Enteroendocrine cells: Neglected players in gastrointestinal disorders?

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Therapeutic Advances in
Gastroenterology
[2008] 1(1) 51–60

DOI: 10.1177/
1756283X08093943

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Los Angeles, London,
New Delhi and Singapore

Abstract: Enteroendocrine cells (EEC) form the basis of the largest endocrine system in the body. They secrete multiple regulatory molecules which control physiological and homeostatic functions, particularly postprandial secretion and motility. Their key purpose is to act as sensors of luminal contents, either in a classical endocrine fashion, or by a paracrine effect on proximate cells, notably vagal afferent fibres. They also play a pivotal role in the control of food intake, and emerging data add roles in mucosal immunity and repair. We propose that EEC are fundamental in several gastrointestinal pathologies, notably Post-infectious Irritable Bowel Syndrome, infectious enteritis, and possibly inflammatory bowel disease. Further work is needed to fully illustrate the importance, detailed biology and therapeutic potential of these frequently overlooked cells.

Keywords: Enteroendocrine cells, innate immunity, inflammation, inflammatory bowel disease, irritable bowel syndrome, anendocrinosis

Introduction

The largest endocrine system, in terms of total number of cells, is the enteroendocrine system of the digestive tract. In contrast to other endocrine systems, enteroendocrine cells (EEC) are scattered as single cells throughout the intestinal tract, located within the intestinal crypts and villi, and comprising ~1% of the epithelial cell population [Sternini *et al.* 2008]. There are at least 15 subtypes of EEC, secreting multiple peptide hormones which elaborately control physiological and homeostatic functions in the digestive tract, particularly postprandial secretion and motility. A set of biogenic amines are also synthesized and secreted, particularly 5-HT from the extremely diffuse enterochromaffin cell (EC) type. As with extra-gastrointestinal endocrine systems, co-expression, and presumably co-secretion, of more than one mediator is widely observed. Recent research has centered upon the potential role of EEC in immune regulation, a novel theme which will be discussed in detail below.

It has become clear that the key function of EEC is to act as sensors of luminal contents, particularly nutrients. Those EEC 'open' to the lumen thereby function as transepithelial signal transduction conduits, with apical physiochemical signals resulting in basolateral exocytosis of biological mediators. These act either in a classical

endocrine fashion or by a paracrine effect on proximate cells, notably vagal afferent fibers. Their diffuse distribution is thus functionally appropriate, the EEC network forming an intimate first-line component of the brain-gut axis. For example, EEC are central to the gastrointestinal (GI) regulation of food intake via nutrient sensing, and have recently been shown to express 'taste' G-protein coupled receptors (the T1 sweet-sensing and T2 bitter-sensing families) and the associated small G-protein α -gustducin [Sternini *et al.* 2008; Cummings and Overduin, 2007]. Non-nutrient chemical factors also regulate EEC activity, for example secretin is secreted in response to duodenal acidification, and 5-HT secreting EC have mechanosensory properties [Lal *et al.* 2004; Bozkurt *et al.* 1999; Grundy *et al.* 1995; Dockray *et al.* 1985].

EEC biology

Unlike other endocrine systems, there is rapid cell turnover in the EEC population. Notch signaling plays a central role in development and proliferation in several systems, including the nervous system, pancreas and EEC, in a manner involving lateral inhibition. This cell-cell interaction allows a differentiating cell to inhibit neighboring cells from doing the same and is mediated by the transmembrane receptor Notch, and by Delta,

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its transmembrane ligand. The transcription factor Neurogenin-3 (ngn3) is at the apex of this pathway. It can stimulate Neuro D (both ngn-3 and Neuro D are part of the bHLH family transcription factors, also involved in pancreatic endocrine cell differentiation) which stimulates cellular expression and differentiation, and it can stimulate Delta to activate Notch. This downregulates cellular expression of Hes-1 stimulation, the key transcription factor favoring the absorptive enterocyte pathway [Bjerknes and Cheng, 2006; Skipper *et al.* 2000].

