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Aspects of the biology of regeneration and repair in the human gastrointestinal tract

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The main pathways of epithelial differentiation in the intestine, Paneth, mucous, endocrine and columnar cell lineages are well recognized. However, in abnormal circumstances, for example in mucosal ulceration, a cell lineage with features distinct from these emerges, which has often been dismissed in the past as 'pyloric' metaplasia, because of its morphological resemblance to the pyloric mucosa in the stomach. However, we can conclude that this cell lineage has a defined phenotype unique in gastrointestinal epithelia, has a histogenesis that resembles that of Brunner's glands, but acquires a proliferative organization similar to that of the gastric gland. It expresses several peptides of particular interest, including epidermal growth factor, the trefoil peptides TFF1, TFF2, TFF3, lysozyme and PSTI. The presence of this lineage also appears to cause altered gene expression in adjacent indigenous cell lineages. We propose that this cell lineage is induced in gastrointestinal stem cells as a result of chronic mucosal ulceration, and plays an important part in ulcer healing; it should therefore be added to the repertoire of gastrointestinal stem cells.

Keywords: gastrointestinal mucosa; clonality; trefoil peptides

1. INTRODUCTION

There are now numerous models for the study of intestinal epithelial organization, and indeed for the study of intestinal disease; these range from *Drosophila*, *Caenorhabditis elegans*, through zebrafish to the mouse, and a good deal of important information has been gleaned from them. However, from time to time, it is worth re-visiting the human model, if only to examine the consistency of such experimental systems. But of course, in terms of flexibility, and the ability to manipulate the system, the human model is most limited. It is possible, however, through taking advantage of so-called 'experiments of nature' and the study of the changes that occur in disease processes, to obtain a degree of insight into the organization of the human intestinal mucosa, and the way in which it responds to damage in disease.

For example, work with allophenic tetraparental mice, using a polymorphism for the binding of the lectin *Dolichos biflorus* agglutinin (Dlb-1) (Schmidt *et al.* 1988), or with mice heterozygous for an X-linked gene, glucose-6-phosphate dehydrogenase (Griffiths *et al.* 1988), has shown that intestinal crypts are clonal populations, derived from a single stem cell during gut morphogenesis. This clonality has been shown to extend to gastric gland units in the mouse, and also to gut endocrine cell lineage (Thompson *et al.* 1990). In the human, the only indication that colonic crypts are clonal is the occurrence of crypt-restricted loss of the ability to O-acylate the acid sialomucin in colonic goblet cells. Although this has been used to imply crypt monoclonality (Williams 1997), it is clear that it applies only to the goblet cell lineage; a far cry from showing that clonality extends to the other contained cell lineages.

The first such definitive evidence comes from a study of an individual with the genotype XO/XY, in which the proportion of XO lymphocytes in the peripheral blood approached 20%; in the XY cells, the Y chromosome was dicentric (Novelli *et al.* 1996). In the colonic and small intestinal mucosa of this individual, the crypts were uniformly XO or XY; at the patches of boundaries between XO and XY crypts, mixed crypts were never seen. This means that human crypts are clonal populations, and it was shown that this clonality included all cell lineages in the crypt, including the endocrine cells (figure 1). More controversially, this patient also had familial polyposis coli, and observations indicated that 76% of the microadenomas were polyclonal. Calculations made from the density of microadenomas suggested that only a small proportion of these polyclonal proliferations could be attributed to collision between adjacent adenomas. In this respect, it is interesting that studies on chimaeras of *Min* mice also indicate a polyclonal origin for adenomas in this mouse FAP model (Dove, this volume).

Thus it is fairly clear that human colonic crypts are indeed clonal, and indeed that, in general, organization of human crypts follows the same rules as those derived from studies in experimental systems.

2. COOPERATION BETWEEN CRYPTS: THE DEVELOPMENT OF THE ULCER-ASSOCIATED CELL LINEAGE (UACL)

In chronic intestinal ulcers in the human gut, tubular structures are induced which contain mucin-producing cells that are quite dissimilar from the indigenous cell lineages (Liber 1951; Kawel & Tesluk 1955; Lee 1964;

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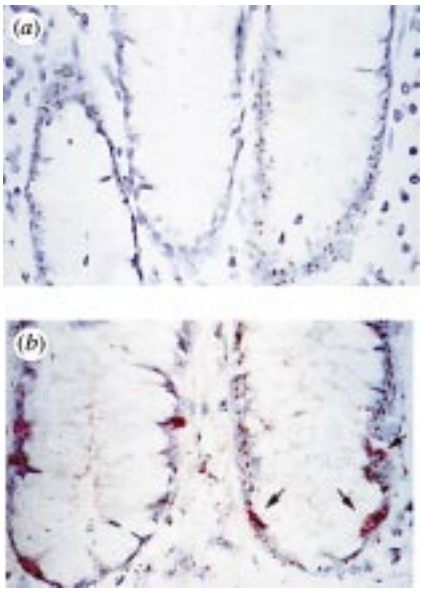

Figure 1. Crypts from an individual with the genotype XO/XY. (*a*) Section of colon stained with probes for the Y chromosome cY97 and pDP105; crypts are either all positive (dark spot over the nucleus) or all negative. (*b*) XY colonic crypt with Y-positive neuroendocrine cells (arrows) and an adjacent XO crypt with Y-negative neuroendocrine cells. (Reproduced from Novelli *et al.* (1996), with permission.)

