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Migration of the Ductular Elements of Gut-Associated Glands Gives Clues to the Histogenesis of Structures Associated with Responses to Acid Hypersecretory State:

The Origins of "Gastric Metaplasia" in the Duodenum of the Specialized Mucosa of Barrett's Esophagus and of Pseudopyloric Metaplasia

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This article suggests that cell lineages of defined phenotype arise within gastrointestinal epithelia exposed to acid hypersecretion — the ulcer-associated cell lineage (UACL), "gastric metaplasia" and that of Barrett's esophagus. Detailed study of both the histogenesis and secretory peptide phenotype of the UACL and gastric metaplasia reveal an origin from newly-formed ducts and Brunner's gland ducts, respectively. It is suggested that Barrett's epithelium arises directly from the epithelium of the cardiac esophageal glands, and that these three ductal epithelia are the origins of these three important adaptive phenomena to gastric hypersecretion.

INTRODUCTION

Gastric acid hypersecretion is associated with a number of morphological appearances: in acid reflux disease, there are changes in the oesophagus, which are usually designated as Barrett's esophagus, where the usual squamous mucosal lining becomes replaced by columnar epithelial cells of putative specific aspect. In the duodenum, the villi become covered with mucus-secreting cells, which powerfully resemble the cells that line the gastric foveolae, so much so that they are often referred to as "gastric metaplasia." Lastly, in both the duodenum in peptic ulceration, with its associated excess secretion of acid, and in the gastric mucosa in chronic atrophic gastritis, often associated with peptic ulceration and *Helicobacter pylori* infection, there evolves a lineage that also resembles gastric epithelium, often seen occupying the *lamina propria*, but also growing onto the villi in the duodenum and replacing the indigenous surface lineages. This has, in the past, been referred to "pyloric" or "pseudopyloric" metaplasia [1, 2], or even as Brunner's gland metaplasia because of morphological similarities with these cells [2]. More recently, these structures have been given the rather pretentious, but perhaps more accurate name, ulcer-associated cell lineage (UACL)[b] [3].

Thus, we have three situations where abnormal secretion of acid apparently leads to the evolution of three discrete morphological states. Here we shall make the case that each of these morphological phenomena are caused by the upward growth and migration of ductular cells: in the case of Barrett's esophagus, the cells grow from the ducts of

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[b] *Abbreviations:* UACL, ulcer-associated cell lineage; EGF/URO, epidermal growth factor/urogastrone; EGFR, EGF/URO receptor.

esophageal glands upwards onto the esophageal surface, while in the case of gastric metaplasia of the duodenum, the surface cells are derived from the ductular cells of Brunner's glands. Thus, the stomach is confined by two structures that have the capacity to respond morphologically to the presence of excess acid: proximally the esophageal glands and distally, the Brunner's glands. Where no pre-existing structures with ducts are present, then a new gland develops, the UACL, whose ducts grow to make contact with the gut lumen in the duodenum via a nearby villus, and cells migrate through this duct to reach and clothe the adjacent villus [3].

Moreover, recent morphological, immunohistochemical and *in situ* hybridization studies have shown that, far from being mere metaplasias, these cell lineages do, in fact, have novel functional properties: they have a definable life history during which they sequentially acquire differentiation antigens constituting a distinct phenotype and synthesize and secrete large amounts of regulatory peptides of considerable interest. In some instances, the presence of these cells in the ulcerated mucosa also appears to induce peptide gene expression in the local intestinal cells.

THE HISTOGENESIS OF THE ULCER-ASSOCIATED CELL LINEAGE

In the duodenum, the UACL appears first as a small, intensely D/PAS positive bud at the base of the intestinal crypts adjacent to a peptic ulcer, and these buds push outwards into the surrounding stroma of the *lamina propria* as small tubules, which quickly coalesce with tubules from other crypts to form a more or less complex acinar arrangement. They also lie within a distinctive, immature, acid mucopolysaccharide-rich stroma. In larger gland formations, a single duct is formed by the joining of two or more smaller ductules, and this duct grows upwards through the core of an adjacent villus towards the epithelial surface. At the epithelial surface, the duct emerges through a distinct pore. The UACL moves out of the tubule and onto the villus surface, where it replaces the

