, Hôpital Bichat, 170 Boulevard Ney, F-75018 Paris, France

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Summary. The presence in human gastric juice of a lipase secreted by the gastric mucosae has been reported previously, but its exact cellular origin has not yet been established. Polyclonal antibodies specific to human gastric lipase (HGL) were prepared, and used by an immunofluorescence technique to label cells producing HGL. This immunocytolocalization was correlated with that of pepsin (chief cells) and parietal cells using specific polyclonal or monoclonal antibodies.

Our results clearly establish that HGL is exclusively located in the chief cells of fundic mucosa; furthermore, it was found to be always co-located with pepsin. No HGL was observed in the parietal or mucus cells. HGL was always detected intracellularly, either in secretory granules of the apical region of the chief cells, or revealed by more diffuse cytoplasmic labelling.

Introduction

In humans, the hydrolysis of dietary triglycerides begins in the stomach. This hydrolysis is catalyzed by HGL, the main acid-stable lipase present in the gastric juice (Schoneyder and Volquartz 1946; Tiruppathi and Balasubramanian 1982). Studies on dispersed human gastric glands (De Nigris et al. 1985), genetic recombination experiments (Bodmer et al. 1987) or measurement of lypolytic activity (Moreau et al. 1987; Abrams et al. 1986) using gastric mucosal biopsy samples have shown that gastric tissue secretes a true lipase. Recently we clearly demonstrated, by means of gastric biopsies, that HGL activity was exclusively located in human fundic mucosa (Moreau et al. 1988 b, c). Furthermore Szafran (Szafran et al. 1978) has reported that lipase and pepsin activity outputs were correlated in humans when a pentagastrin stimulation was used. However the exact cellular location of HGL has never been clearly established. In the rat, a gastric location of preduodenal lipase activity, after induction by a fat meal, has been reported on the basis of histochemical data (Barrowman and Darnton 1970). However this conclusion is controversial since other authors observed no lipase activity in the rat stomach (Perret 1982; De Nigris et al. 1985; Hamosh 1986; Moreau et al. 1988a). On the other hand, using polyclonal antibodies specific to rat lingual lipase, Roberts and Jaffe (1986) have demonstrated the existence of a true rat lingual lipase located in demilune cells of the serous Von Ebner glands. These authors also failed to detect any gastric lipase in the rat.

The aim of the present study was to determine what cells are responsible for HGL secretion, using polyclonal antibodies specific to HGL as cell markers, and comparing the results with those obtained with polyclonal or monoclonal antibodies specific to pepsin or parietal cells.

* To whom offprint requests should be sent

Abbreviations: HGL: Human gastric lipase. SDS PAGE: Sodium dodecyl sulfate – polyAcrylamid gel electrophoresis. PBS: Phosphate buffer saline.

Materials and methods

Antibodies. Antibodies against HGL were raised in the guinea pig using the method described in (Feracci and Maroux 1980). Briefly, 350 µg of purified HGL (Tiruppathi and Balasubramanian 1982) in 0.5 ml of water was emulsified with an equal volume of complete Freund's adjuvant and was injected intradermally at 4–5 locations around the neck of the guinea pig. A second injection was performed intramuscularly 8 days later with an identical amount of HGL without adjuvant. A third injection was performed under similar conditions 10 days later with 175 µg of HGL, and the last boost was carried out sub-cutaneously the next day. The guinea pigs were bled 8 days after the last injection. The antiserum obtained inhibited HGL activity by 50% when 4 µl of total serum was added to 10 µg of purified HGL; whereas control guinea pig serum had no inhibitory effect. Guinea pig antiserum to HGL was depleted three times against a mucus rich fraction from rabbit antrum containing no detectable lipase activity.

Rabbit antiserum to porcine pepsin was purchased from Behring, and a fraction was depleted three times against pure HGL.

Mouse monoclonal immunoglobulins to parietal cells were kindly provided by Dr. F. Benkouka (INSERM U10, Hôpital Bichat, Paris, France).