Wright *et al.* 1990*a*). These complex structures are confined to the lamina propria, close to the ulcer margins, and produce neutral mucin, staining positively with the diastase periodic acid Schiff (D/PAS) method, unlike the acid mucin-producing, alcianophilic intestinal goblet cells (Liber 1951). They are usually regarded, on morphological grounds, as 'pyloric' or 'pseudopyloric' metaplasia (Liber 1951; Lee 1964), or even as 'Brunner's gland' metaplasia on the same grounds (Kawel & Tesluk 1955), and the suggestion was made that the production of these cells in some way 'protected' the mucosa. However, Kawel & Tesluk maintained that, until the function of these cells was established, it would be fruitless to speculate on their nature.

However, recent morphological, immunohistochemical and *in situ* hybridization studies have shown that, far from simply being a metaplasia, these cells have novel properties: (i) 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; (ii) the presence of these cells in the ulcerated mucosa appears to induce peptide gene expression in the local intestinal cells; and (iii) they also develop their own proliferative organization (Wright *et al.* 1990*a–c*, 1993; Ahnen *et al.* 1991, 1994; Patel *et al.* 1994; May & Westley 1997). For these reasons, it is proposed that these cells, although being differentiation progeny of intestinal stem cells, constitute a cell lineage in their own right: the 'ulcer-associated cell lineage' (UACL).

3. THE ORIGINS AND LIFE HISTORY OF THE UACL

The UACL is found in conditions that cause chronic intestinal ulceration. It appears first as a small, intensely

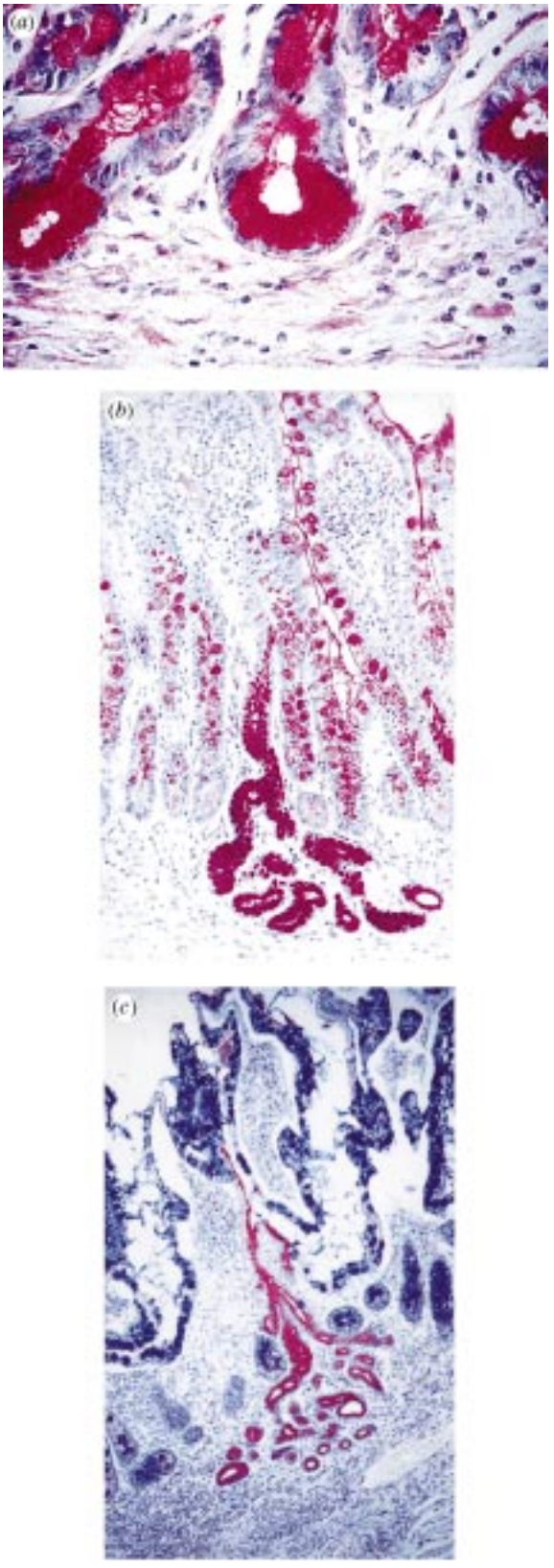

Figure 2. (*a*) Early buds of the UACL growing out of parent crypts close to an ulcerated area of the mucosa in Crohn's disease. Note the abrupt origin of the UACL cells, from the stem cell zone, and the absence of mitotic activity in the UACL cells. (*b*) A more developed acinar complex, growing as a newly formed gland in the lamina propria. (*c*) A mature UACL complex, showing the acinar area, the duct and the UACL cells clothing the surface of a villus, displacing the indigenous cell lineages; note the single goblet cell in the duct area. (Reproduced from Wright *et al.* (1990*a*), with permission.)

