ORIGIN OF THE CHIEF CELLS OF THE HUMAN FUNDIC GLANDS

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The human gastric chief cells are located in the bottom of the fundic glands. They contain a well developed and basal rough endoplasmic reticulum, basal nucleus, Golgi apparatus, and abundant apical secretory granules. These secretory granules are electrondense and contain several proteins, e.g., pepsinogen, prochymosin and phospholipase A2 (Waalewijn et al., 1991). The origin of these chief cells during tissue renewal is controversial; neither mitosis nor ^{3}H-thymidine uptake is observed in these cells. Thus, the origin of the new chief cells by division from the previous ones, seems to be discounted. Some studies suggest that a part of the mucous neck cells transforms into chief cells, or even that the mucous neck cells are precursors of chief cells (Suzuki et al., 1983). The main characteristic of the mucous neck cells is their secretory granules, they are electronlucent but with a small electrondense core. The mucous neck cells contain few rough endoplasmic reticulum cisternae. Transitional cells between mucous neck and chief cells have also been described (Waalewijn et al., 1991; Madrid et al., 1990). They are characterized by their biphasic granules, which contain an electronlucent area and an electrondense region of variable size.

Lectins are proteins or glycoproteins which specifically label to a single carbohydrate or to an oligosaccharidic sequence. They have been widely used to investigate the oligosaccharidic composition of the mucin-type glycoproteins of the mucous secretions. On the other hand, lysozyme is a protein localized in some serous cells, e.g., the Paneth cells. The distribution of this protein in the human stomach has not been so far described.

The aim of the present work is the obtention of new data to support the developmental relationship between the mucous neck, transitional and chief cells of the human fundic glands. For this, lectins and lysozyme will be used as markers of mucous and serous secretions, respectively.

Samples of histologically normal human fundic mucosa were obtained from ten cases of total or partial gastrectomy due to duodenal ulcer. For light microscopy, tissue samples were fixed in 10% formalin in PBS and embedded in paraffin. For electron microscopy the tissues were fixed in 2% glutaraldehyde and embedded in Lowicryl K4M.

Paraffin sections were stained with: 1) HRP-labeled lectins: *Ulex europaeus*-I agglutinin (UEA-I), *Lotus tetragonolobus* agglutinin (LTA), *Canavalia ensiformis* agglutinin (Con-A), wheat germ agglutinin (WGA), and *Helix pomatia* agglutinin (HPA); or 2) *Aleuria aurantia* agglutinin- (AAA-) digoxigenin (DIG) labeled. After AAA-DIG a mouse anti-DIG antibody HRP-labeled was used.

For electron microscopical studies three methods were used: 1) one-step method: the grids were directly incubated with HPA-, or UEA-I-gold conjugates; 2) two-step method: the grids were labeled first with Con-A or WGA, and then with HRP- or ovomucoid-gold complex, respectively; or 3) a three-step method: the first layer was a dilution of LTA-DIG or AAA-DIG; as second layer, a mouse anti-DIG antibody was used, and subsequently a goat anti-mouse IgG+H antibody gold-labeled.

Since WGA can label both to N-acetylglucosamine (GlcNAc) and sialic acid, neuraminidase pre-treatments were also used to remove sialic acid.

The following controls were used: 1) substitution of the different molecules by buffer; and 2) pre-incubation of the lectins with the corresponding hapten sugars.

Since the blood group of the individuals can be expressed in the tissues and can modify the binding pattern of some lectins; the secretor status was