After sodium dodecyl sulfate polyacrylamide gel electrophoresis of purified HGL or human gastric juice, the specificity of HGL and pepsin antisera was established by protein blotting onto nitrocellulose membrane by the method described in (Towbin et al. 1979). After incubation with the primary antibody, an antispecies antibody (goat anti-rabbit or anti-guinea pig immunoglobulin G) labelled with horseradish peroxidase was applied to reveal primary antibodies binding sites.

Biopsy specimen sampling. Four patients were used in this study; they presented with clinical signs of gastro oesophagus reflux. They showed no symptom of functional gastric disorders nor any endoscopically detectable anomaly in stomach or duodenum. Biopsy specimens were sampled with the subjects' prior agreement in ac-

cordance with the human experimental committee's recommendations.

Three paired biopsy specimens were sampled from each of the four patients (two men aged 32 and 44 years, and two women aged 31 and 63 years) at Sainte Marguerite Hospital (Pr. H. Sarles). The three sites of these paired biopsies were as follows: the first in the fundic mucosa (at the level of the base of the great tuberosity), the second in the prepyloric antral mucosa, and the third in the duodenal mucosa. One of each pair of biopsies was immediately fixed for 4 h in PBS containing 4% formaldehyde freshly prepared from paraformaldehyde. The other biopsies were homogenized and assayed for lipase activity using the method described previously with tributyrin as substrate (Gargouri et al. 1986). One unit corresponds to 1 μM of fatty acid released per minute.

Immunofluorescence microscopy on ultrathin frozen sections. Excess formaldehyde was removed from biopsies by washing several times with buffer during at least 24 h. Fixed blocks were cut into smaller fragments and infused for 30 min at 4° C in a 0.1 *M* phosphate buffer (pH 7.4), containing 1 *M* sucrose. These fragments, frozen in liquid nitrogen, were sectioned in an Ultrotome III (LKB Instruments, Rockville, MD) equipped with a cryokit attachment. Section thickness was estimated by comparing the interference spectrum with the conventional scale used in ultramicrotomy. Purple sections (1500–2000 Å) were picked up with a drop of 2.3 *M* sucrose as described by Tokuyasu (1973) and deposited on a glass slide. After several washes with PBS, the sections were incubated during 10 min in a 5% (w/v) bovine serum albumin solution in PBS in order to minimize nonspecific labelling, and washed again with PBS. The following immunocytochemical reactions were then carried out at room temperature:

(*a*) Incubation of the sections for 30 min with various concentrations of guinea pig antibody to HGL in a 0.5% (w/v) BSA solution in PBS.
(*b*) Five 3-min washes with PBS.
(*c*) Incubation for 30 min with rhodamin labelled sheep anti-guinea pig immunoglobulin (100 µg/ml) in a 0.5% (w/v) BSA solution in PBS.
(*d*) Five 3-min washes with PBS.
(*e*) Incubation for 30 min with various concentrations of rabbit antibody to porcine pepsin in a 0.5% (w/v) BSA solution in PBS, or with mouse monoclonal antibody to parietal cells (culture supernatant).
(*f*) Five 3-min washes with PBS.
(*g*) Incubation for 30 min with fluorescein labelled goat anti-mouse or anti-rabbit immunoglobulin (100 µg/ml) in 0.5% (w/v) BSA solution in PBS.

Finally the sections were given five 3-min washes with PBS, then mounted in 50% glycerol, and examined with a Zeiss Photomicroscope III equipped with a III RS fluorescence epicondenser.

Results

Immunoblotting controls

Human gastric juice was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by immunoblotting using nitrocellulose membranes (Fig. 1, lanes A and B). In Fig. 1, the guinea pig antibody to HGL used as first antibody can be seen reacting to the lipase band (49 kDa). Control non immunized guinea pig serum showed no reaction. Using crude guinea pig HGL antiserum, a slight cross-reaction with human gastric juice was observed with a band of 30 kDa (Fig. 1, lane A). This cross-reaction disappeared after depletion of the anti serum with rabbit mucus rich fractions (Fig. 1, lane B). On the other hand, using the depleted antibody, no cross reaction was observed with pure porcine pancreatic lipase (Fig. 1, lane C).