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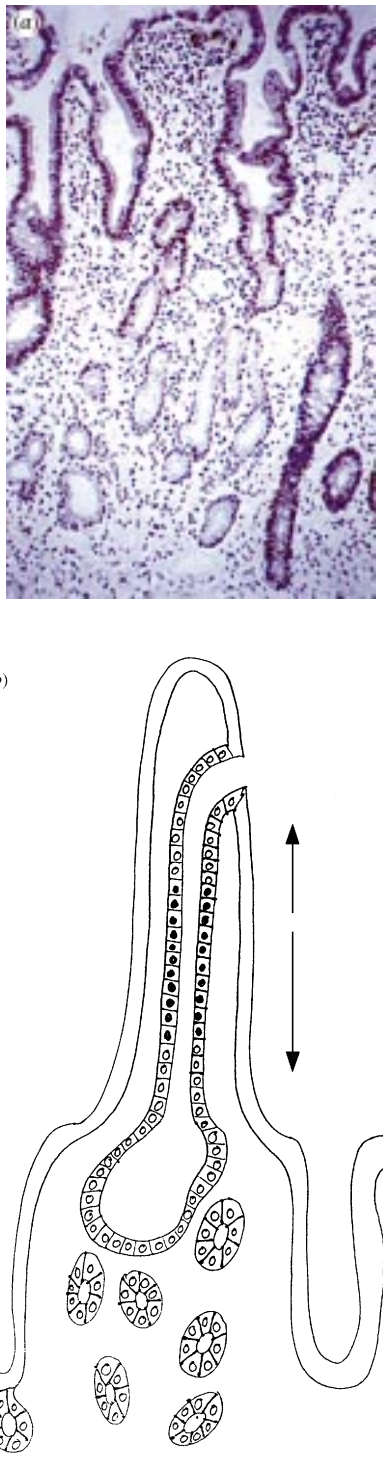

Figure 3. (*a*) A mature UACL complex, showing the proliferative zone staining positively for PCNA in the duct. (*b*) A diagrammatic representation of the proliferative zone in the duct of the UACL, with the possible pathways of migration indicated.

D/PAS-positive bud at the base of the intestinal crypts adjacent to the ulcer (figure 2*a*). 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 (figure 2*b*). Thus, notwithstanding the clonal nature of intestinal crypts, there is clear evidence that, during induction of this lineage, monoclonal crypts contribute their progeny to form a polyclonal glandular network. It is interesting to note that, at this stage, the buds and acini are devoid of mitotic figures, or indeed of proliferative activity as assessed by Ki67 (Wright *et al.* 1990*a*; Patel *et al.* 1994) or PCNA (Patel *et al.* 1994) staining. In larger gland formations, ducts are 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 (figure 2*c*). At the epithelial surface the duct emerges through a distinct pore. The UACL also moves out of the tubule and onto the villus surface, where it replaces the indigenous surface cell lineages (figure 2*c*). The entire villus surface can become covered with the UACL cells.

Although most commonly seen in the small intestine, particularly in Crohn's disease and duodenal ulcer disease, the UACL also appears in the colon, although by no means as commonly. Nor is the UACL confined to the luminal gastrointestinal tract. Its presence has been detected in pancreatic ducts in chronic pancreatitis, in the gall bladder in chronic cholecystitis, and even in the fallopian tube in chronic salpingitis and in inflammatory nasal polypi (Wright *et al.* 1990*c*), so the appearance of this lineage is indeed widespread after mucosal damage.

4. THE ORGANIZATION OF THE UACL

Mitotic figures, and indeed cycling cells as indicated by Ki67 or PCNA staining, are not found in the buds or acini of the UACL (Ahnen *et al.* 1994; Patel *et al.* 1994). Once the duct is formed, then the UACL develops its own proliferative architecture: about two-thirds of the way up the duct, a clearly defined zone of proliferative cells appears (figure 3*a*), in which mitotic figures can also sometimes be seen. This zone is quite discrete, and ends well before the duct emerges onto the surface. This pattern, with the proliferative compartment towards the top of the duct, is similar to that found in the gastric gland in the antral gastric mucosa. However, this mode of histogenesis is not unique. It is now generally appreciated that Brunner's gland primordia in the duodenum begin as small buds which 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 (Ahnen *et al.* 1994). However, in the adult Brunner's gland there is no defined proliferative organization as in the UACL. It thus appears as if the UACL reiterates the histogenetic program of the Brunners glands, but then develops the proliferative organization of gastric gland tubules (Ahnen *et al.* 1994).