Experiments utilizing ngn3 null mice have however provided evidence for the existence of both ngn3 $-/-$ dependent and independent EEC lineages [Lee *et al.* 2002]. Two models have been proposed. The first suggests that ngn3 is initially needed for the proliferation of all EEC, and subsequently required for terminal differentiation of a restricted subset e.g., A, D, and G cells, but not serotonergic EC cells. Thus ngn3 deficiency provides a smaller pool of precursors from which EC can differentiate. The other model suggests that ngn3 is required for the terminal differentiation of A, D, and G cells.

Loss of Neuro D results in diminished α and β cells in the pancreas, as well as decreased secretin and CCK expression in the gut. Ngn3 deletion results in total absence of the four pancreatic endocrine cell types. Very recent data have also identified a role for the transcription factor

NKx2.2 in EEC cell type determination [Desai *et al.* 2008]. In contrast, Hes-1 ablation leads to excessive differentiation of EEC in the gut. More broadly, bHLH transcription factors play a vital role in the determination of various cell fates. For example, loss of Math-1 leads to deletion of all three secretory cell lineages in the intestine, i.e. Goblet, Paneth and EEC [Yang *et al.* 2001] (Figure 1).

Over expression of the pancreatic-duodenal homeobox 1 gene (pdx-1) has been shown *in vitro* to induce the differentiation of intestinal epithelial cells into hormone producing EEC, although this gene plays a minor role when compared to Notch signaling [Yamada *et al.* 2001].

EEC are terminally differentiated cells, which together with Goblet cells and Paneth cells constitute the secretory cell types in the small intestine lineages, whilst ~90% of the healthy epithelium is lined by absorptive columnar enterocytes. It is now clear that all lineages share a common crypt stem cell origin. Notch signaling and lateral inhibition lead to a Hes-1 directed population following the absorptive pathway, whilst the secretory lineages are directed by Math-1, with EEC progenitor differentiation further regulated by ngn3 [Bjerknes and Cheng, 2006]. EEC also turnover very rapidly, with a lifespan in the order of 4–6 days, in distinction to nondiffuse endocrine organs, perhaps suggesting more plasticity and acute

Figure 1. Intestinal stem cell pathways for terminal differentiation into absorptive enterocytes and secretory (EEC, Goblet and Paneth) cell lineages.

reactivity applies in this archeobiologically more ancient system.

In view of the size, diversity and functional complexity of the enteroendocrine system it is therefore surprising how little is known about their roles in GI diseases. Given the multiple hypo- and hypersecretory states that are well characterized in nonGI endocrine systems, it seems barely conceivable that similar disorders will not exist in the GI tract. However, with the exception of rare endocrine tumors, which will not be discussed, there are very few reports of primary functional abnormalities of these cells. These extreme cases will be discussed below, but it is timely to speculate what the clinical phenotype of more subtle enteroendocrinopathies would be. Functional gastrointestinal symptoms, appetite disorders or altered bowel habit? How would these be recognized and diagnosed? It is probable that gut hormone profiles in peripheral blood are far too insensitive to detect moderate but important secretory dysfunction in the paracrine environment or the splanchnic endocrine compartment. Recent research has however begun to look at their potential roles in well known GI disorders, particularly irritable bowel syndrome (IBS), GI infection and inflammatory bowel disease (IBD). EEC are also attractive targets in the regulation and dysregulation of GI physiology in disease, and in the control of energy homeostasis.

The evidence for, and potential applications of, this evolving story will be reviewed. Since a key physiological role of gut hormones likely to be pertinent to GI disease is in the control of food intake, this will be addressed initially. This will be followed by a review of pathophysiological situations in which EEC may be relevant. This is a field in its infancy, likely to generate novel pathobiological understandings in gastroenterology, leading to tractable therapeutic approaches.