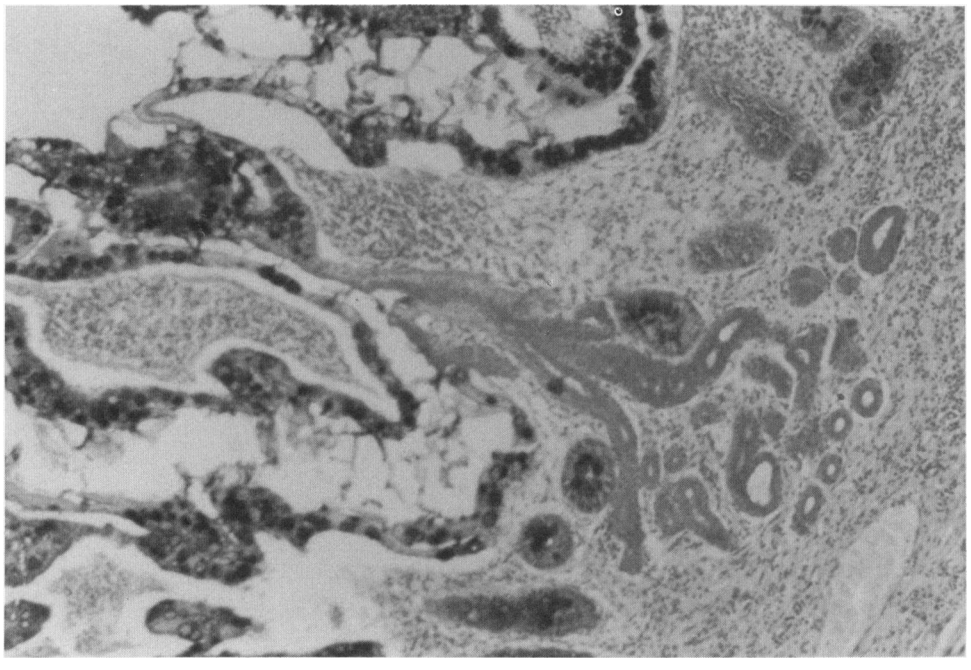

Figure 1. A mature complex of the ulcer-associated cell lineage, showing the basal acini, the duct system and the cells that migrate out onto the surface to clothe it, replacing the indigenous cell lineages.

indigenous surface cell lineages. The entire villous surface can thus become covered with the UACL cells. Figure 1 shows a mature UACL complex, with the glandular portion giving rise to the duct, from whence cells migrate onto the villous surface to cover it.

In the UACL, once the duct is formed, about two-thirds of the way up the duct, a clearly defined zone of proliferating-cell-nuclear-antigen (PCNA)-positive cells appears in which mitotic figures can also sometimes be seen. This zone is quite discrete and ends well before the duct emerges onto the surface. Thus, the important duct area in this lineage is also the site of the proliferative zone [4]. It is interesting and relevant that Brunner's gland primordia also begin as small buds that grow out of the duodenal crypts at about 16 weeks of intrauterine life in the human, grow as tubules in the submucosa and by 36 weeks achieve the familiar adult tubuloalveolar pattern [4]. Thus, the histogenesis of the duct in the UACL is similar to that of Brunner's gland duct in embryonic development. In practical terms, it means that ductular cells are available to migrate onto the surface, even where Brunner's gland ducts are not available.

The UACL also expresses similar secretory proteins to the ones we have seen in the lineages discussed above; again there is a very distinctive pattern of synthesis within the organized structure. The acinar portion contains abundant immunoreactive epidermal growth factor/urogastrone (EGF/URO), which is also seen in the secretions [5]. In normal conditions, EGF/URO is produced by gut-associated salivary, esophageal and Brunner's glands, but not by other cell lineages in the gut [6]. The location of EGF/URO receptors (EGFR) in gastrointestinal cells is currently under dispute. There is evidence that EGFR in the gut are polarized to the laterobasal membranes in the rat [7], but Thompson [8] has shown apically-sited EGFR in the neonate. However, these EGF receptors on the microvillar membrane are apparently not associated with phosphorylation of membrane proteins after ligand:receptor binding [9]. Radiolabeled EGF/URO given orally to normal rats does not bind to the intact mucosa but readily binds locally when a mucosal defect is present [10]. Moreover, EGF/URO has been reported to be mitogenically active parenterally but not when given directly into the intestine [11], although this too has been disputed [12]. Whatever the exact binding mechanism, the secretion of EGF/URO by the UACL ensures that the peptide is available locally to stimulate repair and regeneration in the local ulcer environment, and we have suggested that this is an important *in vivo* role for EGF/URO [5].