Thus the life history of the UACL is complex: certainly, in the absence of cell division, the buds and early tubules appear to be direct differentiation progeny of the stem cells from the parent crypts and migrate downwards into the evolving tubule. This proposal is supported by the presence, within the UACL tubules and even the duct, of other stem cell derivatives: Paneth, goblet and neuroendocrine cells (figure 2*c*). This concept of downward migration in the intestinal crypt is not novel: Bjerknes & Cheng (1981) proposed that beneath cell position 5 in the crypt, cells that are destined to differentiate into all lineages

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migrate downwards into the base of the crypt, and that the only cells that divide in this zone are the crypt stem cells. In the case of the UACL, once differentiation to this pathway is triggered, most stem cell production destined for downward migration is directed into the UACL pathway, although with an admixture of other intestinal cell lineages.

Once the duct is formed, a defined proliferative zone develops. In the gastric gland, cells born in the isthmus-neck region migrate upwards to renew the foveolar and surface mucous cells, and downwards to replace the parietal and chief cell populations, i.e. there is a bi-directional flux of cells. If this were to happen in the UACL, then the proliferative zone would take over replacement in the entire UACL, producing the surface cells and also cells to migrate downwards to expand the acinar cell population (figure 3*b*). If this is the case, how the proliferative zone interrelates with the parent crypts is obscure.

5. GENE EXPRESSION IN THE UACL

(a) *Genes related to differentiation*

The UACL itself is a differentiating cell lineage: that is to say, in moving from the basal buds to the surface, cells express different proteins depending on their position within the UACL. All parts of the UACL secrete neutral mucin, which is intensely D/PAS positive. However, the pattern of staining varies using monoclonal antibodies that recognize different but overlapping epitopes in the polymorphic core protein in epithelial mucin. Thus, the acinar cells show membrane staining only with HMFG1, whereas the surface cells express membrane and cytoplasmic staining with HMFG2 (Wright *et al.* 1990*a*), possibly reflecting differences in glycosylation patterns of the MUC1 molecule in these parts of the lineage. The positivity with these antibodies indicate that the UACL shows aberrant expression of the *MUC1* gene, which is not usually seen in the intestine. Moreover, the surface cells stain intensely with *Lens culinaris* lectin (which recognizes binding sites for α-mannose, α-galactose and N-acetyl-galactosamine, in that order of preference), while the acinar cell are negative (Wright *et al.* 1990*a*). The surface cells also stain with PR3B10, a monoclonal antibody that recognizes a 150 kDa glycoprotein related to CEA, the cell adhesion molecule; the acinar cells are negative (Wright *et al.* 1990*a*). Thus the UACL cells express different secretory conjugates according to their position within the UACL, while the adhesion proteins expressed by the surface cells are different to the acinar cells.

(b) *Regulatory peptide gene expression in the UACL*

(i) *Epidermal growth factor*

The UACL also expresses different secretory proteins, again dependent upon position. The acinar portion contains abundant immunoreactive epidermal growth factor (EGF), which is also seen in the secretions (Wright *et al.* 1990*a*). It is significant that EGF should be produced locally around ulcers in the intestine, for several reasons. EGF is a very potent stimulator of cell proliferation in the rodent and human gastrointestinal mucosa (Walker-Smith *et al.* 1985; Goodlad *et al.* 1987), and also modulates intestinal epithelial cell differentiation (Goodlad *et al.* 1991). In normal conditions, EGF is produced by gut-associated salivary and Brunner's glands (Heitz *et al.* 1978). There is evidence that EGF receptors in the gut are polarized to the laterobasal membranes (Scheving *et al.* 1989). The secretion of EGF by the UACL ensures that the peptide is available locally to stimulate repair and regeneration in the local ulcer environment. We have suggested that this is an important *in vivo* role for EGF (Wright *et al.* 1990*a*).

(ii) *Trefoil peptides*

Trefoil peptides, or trefoil family factors (TFFs), are small, stable, secretory peptides, bearing one or more trefoil structural motifs (figure 4). They are expressed mainly in the gut, in association with mucin-secreting cells. Three trefoil peptides are known in man: TFF1 (pS2), TFF2 (spasmolytic peptide, SP), and TFF3 (intestinal trefoil factor, ITF). Each is expressed within specific compartments in the gut, in patterns approximately conserved between mammalian species. A number of more complex trefoil-motif-containing peptides are known in amphibians (Hoffmann & Hauser 1993).