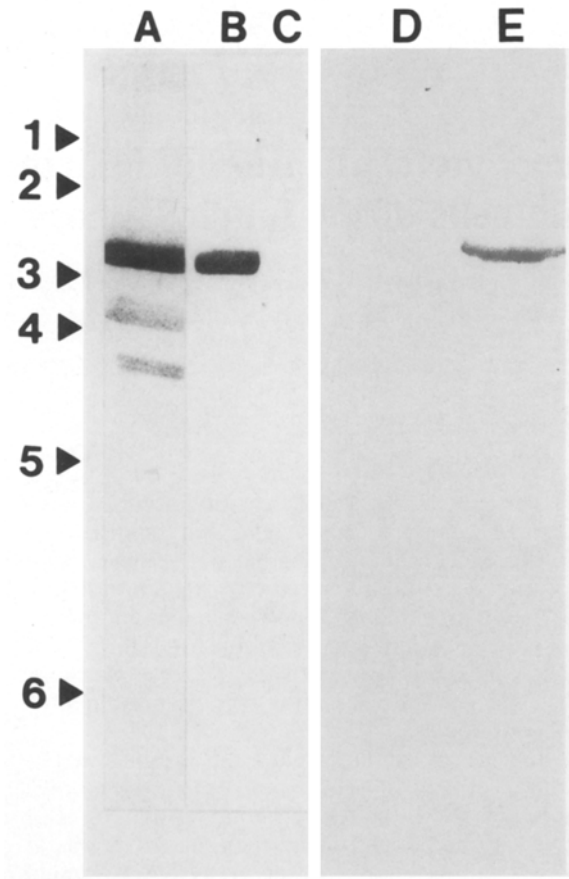

Fig. 1. Immunoblotting of HGL. Human gastric juice (Lanes *A* and *B* and porcine pancreatic lipase (Lane *C*) were subjected to the SDS PAGE followed by immunoblotting using nitrocellulose membrane. Crude guinea pig antibodies to HGL were found to react mainly with a Mr = 49 kDa band corresponding to that of HGL, and slightly with two bands corresponding to a Mr of about 32 and 27 kDa (Lane *A*). Depleted guinea pig antibodies to HGL were found to react specifically with Mr = 49 kDa band (Lane *B*), and not with porcine pancreatic lipase (Lane *C*). Supernatant of fundic (Lane *E*) and antral (Lane *D*) biopsy homogenates in Triton × 100 were subjected to the SDS PAGE followed by immunoblotting. Depleted guinea pig antibodies to HGL were found to react only with the HGL band in the fundic biopsy (Lane *E*), and not in the antral biopsy (Lane *D*). *Arrows* on the left side indicate the position of protein standards: *1* Phosphorylase b, Mr = 94 kDa. *2*. Bovine serum albumin, Mr = 67 kDa. *3*. Ovalbumin, Mr = 43 kDa. *4*. Carbonic anhydrase, Mr = 30 kDa. *5*. Soybean trypsin inhibitor, Mr = 20 kDa. *6*. Lactalbumin, Mr = 14.4 kDa

Reactivity to a band of 30 kDa was also observed with human gastric juice when rabbit antibodies to porcine pepsin were used as first antibody (data not shown). A slight cross-reactivity with the band corresponding to HGL was also observed using crude antibody, and this cross-reactivity disappeared when antibodies were depleted with rabbit mucus rich fractions (data not shown). Furthermore supernatants of fundic and antral biopsy homogenates in Triton × 100 or SDS (Moreau et al. 1988c) were subjected to SDS PAGE electrophoresis, followed by immunoblotting. From Fig. 1, lane E, it is clear that depleted guinea pig antibodies to HGL react only with the HGL band (49 kDa) present in the fundic biopsy, and not in the antral biopsy (Fig. 1, lane D). Depleted rabbit antibodies to porcine pepsin revealed only one band of 30 kDa present in the fundic biopsy