EEC roles in food intake and energy homeostasis

Satiation, the process by which a meal is terminated, has two main GI components: mechanical gastric distension, mediated via vagal afferent stimulation and EEC peptide hormone secretion. The hindbrain is the principal inputting site for short-acting satiation signals which are transmitted vagally, with signals relayed to the nucleus of the solitary tract, and hormonally, the *area postrema* being sited outside the

blood–brain barrier. Long-term weight stability is achieved with adiposity hormones such as leptin, adiponectin and the downstream targets of insulin [Cummings *et al.* 2007].

The regulatory peptide cholecystokinin (CCK) plays a role as the primary proximal intestinal signal reducing food intake. It is secreted by the I cells of the duodenum and jejunum in response to luminal nutrients especially lipids and proteins [Furness *et al.* 1999]. CCK causes a delay in gastric emptying, therefore potentiating mechanical satiety signals [Lal *et al.* 2004]. When CCK is injected peripherally before meals it decreases meal size through CCK₁ receptor stimulation [Gibbs *et al.* 1973]. Rats lacking CCK_{1R} show increased meal size and gradually become obese, a phenotype possibly driven by concomitant neuropeptide Y over-expression [Moran *et al.* 2006, 2008]. The obesity is mild and inconsistent, possibly reflecting the role of CCK in short term satiety signaling. CCK has also been shown to play a role in human satiety induced by fat [Lal *et al.* 2004; Matzinger *et al.* 1999].

Two important EEC products of the preproglucagon gene are glucagon-like peptide-1 (GLP-1) and oxyntomodulin. These gut hormones play a role in distal intestinal satiety signaling, and are secreted by L cells in the distal ileum and colon secondary to ingested fats and carbohydrates. Injected peripherally, they elicit satiety in normal weight and obese subjects through stimulation of GLP1 receptor. GLP1 enhances insulin secretion as well, this incretin effect contributing further to energy homeostasis [Drucker *et al.* 2006]. An anorectic effect of peptide YY (PYY) has been shown in animal and human studies [Le Roux *et al.* 2008; Pironi *et al.* 1993; Baynes, *et al.* 2006]. Interestingly, PYY secretion is reported to be attenuated in obesity lean persons [Cummings *et al.* 2007]. Although, intravenous infusions of biologically active PYY3-36 reduce food intake in humans, supraphysiological levels induced nausea [Le roux *et al.* 2008]. The same has been reported for CCK [Chua *et al.* 1994], and release of 5-HT by chemotherapy drugs underpins the severe nausea and emesis they elicit [Cubeddu *et al.* 1990]. These observations therefore add credence to the possibility that unrecognized EEC dysfunction may be present in unexplained nausea or appetite disturbance.

Ghrelin, to date the sole GI orexigenic peptide, secreted by gastric endocrine cells, conversely

increases food intake in humans and animals. It has a role in both short-and long-term food control, and genomic deletion of the murine peptide or its receptor protects against weight gain on a high fat diet [Wortley *et al.* 2005; Zigman *et al.* 2005; Baynes, *et al.* 2006].

Further evidence that EEC and their products are biologically central to food and weight regulation in humans comes from bariatric surgery studies. Human subjects with severe obesity are increasingly treated thus to promote weight loss, simplistically considered to reflect either decreased gastric volume or small intestinal surface area. Increased plasma levels of PYY and GLP-1 in Roux-en-Y gastric bypass patients and in jejunointestinal bypass rats have been noted [Le Roux *et al.* 2006]. Altered gut anatomy, innervation or motility certainly plays a role in the aetiology of these hormonal changes. An increased exposure of the distal gut to partially undigested food is associated with decreased release of proximally secreted hormones and increased secretion of distally secreted anorectic hormones such as PYY, oxyntomodulin and GLP-1 [LeRoux *et al.* 2006; Sarson *et al.* 1981]. An increase in GLP-1 has been attributed to the sudden amelioration of diabetes control in postoperative patients [LeRoux *et al.* 2006], therefore preceding weight loss and peripheral resensitization to insulin. Hypoglycaemic episodes in patients post Roux-en-Y gastric bypass surgery has also been attributed to GLP-1 hypersecretion, stimulating beta cells [Patti *et al.* 2005].