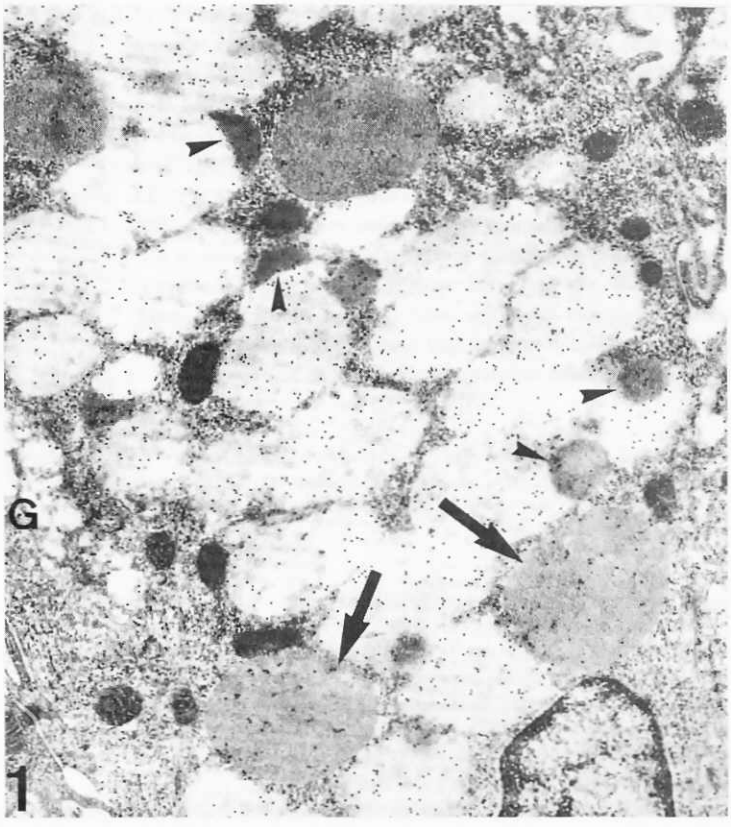

Fig. 1. AAA. Transitional cell. The granules show electronlucent and electrondense regions (arrowheads). The labeling is greater in the electronlucent areas. Some granules are completely electondense (arrows). Note the labeling inthe Golgi apparatus (G). x 18,600.

determined by the immunocytochemical localization of the blood group antigens, both at light and electron microscopical level. For light microscopy, the paraffin sections were incubated with anti-A, anti-B or anti-H monoclonal antibodies, and then with anti-mouse IgG+H HRP-labeled. For electron microscopy, the second antibody was a goat anti-mouse IgG+H gold-labeled.

The mucous neck cells contained abundant granules with a large electronlucent area which was labeled by WGA, HPA, LTA, UEA-I and AAA, and slightly by Con-A. The intermediate cells contained secretory granules with electronlucent areas which decreased in size as these cells were localized in lower positions in the fundic glands. This electronlucent area showed a similar lectin binding pattern to that of the mucous neck cell granules (Figs. 1, 2). The electrondense regions of both the mucous neck and transitional cells were only labelled with Con-A and AAA (Figs. 1, 2). The chief cells were only positive in their electrondense granules, to Con-A and AAA (Fig. 2). Moreover, some transitional cells located close to the bottom of the glands showed some completely electrondense granules (Fig. 1). Reactivity to lectins was also found in the Golgi apparatus. Basically LTA, UEA-I, HPA and WGA mainly labelled to the trans face in the mucous neck and transitional cells, while Con-A and AAA (Fig. 1) labeled from cis to trans faces in the three cell types. HPA also showed a slight labelling in the cis face of the Golgi apparatus. Lysozyme reactivity in the mucous neck cells was restricted to the electrondense core of the granules, and in the upper transitional cells, lysozime appeared only slightly in the electrondense regions of the granules. Neuraminidase treatment before WGA labeling did not modify the lectin reactivity.