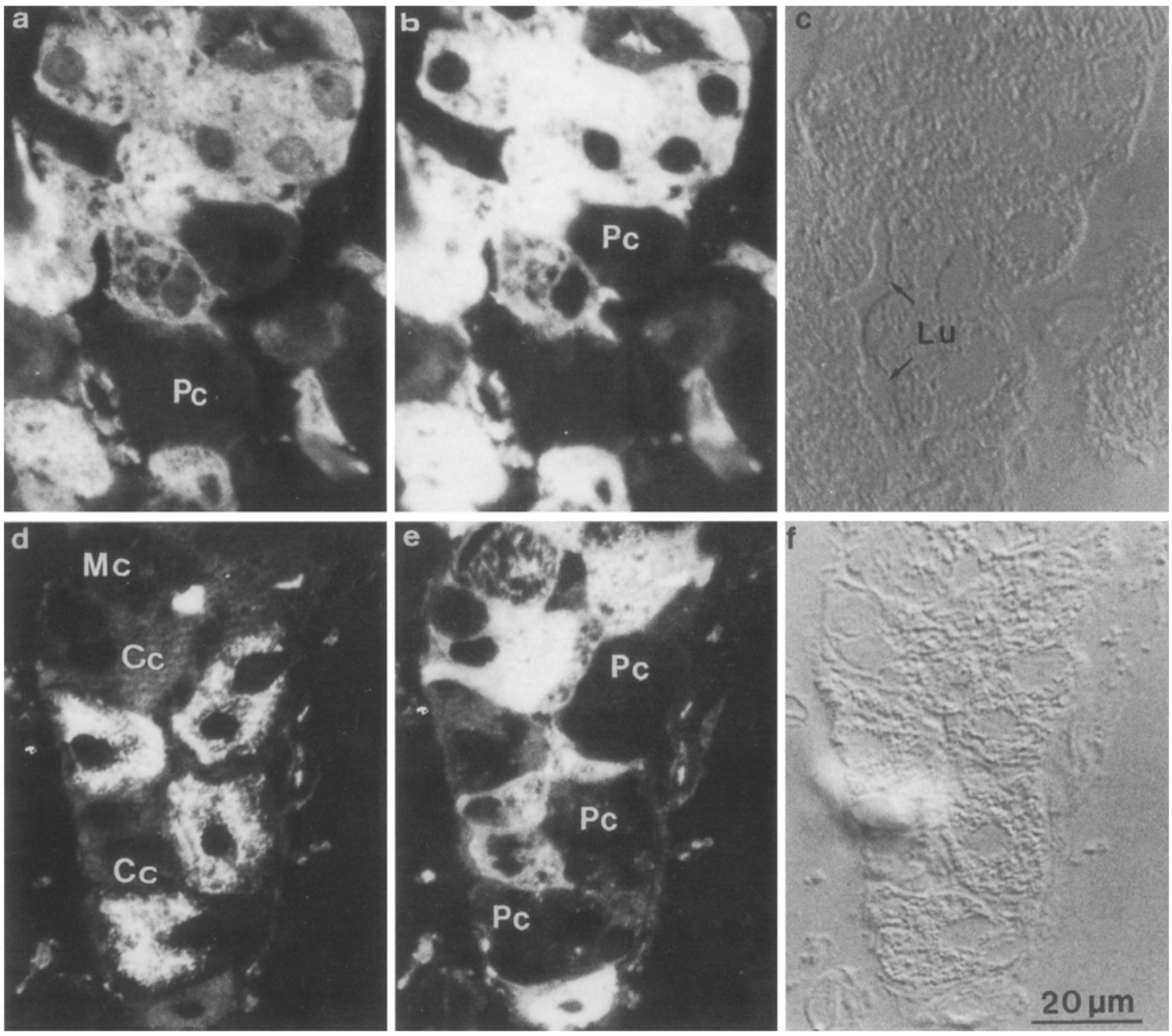

Fig. 2. Human fundic mucosa immunolabelling. One section was first stained using guinea pig antibodies to HGL (1/200) revealed by rhodamin labelled immunoglobulins to guinea pig (100 µg/ml) (**b**), followed by rabbit antibodies to porcine pepsin (1/200) revealed by fluorescein labelled immunoglobulins to rabbit (100 µg/ml) (**a**). Nomarski interference optics (**c**). ×800. Another section was first stained using guinea pig antibodies to HGL (1/200) revealed by rhodamin labelled immunoglobulins to guinea pig (100 µg/ml) (**e**), followed by mouse monoclonal immunoglobulins to parietal cells (without dilution) revealed by fluorescein labelled immunoglobulins to mouse (100 µg/ml) (**d**). Nomarski interference optic (*f*). ×800. *Mc:* mucus cell. *Cc:* chief cell. *Pc:* parietal cell. *Lu:* lumen of the gland

homogenate (data not shown), and not in the antral biopsy. These results correlate with lipase activity measurements performed on the Triton ×100 supernatant: 6000 lipase units/g of fresh mucosae in fundic biopsy, and no lipase activity found in antral biopsy.

Immunocytochemical localization of HGL

Lipase assays carried out on paired biopsies showed that only the fundic biopsy contained lipase activity (average of 4200 U/g of fresh mucosae), and no lipase activity was observed in either the antral or duodenal biopsies.