Other regulatory peptide genes are also expressed by the UACL in a site-specific manner. hSP and pS2 are members of the trefoil peptide family, a growing group of proteins that share the unique "trefoil" motif, a three-leafed domain held by disulfide bonds based on cysteine residues [13]. The canonical molecule is pS2, a 60 amino acid secretory protein that was originally found by differentially screening a cDNA library from the human breast carcinoma cell line MCF-7 [14]. The function of pS2 is as yet unknown, but it is highly homologous with spasmolytic polypeptide, a known gastrointestinal regulatory peptide, in which the trefoil domain is tandomly repeated [15]. It has been claimed that porcine spasmolytic polypeptide inhibits gastric acid secretion and also intestinal motility [16, 17] and is mitogenic for MCF-7 and colorectal carcinoma cells *in vitro* [18], and ligand:receptor binding results in inhibition of adenylate cyclase [16]. However, these claims have now been disputed, and the trefoil peptides are now regarded as important motogens for gastrointestinal epithelial cells (see Poulsom in this issue [page 137]). In the normal stomach, pS2 and hSP are secreted and are co-expressed by the foveolar and surface cells [19], although they are products of different genes [20]. Moreover, hSP is expressed in large amounts in the pyloric glands of the gastric antrum and in Brunner's gland acini. hSP and pS2 mRNA expression can be readily demonstrated by hybridization *in situ* using ^{35}S labeled riboprobes. hSP mRNA is found in the acini and lower duct cells, whereas pS2 mRNA and protein are localized in large amounts in the upper duct and all

surface cells. Moreover, it has recently been shown that the UACL expresses a third member of the trefoil peptide family, intestinal trefoil factor (ITF), a single trefoil domain peptide [9], constitutively expressed by intestinal goblet cell, but not by gastric mucous cells [21]. ITF is expressed throughout the UACL. Addition of recombinant rat ITF to the basolateral surface of rat small intestine results in an increase in short-circuit current, associated with increased chloride secretion, and binding sites, have been demonstrated on the surface of the rat small intestinal mucosa [21]. These peptides also have powerful motogenic properties [18a]. Thus, the UACL secretes at least four peptides with potentially important biological effects. A further protein of significance is produced by the UACL: lysozyme, which has antibacterial and putative immunoregulatory function. Both lysozyme mRNA and protein are found in abundance in the UACL [10].

In addition, it is becoming clear that the UACL is also associated with trefoil peptide gene expression in the indigenous cell lineages in the adjacent mucosa [22]. The normal cell lineages in the intestine include the mucin-producing goblet cells, neuroendocrine cells and enterocytes. The mucous cells in the vicinity of the UACL express abundant immunoreactive pS2 in the basal parts of the cytoplasm in formalin-fixed paraffin-embedded sections. In glutaraldehyde-fixed, resin-embedded sections, in addition to labeling in the Golgi area, pS2 is seen within the theca also. *In situ* hybridization with an ^{35}S-labeled antisense riboprobe shows pS2 mRNA localized in considerable concentration in the cytoplasm beneath the mucus-filled theca. Ultrastructural immunocytochemistry confirms that immunoreactive pS2 is found in the rough endoplasmic reticulum of these cells and is also co-packaged via the Golgi complex into the mucous granules. Thus, the mucous cells adjacent to peptic ulcers where the UACL is seen show pS2 expression and also co-secrete pS2 with the liberated mucus into the intestinal lumen. In addition, neuroendocrine cells adjacent to the UACL also express pS2 protein [22]. The finding of the same peptide co-packaged in both mucous granules and neuroendocrine granules is highly unusual: mucous granules are secreted into the lumen, whereas neuroendocrine granules are released into the basal and lateral membranes. The function of these granules are, of course, very different. Mucus has lubricating and protective functions in the gut, but endocrine secretions release various regulatory peptides that act via paracrine or autocrine mechanisms to produce manifold effects on the gut [23].