TFF1 (*pS2*)

TFF1 is a single-trefoil protein whose secreted length is 60 amino acids: the mature peptide is compact, and extremely resistant to proteases (Rio *et al.* 1988*a*). TFF1 is produced by the normal stomach (Rio *et al.* 1988*b*), a small proportion of normal breast epithelial cells, 60% of breast carcinomas and a number of other tumours (see Poulsom (1997) for review). There is some evidence that TFF1 can exist as a dimer bridging through the seventh cysteine residue either to another molecule (Chadwick *et al.* 1995), or perhaps TFF3 (Poulsom 1997). The *TFF1* locus is on chromosome 21 in the region of q22.3 (Tomasetto *et al.* 1992); the gene has three exons, one principal transcription start site and a poly A addition signal, giving a single mRNA of *ca.* 600 bases in length (Rio & Chambon 1990). Several polymorphisms have been suggested for the gene, but none apparently affect the peptide sequence (Mori *et al.* 1990). In gastric, breast and pancreatic tumours, the gene appears largely intact (see Poulsom (1997) for review).

TFF1 is normally expressed in the mucous cell of the gastric foveolae throughout the stomach and in the duct cells of Brunner's glands in the duodenum. The small bowel appears not to normally express TFF1, although there are reports of some goblet cells being positive in the distal colon (Poulsom 1997). Human gastric juice contains 30–100 $\mu g\,l^{-1}$ of TFF1 (Rio *et al.* 1988*b*), levels similar to those seen in conditioned medium from the TFF1-producing breast cancer cell line MCF-7. Excretion of TFF1 in urine averages 13.6 $ng\,mg^{-1}$ creatinine, whereas levels of 494 $pg\,ml^{-1}$ have been reported in serum (Miyashita *et al.* 1994). One report states that normal serum levels of TFF1 are 186 $pg\,ml^{-1}$ and are elevated during active episodes of inflammatory bowel disease (Duclos *et al.* 1991).

The upstream sequences of the *TFF1* gene contains a number of possible regulatory sequences, some of which do appear functional; for example, TFF1 secretion from MCF-7 cells is stimulated by oestrogens (Takahashi *et al.* 1990). Other possible controls have been reviewed

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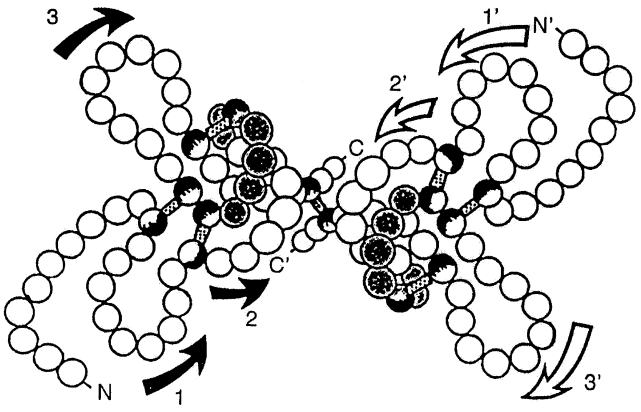

Figure 4. A diagrammatic representation of the three-leafed 'trefoil' domain in a dimer of intestinal trefoil factor. Two molecules of TFF3(ITF) are shown with their N- and C-termini labelled. Cysteine residues are half-shaded. In each monomer of TFF3, seven residues (shaded) are thought to form an α-helix and residues at the base of loop 3 are thought to be in an anti-parallel β-sheet arrangement. (Reproduced from Chadwick *et al.* (1995), with permission.)

(Poulsom & Wright 1993), and those of possible importance in the gut include bile acids (Baker *et al.* 1992), epidermal growth factor and fibroblast growth factor (Cavailles *et al.* 1989), and several cytokines (IL-6, IL-7 and TNFα; Hirota *et al.* 1994), all of which upregulate *TFF1* gene expression, albeit in cell lines in the *in vitro* situation.

Despite early ideas that trefoil factors had mitogenic properties, TFF1 extracted from MCF-7 cell-culture supernatants is not mitogenic for a range of gastric carcinoma cell lines, MCF-7 cells or 3T3 fibroblasts when used at concentrations up to 100 μg ml^{-1} (Kida *et al.* 1989). However it has recently been observed that transfection of mouse mammary adenocarcinoma cells with a human *TFF1* cDNA causes the cells to develop a branched appearance rather than remain in the usual spheroidal format in collagen gels (Williams *et al.* 1996), and thus trefoil molecules may influence morphogenesis generally. *TFF1* knockout mice develop gastric adenomas and some carcinomas, together with small bowel inflammation, indicating either a tumour suppressor role for the gene, or a breakdown in mucosal protection (Lefebre *et al.* 1996).