These physiological and allied observations therefore place EEC and their targets, especially the vagus, centrally in the frame for emergent research into the mechanisms of abnormal appetite and satiety observed in GI disorders.

EEC roles in innate immunity and repair

Host defence against invading microbial organisms is maintained by an intact epithelial barrier and by the immune system. Immunity has innate and acquired components, recognizing micro-organisms as nonself and triggering an immune response. Cells of the innate immune system principally sense microbial presence via activation of Toll-like receptors (TLR). TLR are differentially distributed in multiple cell types, but are chiefly expressed by dendritic cells, macrophages, and myofibroblasts [Otte *et al.* 2004].

TLRs recognize a broad range of pathogen-derived components, signaling to induce the expression of pro-inflammatory genes and cytokines as a co-ordinated immune response. This, in conjunction with phagocytosis-mediated antigen presentation, instructs the development of antigen-specific adaptive immunity, especially via Th1 cells [Takeda and Akira 2005]. Recent exciting data have demonstrated that TLRs are also found on EEC. This assigns a novel role to EEC as innate immunity sensors, in addition to their canonical role as nutrient sensors. Whether this is a constitutive role or active only in certain circumstances is not yet known. In an elegant immunohistochemistry study by Bogunovic *et al.* scattered intestinal epithelial cells (IEC) were shown to co-express TLR1, TLR2, TLR4, co-localizing with multicytokeratin (a marker for IEC) in cells immunoreactive to TLR2 antibodies; and serotonin (a marker for EEC) in IEC immunoreactive to TLR1, TLR2, TLR4 antibodies. A murine EEC line, STC-1, was also shown to express mRNA transcripts for TLR 1, TLR 2, TLR 4, TLR 6 and the co-receptor MD-2. In the same cell line, the TLR4 ligand lipopolysaccharide (LPS) induced the phosphorylation of ERK1/2 and MAPK and activated NF- κ B activation, the pivotal downstream signaling target of TLR occupancy. STC-1 exposure to LPS also triggered a calcium flux and induced the expression of the proinflammatory cytokine TNF α and the anti-inflammatory cytokine TGF- β , in conjunction with increased CCK secretion [Bogunovic *et al.* 2007]. Similar results have been shown by Palazzo *et al.* with TLR 5 and 9 also evident [Palazzo *et al.* 2007].

CCK may also have immunomodulatory properties, increasing the translocation of secretory IgA into the lumen and lymph [Freier *et al.* 1991; Imaeda *et al.* 1993; Freier *et al.* 1989]. It has also been reported that corticosteroids reduce CCK activity, which may contribute to increased bacterial adherence and impaired IgA secretion [Alverdy *et al.* 1991, 1997]. This may also be important in the reduced mucosal immunity observed in TPN-fed mice [Keith Hannah *et al.* 2000].

A further key development was the recent description of a 'hard-wired' CCK-vagal-immune reflex circuit. Nutrient-stimulated CCK secretion (a high fat diet) was shown to ameliorate the inflammatory response and to abrogate the increased epithelial permeability triggered by

haemorrhagic shock [Luyer *et al.* 2005; Tracey *et al.* 2005]. Taken together with the above new data demonstrating that EEC can operate as innate immune sensors, it seems likely that inherent or acquired abnormalities in EEC function (or simple nutrient-privation), would predispose to poorer defensive responses to an infective or inflammatory challenge. In the gut, such challenges are of course the permanent norm. Whether similar biology exists for EEC types other than CCK now needs to be urgently explored.