The pattern of peptide expression in the UACL also points to its histogenesis: Brunner's gland primordia begin as bud-like outgrowths from the bases of duodenal crypts, which form tubules that by 28 weeks of intrauterine life show the familiar tubuloalveolar pattern of the adult Brunner's glands. At 18 weeks, immunoreactive EGF/URO is present in abundance, while pS2 peptide is confined to the developing ducts. However, hSP mRNA is present in considerable concentration throughout Brunner's gland acini and ducts. This pattern of trefoil gene expression is maintained throughout fetal life, and moreover, in the adult, pS2 peptide and transcripts are expressed by the ductal cells, and large amounts of hSP protein and mRNA are present in the acini [4, 10]. These observations indicate that the UACL reiterates the developmental program of Brunner's glands.

These observations indicate that the UACL is not a metaplasia, which formally can be defined as a change from one defined differentiated phenotype to another. The UACL, though sharing several phenotypic features with other cell lineages in the gut, does have a unique structure and function. Since it is induced only in chronic ulcerative conditions, it would certainly appear to be a primary defense reaction, producing a cocktail of active peptides and proteins, EGF/URO, hSP, pS2 and lysozyme, which would be expected to advance mucosal healing. There are also early indications that other peptides may be produced by the UACL, and immunoreactive TGF-α has been detected.

THE HISTOGENESIS OF DUODENAL GASTRIC METAPLASIA

In duodenitis and duodenal ulcer disease, cells with the apparent phenotype of gastric foveolar epithelial cells appear on the surface of duodenal villi. These have been called gastric metaplasia with the implication that these cells arise by a metaplastic process from villus epithelial cells, or more believably, from the duodenal crypt cells. This is despite numerous studies, both by ourselves [24] and others, which show fairly unequivocally that these cells arise in continuity with Brunner's gland ducts. It is well known that Brunner's gland acini contain immunoreactive EGF (and also hSP mRNA and peptide), and recent studies have also drawn parallels between the trefoil peptide status of the ductal epithelium and of the gastric metaplastic epithelium; they both contain pS2 and hSP mRNA and pS2 peptides, although hSP peptides not always demonstrable. The presence of EGF in the acini, and trefoil peptide gene expression in the duct is, of course, not dissimilar to the UACL, although the topography differs. The Brunner's gland duct epithelium is thus considered to be the source of gastric metaplasia.

THE HISTOGENESIS OF BARRETT'S ESOPHAGUS

The distal esophagus is lined by squamous epithelium, which, when exposed to chronic reflux of gastroduodenal contents, is sometimes replaced by the glandular epithelium of Barrett's esophagus. There are usually three types of epithelium that line Barrett's oesophagus: rather atrophic fundic mucosa, mucosa with complete or type 1 intestinal metaplasia and so-called specialized Barrett's epithelium, resembling the incomplete or type 2 intestinal metaplasia of the stomach. It is usually assumed that such epithelium arises from ingrowth by the cardiac epithelium, which grows in to cover the ulcerated and largely destroyed squamous epithelium. However, there are several observations that are not in accord with this concept: when the esophageal mucosa of experimental animals is stripped and there is no continuity between the gastric epithelium and the proximal esophageal squamous epithelium, islands of mucin-secreting epithelium appear in the ulcerated area. Similarly, when patients with established Barrett's esophagus are treated with powerful antisecretory therapy such as omeprazole, the proton pump inhibitor, islands of squamous epithelium appear in the glandular epithelium. These observations suggest, not that gastric mucosa grows in from the cardia, or that squamous epithelium grows in to repair the defect from above, but that a stem cell population is present, which gives rise to Barrett's epithelium independently of the gastric mucosa, and conversely, these stem cells remain beneath the Barrett's epithelium to regenerate the squamous epithelium in the repair process. The candidate population of stem cells here is the ducts of the subjacent esophageal glands, which remain under the mucosa, even in Barrett's esophagus.