TFF2 (SP)

Porcine TFF2 is a double-trefoil peptide of 106 amino acid residues. Its compact structure again has marked proteolytic stability (as does hTFF2; Playford *et al.* 1995), and it is the only TFF2 from natural sources that has been purified and sequenced to date (Jørgensen *et al.* 1982). Porcine TFF2 is present in pancreatic preparations used sometimes for cystic fibrosis patients, and is a trace contaminant of porcine insulin (Jørgensen *et al.* 1982). The structure of pTFF2 has been studied in detail (Carr *et al.* 1994; De *et al.* 1994), and regions have been identified on the surface which are candidates for interacting with oligosaccharide chains or with putative receptors. There is a considerable conservation of amino acid residues between TFF2 from different species, and the first trefoil motif is the more strongly related to the single-trefoil motifs present in either TFF1 or TFF3. Threadgill & Womack (1991) predicted that the gene for TFF2 would be on human chromosome 21 when mapping the bovine *TFF2* gene to a syntenic region, and the gene is in fact within 230 kb of the *TFF1* gene and 400 kb of the *TFF3* gene (Chinery *et al.* 1996). There are little or no data concerning the organization or regulatory sequence of *TFF2* genes; the cDNA sequences of rat, mouse and human *TFF2* are sufficiently different to make cross-species *in situ* hybridization difficult.

The principal sites of expression of TFF2 are the stomach and Brunner's glands in the duodenum; TFF2 is also expressed by metaplastic (Hanby *et al.* 1993*a*, 1994) and neoplastic epithelia (see Poulsom (1997) for review). That TFF2 is a major antral peptide (Hanby *et al.* 1993*b*) is supported by the levels of immunoreactive TFF2 in rat gastric antrum (243 pmol g^{-1}), against fundal concentrations of 22 pmol g^{-1} (Taupin *et al.* 1994); the concentration of TFF2 in gastric mucus is between 5 and 10 μM (Jeffrey *et al.* 1994), while in the pig, but not in man, TFF2 is abundantly expressed by the pancreas where the levels can vary from 1 to 180 μg ml^{-1} (Thim *et al.* 1982). However, little is known about the control of TFF2 expression: as a principally exocrine product, secretion is controlled by cholinergic, peptidergic and endocrine mechanisms (Rasmussen *et al.* 1990*a*,*b*, 1993). Whatever the mechanisms controlling expression, response is rapid, with an increase in the relative abundance of rTFF3 mRNA in the rat stomach within 30 min of creating a cryoprobe ulcer (Alison *et al.* 1995).

Recombinant TFF2 at micromolar concentrations is a motogen (Playford *et al.* 1995) and promotes invasion of collagen gels by some cell lines *in vitro* (Lalani *et al.* 1995); *in vivo*, TFF2 protects against indomethacin-induced gastric damage when given subcutaneously or orally (Hanby *et al.* 1993*b*; Babyatsky *et al.* 1994; Playford *et al.* 1995). Recombinant TFF2 modifies the patterns of growth of carcinoma cells in suspension culture, inducing clusters displaying multiple spikes rather than spheroids (Lalani *et al.* 1995). Receptors for TFF2 have been described in epithelial cell membranes that activate adenylate cyclase (Frandsen *et al.* 1986), while recent observations indicate that hTFF2 competes with rTFF3 for binding to putative receptors in the rat colon (Chinery & Cox 1995*a*).

TFF3 (ITF)

TFF3 was first described as a rat cDNA sequence (Suemori *et al.* 1991). Clones for rTFF3 were then identified using oligonucletides based on the sequence of a peptide sequence of a fragment of a *ca.* 55 kDa protein called transformed-growth inhibitory factor (TGIF) (Podolsky *et al.* 1988), although there is no further evidence that rat TFF3 or human TFF3 is related to TGIF (Hauser *et al.* 1993). Sequence analysis indicates that TFF3 from human, mouse and rat are single-trefoil-domain peptides, of predicted size 6558 Da (Hauser *et al.* 1993), and, although there has been some uncertainty over the correct sequence of TFF3, this is now resolved (Poulsom 1997). Whereas monomeric TFF3 has normally been found on extraction of tissues, dimerization of TFF3 appears to occur naturally (Chinery *et al.* 1995) and during its expression in bacteria (Chinery *et al.* 1995) and yeast (Thim *et al.* 1995). Rat TFF3 shows more tendency to dimerize; the seventh

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cysteine (C57) is essential for dimerization, which becomes blocked if the residue is mutated to a lysine residue (Chinery *et al.* 1995) or blocked by a free cysteine (Thim *et al.* 1995). There is some evidence that the biological actions of TFF3 and TFF1 become lost or reduced if dimerization is prevented (Chinery *et al.* 1995; Marchbank *et al.* 1997), and molecular modelling studies suggest that TFF1 and TFF3 could form heterodimers. TFF2 is very resistant to proteolytic digestion (Mashimo *et al.* 1995). *TFF3* contains three exons (Sands *et al.* 1995; Mashimo *et al.* 1995) with single sites for the initiation of transcription, and only one mRNA appears to be present in mouse, rat and human. Although only 153 bases of the upstream sequence supported goblet cell-specific expression, there are no strong homologies in the 5′ upstream sequences, and the oestrogen and EGF response elements seen in *TFF1* appear not to be present. The mouse *TFF3* gene is present on chromosome 17 and *hTFF3* has been localized to chromosome 21, clustered with the *TFF2* and *TFF1* genes (Chinery *et al.* 1996).