Sections of these biopsies (from fundic, antral and duodenal mucosa) were incubated with crude guinea pig antibodies to HGL, and revealed by rhodamine labelled sheep immunoglobulins to guinea pig, and then with crude rabbit antibody to porcine pepsin revealed by fluorescein labelled goat immunoglobulins to rabbit. In this case numerous cells were labelled: chief cells in the fundic mucosa, and mucus cells in the fundic and antrum mucosa. Brush border as well as goblet cells were also labelled in duodenal sections. No cells were labelled in any section with either non immunized control guinea pig or rabbit sera. Pre-incubations of guinea pig antibodies to HGL with HGL and rabbit antibodies to pepsin with pepsin, completely abolished the immunostaining (data not shown). These results clearly indicate the reliable specificity of the antibodies used. On the other hand when the same double labelling procedure was carried out using depleted guinea pig antibodies to HGL and depleted rabbit antibodies to porcine pepsin, the only cell type to be labelled was that of the chief cells located exclusively in fundic mucosa sections, which contain both

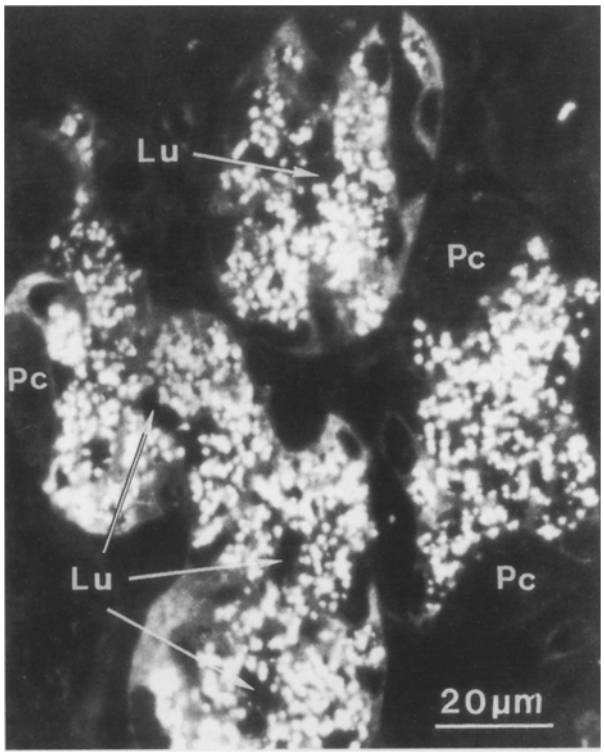

Fig. 3. Human fundic mucosa immunolabelling. The section was stained using guinea pig antibodies to HGL (1/200) revealed by rhodamin labelled immunoglobulins to guinea pig (1/100). Staining in this section was granular and mainly located in the apical portion of chief cells. *Pc:* parietal cell (not stained). *Lu:* lumen of the gland