Classically, there are two types of glands to be found in the esophageal mucosa: esophageal glands proper and the esophageal cardiac glands. The former are small, compound glands with richly-branched tubulo-alveolar secretory portions containing only mucous cells; the ducts are lined by low columnar epithelium in the lower part, and more superficially, with stratified squamous epithelium. The esophageal cardiac glands are found both in the post-cricoid area and in the lower oesophagus and are located in the *lamina propria*. The branched tubules contain cells with granular and mucous cytoplasm. The small ducts, lined with cuboidal epithelium, fuse into a larger, duct, lined with columnar epithelium, which "more or less resembles the mucous epithelium of the gastric foveolae" (Figure 2). It is these gland ducts that are the candidate source of Barrett's esophagus.

There has been little work on the secretory phenotype of the esophageal glands. There is also evidence that the esophageal glands secrete esophageal EGF. There has been no systematic study of the other secretory peptides made by these glands. There is trefoil peptide

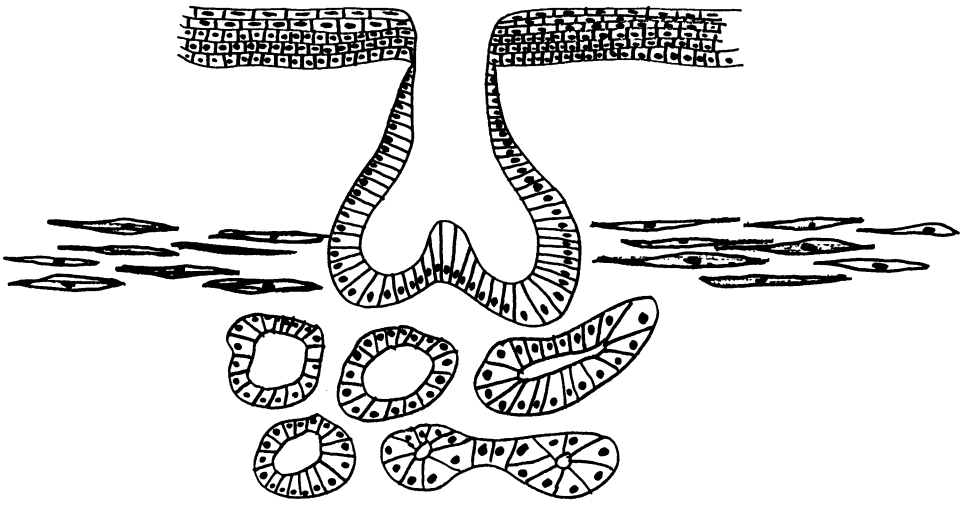

Figure 2. A diagram of a submucosal esophageal gland, showing the gland duct piercing the stratified squamous epithelium and the muscularis mucosae to reach the acini in the submucosa.

gene expression in the epithelium of Barrett's' esophagus. Both hSP and pS2 mRNA was present in the superficial epithelium, although hSP peptides could not always be demonstrated. The deeper epithelium contained only hSP and its message. Thus, an interesting parallel emerges between this epithelium and that of gastric metaplasia: the superficial epithelium contains pS2 and hSP, while hSP and EGF are present in the deeper glands. While this may merely mean similar adaptation to an acid-rich environment, a reasonable hypothesis, which will stand falsification, is that the esophageal gland ducts are the origin of the Barrett's epithelium, as Brunner's gland ducts are the origin of gastric metaplasia and the UACL duct is the origin of the UACL surface lineage. Thus, we can draw a parallel between the rather neglected epithelium of these three ducts: they are of undoubted importance in the histogenesis of gastrointestinal adaptations to gastric acid hypersecretion.

CONCLUSION

It is clear from this discussion that the cells that line the ducts of gut-associated glands are remarkably plastic and, arguably, are able to give rise to a number of important cell lineages in gastrointestinal pathology. The means are available to explore the genotype of the respective lineages and to assess whether these proposals are appropriate.

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