The epithelial barrier is also a key component in host defence. A further preproglucagon splice product, GLP-2, is secreted by enteroendocrine L-cells in the distal small intestine and has been shown to improve intestinal wound healing in a TGF- β mediated process, small bowel responding better than large bowel. Proliferation and migration of epithelial cells in 'wounded' IEC-6 confluent monolayers was enhanced when TGF- β was added in cells lines incubated in GLP-2 [Bulut *et al.* 2004]. GLP-2 has also been shown to ameliorate the barrier dysfunction induced by experimental stress and food allergy [Cameron *et al.* 2005; Cameron *et al.* 2003]. Again, L-cells are activated by luminal nutrients, and the barrier compromise observed in TPN may partly reflect its hyposecretion in the absence of enteral stimuli. Moreover, GLP-2 is also responsible, at least in part for growth and adaptation observed in short-bowel models [Liu *et al.* 2006; Martin *et al.* 2006; Lovshin *et al.* 2000].

Therefore, these data suggest that abnormal EEC function might predispose to GI inflammatory disorders, and advocate that the underlying nutrient-EEC-vagal pathways should be explored as therapeutic targets in the injured gut.

EEC in intestinal infection and inflammation

Intestinal inflammation is associated with increased intestinal secretions and motility, and a parallel decrease in food intake with consequent nutritional imbalances and weight loss [Rigaud *et al.* 1994; McHugh *et al.* 1993; Gee *et al.* 1985]. Early evidence that EEC function was abnormal in GI infection came from veterinary studies, food intake in lambs infected with the parasite *Trichostrongylus colubriformis* was increased by the administration of a specific CCK antagonist loxiglumide [Dynes *et al.* 1998]. Increased plasma CCK has also been

observed in pigs infected with *Ascaris suum* [Yang *et al.* 1990], and in humans infected with *Giardia lamblia* [Leslie *et al.* 2003].

Recent work has sought to study this mechanistically using experimental models of parasitization. Enteritis can be induced in rodents by the nematode *Trichinella spiralis* and colitis by *Trichuris muris*. These offer a more biologically meaningful approach than the more established chemical methods, as in 2,4,6 trinitrobenzene sulfonic acid (TNBS) and in dextran sodium sulphate (DSS) [Oshima *et al.* 1999]. *T.spiralis* was shown to induce CCK and 5-HT hyperplasia and hypersecretion in mice, coinciding with the peak severity of enteritis, at about day nine post-infection. Food intake was reduced by about half at this time point, and was significantly improved by the administration of the same CCK1 receptor antagonist, loxiglumide. It was also shown that immunocompetence was essential, and the CD4 T-lymphocyte population was identified as the key effector cell, inducing secretory cell hyperplasia via an IL-4 receptor alpha subunit-dependent mechanism. The parasite was not the primary driver of the EEC response, since in animals undergoing CD4 immunoneutralization, the nematodes remain in the lumen, yet EEC hyperplasia fails to occur. This model of the immuno-enteroendocrine axis is therefore likely to have broader applicability to other forms of GI infection and inflammation, and offers an excellent platform to explore the biological role of EEC in innate immunity, in crosstalk with other immune cell types, and in the mechanisms linking gut inflammation to abnormal feeding behavior [McDermott *et al.* 2006]. Subsequently, it has been demonstrated that *T.muris*-induced colitis drives colonic EC proliferation by very similar mechanisms. However, this was advanced by the discovery that adoptive transfer of CD4+ cells from immunocompetent infected mice to immunodeficient (SCID) mice induced EC hyperplasia in the recipient [Wang *et al.* 2007]. This was also more marked in Th2-predominant responders and dependent upon STAT-6. Therefore, might ongoing immune responses perpetuate EEC hyperplasia if unregulated or in susceptible genotypes?

