Identification of parietal cells in gastric body mucosa with HMFG-2 monoclonal antibody

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Abstract

Aims—To identify parietal cells in the upper gastrointestinal tract by an immunoperoxidase method, using commercially available monoclonal antibodies.

Methods—Routine surgical biopsy specimens of gastric body mucosa were examined using the avidin-biotin peroxidase method with the monoclonal antibodies HMFG-1 and HMFG-2 to identify parietal cells. Double immunoperoxidase labelling with HK12.18, a well characterised monoclonal antibody directed against an epitope on the α (catalytic) subunit of H⁺ translocating, K⁺ stimulated adenosine triphosphatase (H,K-ATPase), was used to confirm that HMFG-1 and -2 stained parietal cells.

Results—HMFG-1 and HMFG-2 showed consistent parietal cell staining patterns in the gastric body mucosa. HMFG-2 gave a more intense staining pattern of the secretory canaliculi. This was confirmed by double immunolabelling with HK12.18.

Conclusions—HMFG monoclonal antibodies are recommended as highly specific markers of human gastric parietal cells.

Keywords: HMFG, gastric mucosa, parietal cells, antibodies, H,K-ATPase.

Parietal cells are large, round or pyramidal cells found in gastric glands in the mucosal epithelium of the body of the stomach. These cells are located mainly in the mid-region of the gastric glands, surface epithelial cells and mucous neck cells are found in the gastric pit and neck region of the glands, while chief cells are located predominantly in the lower third of the glands. They are also present in antral and duodenal mucosa.

Parietal cells can be identified routinely by light microscopy after staining with haematoxylin and eosin (fig 1). However, if cells are sparse, as in the duodenum, they are not easily identified against a background of other cell types.

Alternative stains may be used—for example, Luxol fast blue, and also more complex techniques, including a five stain combination of alcian blue, periodic acid-Schiff and Masson's trichrome methods. However, these combination techniques are time-consuming. Lectin binding methods have also been used, but these are also difficult to perform and have not been used to identify antral or duodenal parietal cells.

As part of a larger study addressing the distribution and function of sparsely distributed parietal cells in duodenal mucosa, we sought to develop a simple immunocytochemical method which would unambiguously identify parietal cells. A previous study of the novel antigen of epithelial membranes indicated that the monoclonal antibodies directed against human milk fat globule (HFMG) membrane proteins cross-reacted with gastric mucosal cells. Using HMFG-1 and HMFG-2 monoclonal antibodies as immunocytochemical probes on human gastric body mucosa, we have shown that these antibodies are selective for the intracellular canaliculare structures of parietal cells, and have confirmed this selectivity using a well characterised antibody widely used as a gastric parietal cell specific and canalicular membrane H⁺ translocating, K⁺ stimulated adenosine triphosphatase (H,K-ATPase) specific marker.

Methods

Ten consecutive gastric body biopsy specimens from the surgical files were examined. The biopsy specimens had been routinely fixed in formalin and embedded in paraffin wax. Immunocytochemistry was performed using the avidin-biotin peroxidase (ABC) technique. Sections, 3 μm thick, were deparaffinised, rehydrated and incubated for 10 minutes in 3% H₂O₂ to eliminate endogenous peroxidase activity. After rinsing in Tris buffered saline (TBS), the sections were treated with blocking

Figure 1 Parietal cells in gastric body mucosa stained with haematoxylin and eosin.
HMFG-2 for identification of parietal cells in gastric body mucosa

Results
Both HMFG monoclonal antibodies showed consistent staining patterns in all 10 gastric body mucosa biopsy specimens examined.

HMFG-1 labelled the gastric luminal surface and the intracellular secretory canalicular network of parietal cells (fig 2). HMFG-2 showed a similar distribution of labelling but the secretory canaliculi were more intensely stained, with more pronounced and precise delineation of secretory membrane structures (fig 3). On double immunolabelling with HK12.18 and HMFG-1 and HMFG-2, all cells labelled with HMFG antibodies also stained positively with HK12.18, confirming that these were parietal cells (shown for HMFG-2 in fig 4). Furthermore, by the same double immunolabelling criteria, HMFG antibodies stained only parietal cells. Neither HMFG nor H,K-ATPase antibodies stained mucous neck cells or chief cells. Control sections were uniformly negative.

Discussion
The function of parietal cells is to secrete hydrochloric acid, blood group substances and intrinsic factor into the lumen of gastric glands and thence into the stomach. Hydrochloric acid secretion is mediated by H,K-ATPase, which is an integral protein of the apical membrane of the gastric parietal cell. Structurally, the acid secretory apical membrane in a resting (non-secreting) parietal cell is perceived microscopically as numerous tubulovesicular membrane profiles which fill much of the parietal cell cytoplasm. With the onset of acid secretion, tubulovesicles are transformed into an extensively ramified, smooth-surfaced secretory canalicular membrane network, the lumen of which is contiguous with the lumen of the gastric gland. This membranous network is seen on light microscopy as eosinophilic vacuolated cytoplasm surrounding a centrally placed nucleus.

During lactation, membrane bound fat globules are released into the milk. Integral membrane proteins of fat globule membranes have been partially characterised, and include both serum and then incubated with monoclonal antibodies HMFG-1 and HMFG-2 (Unipath), or HK12.18 as a positive control. Negative controls omitted the primary antibody. The sections were incubated for one hour, then rinsed in TBS and incubated with biotinylated anti-mouse immunoglobulin for 30 minutes. After rinsing, sections were incubated for 30 minutes in Dako ABC reagent (Dako, High Wycombe, UK), followed by incubation with peroxidase substrate solution (diaminobenzidine) as specified by the supplier.

To examine the coincidence of parietal cell distribution of HMFG and H,K-ATPase antigens, double labelling with HMFG antibodies and the H,K-ATPase antibody was carried out on the same 10 gastric body biopsy specimens using alkaline phosphatase conjugated secondary antibodies. The sections were photographed by light microscopy (× 400).
large glycoprotein complexes and lower molecular weight components. The monoclonal antibodies HMFG-1 and HMFG-2 are raised against membrane components of this milk fat globule membrane and interact with epitopes on glycoprotein complexes (HMFG-1) and with lower molecular weight constituents (HMFG-2). Application of HMFG monoclonal antibodies to gastric body biopsy specimens in this study resulted in highly selective labelling of parietal cells. The coincidence of intracellular localisation of both HMFG and H,K-ATPase antibodies to secretory canalicular membranes raises interesting questions. The HK12.18 epitope is known to be on the non-glycosylated α subunit of H,K-ATPase, which constitutes up to 90% of the integral membrane protein content of the parietal secretory cell membrane. Co-localisation of HMFG antibodies to the same membrane may indicate cross-reactivity of the antibodies with the highly-glycosylated β subunit of H,K-ATPase. An intriguing possibility is that membranes enclosing milk fat globules possess an H,K-ATPase which establishes K+ and/or H+ gradients across the membrane which have functional roles in accumulation of fat within the globule. Alternatively, the HMFG-like immunoreactivity of parietal cell apical membrane may indicate the presence of non-H,K-ATPase proteins which share epitopes with integral membrane proteins of an unrelated secretory membrane compartment, that of the lactating mammary cell. Further molecular characterisation of the HMFG epitope in parietal cells is in progress.

Compared with parietal cell localisation by complex trichrome histochemistry and lectin binding techniques, HMFG immunocytochemistry offers a higher degree of selectivity and specificity for parietal cells. In terms of identification of small numbers of parietal cells, such as in antral or duodenal mucosa, commercially available HMFG monoclonal antibodies, particularly HMFG-2, are simple and convenient probes.