The absence of modification in the WGA-binding pattern after neuraminidase treatment would indicate that in our tissue, WGA is labelling to GlcNAc. These findings suggest that the electronlucent area of the secretory granules of the mucous neck and transitional cells contained glycoproteins with terminal fucose (Fuc) and GlcNAc residues and, in low quantity, mannose (Man) residues. The dense regions of these secretory granules as well as the granules of the chief cells contained Man and Fuc residues, lacking GlcNAc. Since only AAA, of the three Fuc-labelling lectins used, was reactive in these dense regions, the Fuc residues identified are probably α(1-6) linked to the innermost GlcNAc of N-linked oligosaccharides (Osawa and Tsuji, 1987). Our study demonstrates that the composition of the different compartments of the granules in the transitional and mucous neck cells is different. Previous studies localizing components of the chief cells, have also identified its presence in the electrondense regions of the transitional cells (Waalewijn et al., 1991). The morphological aspect of these granules suggests that as the mucous neck cells descend through the fundic glands, the dense core becomes larger and larger, originating first the transitional cells, and later, the definitive chief cells. Thus, the transitional cells could be considered as migrating and differentiating mucous neck cells. During this migration, the cells progresively decrease the production of mucous glycoproteins (typical of mucous neck cells) while they increase the production of serous components (typical of chief cells), which are stored in the growing electrondense regions of the granules. For this to happen, the cells should gradually change all their secretory machinery. The identification of cells containing biphasic granules which could represent transitional cells between mucous and serous cells, as well as the identification of typical components of mucous cells in typical serous cells, e.g., the Paneth cells, has been previously reported (Leis et al., 1996). All these data suggest that the classical morphological division into mucous and serous cells could be artificial and that one cell type could differentiate into another, as seems to happen in the human fundic glands.

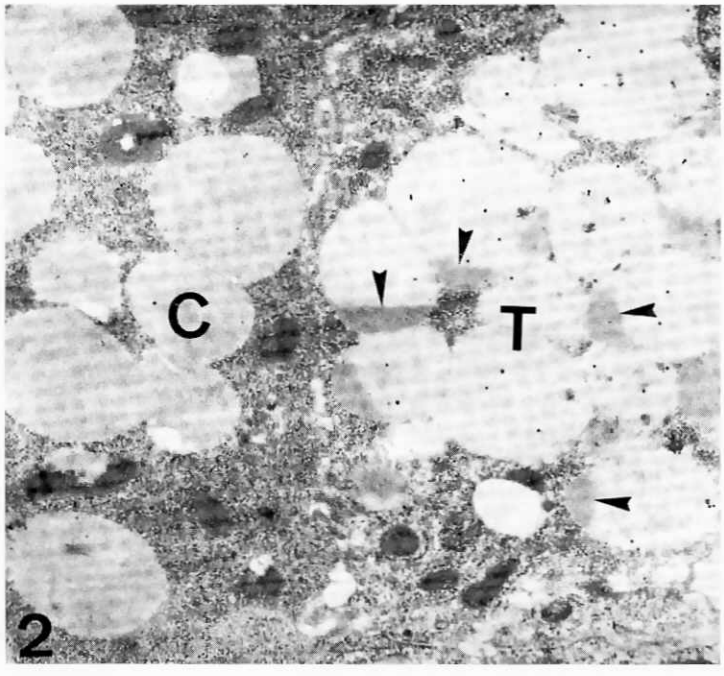

Fig. 2. UEA-I. A negative chief cell (C) is observed, while the transitional cell (T) is only labeled in the electronlucent regions of the granules. Arrowheads: unreactive electrondense regions. x 13,600.

Leis O., Madrid J.F., Ballesta J. and Hernández F. (1996). N- and O-linked oligosaccharides in the secretory granules of rat Paneth cells. An ultrastructural cytochemical study. J. Histochem. Cytochem. (In press).

Madrid J.F., Ballesta J., Castells M.T. and Hernández F. (1990). glycoconjugate distribution in the human fundic mucosa revealed by lectin- and glycoprotein-gold cytochemistry. Histochemistry 95, 179-187.

Osawa T. and Tsuji T. (1987). Fractionation and structural assessment of oligosaccharides and glycopeptides by use of immobilized lectins. Annu. Rev. Biochem. 56, 21-42.

Suzuki S., Furihata C., Tsuyama S. and Murata F. (1983). Cells intermediate between mucous neck cells and chief cells in rat stomach. Histochemistry 79, 167-176.

Waalewijn R.A., Meuwissen S.G.M., Pals G. and Hoefsmit E.C.M. (1991). Localization of pepsinogen (A and C) and cellular differentiation of pepsinogen-synthesizing cells in the human gastric mucosa. Eur. J. Cell Biol. 54, 55-60.

Supported by grants PB 93-1123 from the Spanish DGICYT and UPV 075.327-EC236/95 from the Univeristy of the Basque Country.