Studies using immunohistochemistry have localized TFF3 to goblet cells, enterocytes and colonocytes in the small and large intestine (Suemori *et al.* 1991); however, *in situ* hybridization studies show expression only in the goblet cells (Hauser *et al.* 1993), raising the possibility that TFF3 is absorbed by the columnar cells. TFF3 is not intestine specific (Podolsky *et al.* 1993), being seen in other tissues, notably normal rat stomach (Chinery *et al.* 1992) and the human uterus (Hauser *et al.* 1993), and occurrence in the rat stomach after experimental ulceration has been noted (Alison *et al.* 1995). A recent report has shown that TFF3 is expressed in breast carcinoma (Poulsom *et al.* 1997). Immunoassay has shown that concentrations of TFF3 approaching 29 pmol g^{-1} are found in rat stomach, with 10 μmol g^{-1} and 1.77 μmol g^{-1} seen in the small intestine and colon, respectively (Taupin & Giraud 1994).

Both rat and human TFF3 act as motogens *in vitro*, advancing epithelial cell migration, which is relevant to the rapid restitution that occurs after wounding of the gastrointestinal mucosa (Lacy *et al.* 1993). *In vitro* assays use monolayers of epithelial cells grown to confluence, where artificial wounds are made with a sharp implement: rTFF3 and hTFF3 at 150 nM are active is stimulating migration of IEC6 cells (Dignass *et al.* 1994). However, 10 nM of rTFF3 seems to be as effective as 10 nM of EGF in closing wounds induced in HT29 cells (Chinery & Playford 1995), indicating that TFF3 acts across species. There has been some dispute about the possible proliferative action of TFF3, with the majority opinion indicating that there is no such action (Poulsom 1997).

In addition, TFF3 appears to be active in affecting electrogenic chloride transport; when segments of stripped gastrointestinal mucosa or monolayers of colonic epithelial cells are studied under voltage-clamp conditions, such measurements are possible (Cox *et al.* 1993; Chinery & Cox 1995*b*). Rat jejunum responds to TFF3 with an increase in short-circuit current blocked by piretanide, indicating chloride transport; this action was seen at 100 nM, and was most prominent after application to the serosal aspect, indicating possibly a basolateral distribution of receptors. Synergy between EGF and TFF3 was seen in their action on HT29 cells (Chinery & Cox 1995*b*). Subcutaneous infusion of TFF3 at 250 μg kg^{-1} prevents the development of the gastric damage produced in the rat by restraint and indomethacin injection (Chinery *et al.* 1993); again, a synergy between TFF3 and EGF was seen in this prevention (Chinery & Playford 1995). TFF3 knockout mice are more sensitive to induced colitis than control mice (Matsumo *et al.* 1996).

These actions on migration indicate a possible receptor-mediated action. In this respect, TFF3 induces phosphorylation of β-catenin within 10 s of application to HT29 cells, and there is also evidence that TFF family members upregulate APC expression and downregulate E-cadherin expression, all actions that induce the adoption of the migratory phenotype in epithelial cells (Eftsthanthiou *et al.* 1997). Recent studies (W. Hofmann, personal communication) have shown that TFF3 phosphorylates *erk*1 and *erk*2, indicating activation of MAP kinase: within 8 min there is phosporylation of myosin light-chain kinase, leading to phosporylation of myosin light chain; thus, signal transduction occurs rapidly after TFF3 application. In this respect, recent studies have detected a possible binding protein for TFF3, a *ca.* 28 kDa fragment that became phosphorylated on tyrosine in response to TFF3 (Chinery & Cox 1995*a*). Also, use of iodinated TFF3 showed binding sites in the neck region of gastric glands and jejunal and colonic crypts, similar in location to binding sites demonstrated with a TFF3:β-galactosidase fusion protein (Chinery *et al.* 1993).