lipase and pepsin (Fig. 2a, b, c). Double labelling of HGL and pepsin was always located on the same chief cells, but never on the nucleus of these cells. Double labelling using guinea pig antibodies to HGL and mouse monoclonal antibodies to parietal cells (revealed with their respective antibodies labelled with either rhodamin or fluorescein), confirmed that cells containing HGL were different from parietal cells (Fig. 2d, e, f).

When depleted antibodies to HGL or pepsin were used, no cells were labelled in the antral or duodenal sections. These data are confirmed by the fact that lipase activity measurements carried out on paired biopsy specimens showed that lipase activity was located only in fundic mucosa (average of 4200 units/g of fresh mucosae), and no activity was found in antral and duodenal mucosae.

Further investigation of the HGL and pepsin labelled sections showed that some biopsy specimens presented a fairly uniform cytoplasmic labelling (Fig. 2a, b, e), whereas some other biopsies showed a granular intracellular labelling (Fig. 3). In the later case, the granules were mainly located in the apical portion of the cells, thus strongly resembling the secretory granules.

Discussion

In a previous study we clearly demonstrated that HGL activity is entirely located in the fundic mucosa of the human stomach (Moreau et al. 1988b, c). In the present paper, using specific polyclonal antibodies to HGL, we demonstrate that HGL, like pepsin, is exclusively located in the chief cells of this mucosa. The mucus and parietal cells remained unlabelled. These data are consistent with previous studies showing that parallel secretory stimulation occurs between HGL and pepsin (Szafran et al. 1978). Furthermore, this immunocytolocalization was found to correlate with the results of lipase activity assays performed on paired biopsy specimens.

HGL and porcine pepsin are both glycoproteins (Bodmer et al. 1987; Vatier 1983) and the cross reactions observed using crude antibodies might be due to the immunoreactivity of the glycan moities of these proteins. Depletion of these antibodies with mucus rich fractions, (which are mainly composed of glycoproteins), originating from the antral mucosae, eliminated these nonspecific cross-reactivities. It can be assumed that the immunolabelling of mucus cells obtained when using crude anti-HGL or crude anti-pepsin sera was also due to nonspecific interactions with mucus glycoproteins.

Previous studies have shown differences in structural and catalytic properties between pancreatic and preduodenal lipases (Gargouri et al. 1986; Hamosh 1984), and the results of the present study further demonstrate that these two lipase also differ immunologically. This is in agreement with Roberts and Jaffe's data (Roberts and Jaffe 1986) which showed that antibodies to rat lingual lipase were unable to inhibit rat pancreatic lipolytic activity.

From the granular labelling observed in the apical portion of chief cells, one can conclude that HGL is a secretory enzyme which is fully active in human gastric juice (Tiruppathi and Balasubramanian 1982). The exact reason for the existence of two distinct types of labelling (granular and diffuse cytoplasmic labelling) within the chief cells is not yet clear. This phenomenon might reflect differences in physiological state of the fundic mucosa, but the possibility that an artefact may have been introduced by the microscopic techniques used cannot be totally rulled out. Investigations are in progress to investigate this phenomenon, using fundic mucosa biopsies from other mammalian species.

In conclusion HGL is an enzyme which is colocalized with pepsin and is found exclusively in the chief cells of the fundic mucosa of the human stomach. No specific HGL immunolabelling was observed in either antral or duodenal biopsies. This cellular location correlates with the lipolytic activities measured in paired gastric biopsy samples.

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