(c) *TFF expression in the UACL*

TFF2 and TFF1 mRNA expression can be readily demonstrated by hybridization *in situ* using ^{35}S-labelled riboprobes. TFF2 mRNA is seen in the upper acini and lower duct cells, whereas TFF1 mRNA and protein are seen in large amounts in the upper duct and all surface cells (Wright *et al.* 1990*c*); TFF3 mRNA is found in abundance throughout the UACL (Hauser *et al.* 1993). A further protein of significance is produced by the UACL: lysozyme, which has considerable antibacterial action, is secreted, and both lysozyme mRNA and protein are found in abundance in the UACL (Stamp *et al.* 1992). 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 (Wright *et al.* 1990*b*). 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 TFF1 in the basal parts of the cytoplasm in formalin-fixed, paraffin-embedded sections. In glutaraldehyde-fixed, resin-embedded sections, in addition to labelling in the Golgi area, TFF1 is seen within the theca. *In situ* hybridization with an ^{35}S-labelled antisense riboprobe shows TFF1 mRNA localized in considerable concentration in the cytoplasm beneath the mucous-filled theca. Electron microscopical immunocytochemistry confirms that immunoreactive TFF1 is found in the rough endoplasmic reticulum of these cells, and is also co-packaged via the Golgi 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 mucous into the intestinal lumen.

In addition, neuroendocrine cells adjacent to the UACL also express TFF1 protein. The finding of the same peptide

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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. Mucous has lubricating and protective functions in the gut, whereas endocrine secretion contains large numbers of regulatory peptides that act via paracrine or autocrine mechanisms to produce manifold effects on the gut. Thus, TFF1 may be involved in the secretory mechanism itself, or may be presented to receptors on both apical and basolateral membranes. The EGF-response sequence in the 5′ upstream sequences of the TFF1, described above, might be reacting to the EGF secreted locally by the UACL, inducing TFF1 expression in the adjacent mucosa. However, why the enterocytes do not express TFF1 when they do bear EGF receptors is not explained by this hypothesis.

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 is present in abundance, whereas TFF1 peptide is confined to the developing ducts. However, TFF2 mRNA is present in considerable concentration throughout Brunner's gland acini and ducts. By 36 weeks, the adult pattern of trefoil gene expression has emerged: TFF1 and TFF2 are co-expressed by the ductal cells, and neither peptide nor transcripts are seen in the acini (Ahnen *et al.* 1994). These observations indicate that the UACL reiterates the developmental program of Brunner's glands, although it then acquires the proliferative organization of gastric gland tubules (see above).

6. THE NATURE OF THE UACL

The UACL shows several features that are novel for differentiating gastrointestinal cell lineages: (i) it appears as direct differentiation progeny of crypt stem cells; (ii) it appears in response to injury, growing to form new glandular formations that make their own communication with the surface of the mucosa; (iii) in histogenesis it appears to follow the differentiation program of Brunner's glands primordia; (iv) it develops a proliferative organization very similar to that of the gastric gland; (v) it shows regional differences in differentiation; (vi) it shows a unique pattern of regulatory peptide gene expression, producing EGF and the two trefoil peptides TFF1 and TFF2, again produced in specific parts of the UACL; and (vii) the presence of the UACL modifies the behaviour of the indigenous cell lineages in the vicinity: goblet cells actively synthesize and secrete TFF1, while neuroendocrine cell granules also contain co-packaged TFF2.

These observations indicate that the UACL is unlikely to be a metaplasia, which can formally be defined as a change from one defined differentiated phenotype to another. The UACL, although sharing several phenotypic features with other cell lineages in the gut, does have a unique phenotype, and we are therefore justified in considering it a new pathway of gastrointestinal differentiation. Its function seems to be clear. Because it is induced only in chronic inflammatory and ulcerative conditions, it would certainly appear to be a primary defence reaction, producing a cocktail of active peptides and proteins, EGF, TFF2, TFF1, TFF3, PSTI and lysozyme, which would be expected to advance mucosal healing. There are also early indications that other peptides may be produced by the UACL, e.g. immunoreactive TGFa has been detected (Ahnen *et al.* 1991).

7. CONCLUSION

The morphological organization and function of the UACL is now being worked out, and its phenotype fully defined. However, the above observations raise as many questions as they answer. What is the stimulus for the induction of the UACL? Possibly mesenchymal induction, as profound myofibroblast proliferation—cells usually confined to the pericryptal myofibroblast sheath—is seen around the early UACL buds. Stem cell differentiation certainly seems to be switched on directly, and wholly into the UACL pathway (figure 2*a*). How does growth of the bud continue in the absence of cell division? If cells are being directed downwards from the crypt stem cell compartment, then how does the tubule divide, in the absence of cell division, if not by mesenchymal direction? After the morphogenesis of the new gland and duct, the new proliferative compartment develops. What, then, are the migration pathways of the cells emerging from the new compartment, and what is the relationship between the new proliferative compartment and the parent crypts?

There also seems to be a very rigid positional organization of peptide gene expression, with cells changing expression as they migrate through the lineage. The abundance of message and protein for the trefoil peptides TFF2 and TFF1, and the induction of TFF1 in surrounding cells, possibly by the EGF secreted by the UACL, indicates an important role for these molecules, possibly in mucosal defence. The discovery of their function in humans then becomes important in respect of novel therapeutic strategies.

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