TNBS-induced ileitis in mice also causes an increase in 5HT-immunoreactive EEC, a decrease in serotonin reuptake transporter (SERT) and an increase in 5-HT release [O'Hara *et al.* 2004]. Neurotensin (N) cells and

Somatostatin (D) cells were also changed, but the mucosal content of other gut peptides was not significantly altered. In human colitis, data are surprisingly sparse. There has been a single report that chromogranin A and 5-HT cells positive are increased in both Crohn's disease and ulcerative colitis [El Salhy *et al.* 1997]. In Crohn's colitis only, PYY and PP cells were reduced whilst enteroglucagon cells were increased. However, our recent studies (unpublished data) suggest that all endocrine cell types are reduced in active proctitis, and that this persists when the inflammation resolves. Increased 5-HT EC cells have also been demonstrated in a single study of Crohn's ileitis [Bishop *et al.* 1987]. A newly ascribed Crohn's-associated gene, *Phox2b*, is a homeodomain transcription factor predominantly expressed in differentiated neurones. Work using Genome Wide Analysis (GWA) has shown a close association between this single nuclear polymorphism and Crohn's disease [Rioux *et al.* 2007]. It is potentially mechanistically relevant here: immunostaining suggests it is expressed by epithelial neuroendocrine cells, and this is known to be linked to Delta-Notch signaling. The role of T cells in EEC proliferation is further supported by studies in T-cell receptor α $-/-$ mice, which express reduced cytokines (IL-2, IL-10, TGF- β) and display decreased CCK, 5-HT, and NT immunostaining cell numbers whilst developing an ulcerative colitis-like phenotype [Rubin *et al.* 2000].

Taken together, these results suggest that the complex regulation of EEC development and differentiation operates under immune control, so may be dynamically altered by the state of inflammation. Given that the likely switch takes place along the lineage commitment pathway from the stem cell precursor niche, the time lag of 3–7 days is appropriate. The increased EEC population may have biological consequences, most simply in mediating altered gut motility, sensation and secretory activity noted in inflammatory and infective bowel disease. It may also be advantageous to inhibit food intake in the short term. More specifically, the recent assignment of EEC as part of the innate immune system suggests that up-regulating these cells may be a programmed and adaptive response, enhancing mucosal immunity and barrier function whilst contributing to repair mechanisms. Therefore, careful consideration and dissection of these roles is necessary before advocating these as therapeutic targets. Finally, recent data

have suggested that EEC may also be an autoimmune target in Crohn's disease. An ileal cDNA library approach was used to identify ubiquitination factor 4A, a U-box-type ubiquitin-protein ligase, as a target for antibodies in serum from Crohn's patients. Subsequent immunolocalization suggested that the target protein was upregulated in EEC of Crohn's patients, and these patients particularly displayed an increased predisposition for active, penetrative and structuring disease, and increased needs for surgery [Sakiyama *et al.* 2007].

EEC and post-infectious irritable bowel syndrome (PI-IBS)

The last section dealt with abnormal EEC in overtly diseased gut. However, most clinical practitioners find that a larger proportion of their practice constitutes patients with IBS. From 6–17% of hospital referrals to gastroenterologists in the US and the UK, respectively, are IBS-related [Longstreth *et al.* 2000]. A small, but significant subgroup of patients report a sudden onset of IBS post gastroenteritis. It has been noted that infections caused by *Campylobacter* sp., and to a lesser extent *Shigella*, are associated with a high chance of developing IBS-like symptoms. In addition to the infective agent and the ambient psychosocial state of the index case, there are some data to suggest that the host response to the infective agent plays a major role in developing IBS. Polymorphisms associated with an overproduction of anti-inflammatory cytokines such as interleukin-10 (IL-10) and TGF- β were underrepresented in a cohort of IBS patients [Chan *et al.* 2000]. Although, conventional biopsies are all by definition normal in IBS, more rigorous analysis suggests that subtle inflammatory abnormalities and altered inflammatory cell populations exist [Chadwick *et al.* 2002; Spiller *et al.* 2000].

The seminal supportive data published by Spiller show that a low grade, long-lasting T-cell mediated inflammation is present in patients with post-infectious IBS. Mucosal biopsies taken early post-*Campylobacter* infection show acute superficial ulcerations. This rapidly regresses however on serial rectal biopsies. In most cases, the mucosa looked normal at 2 weeks; however quantitative histology showed a persistently high T cell lymphocyte count (CD3 subtype) and newly recruited calprotectin-positive macrophages. This gradually decreased by three months. However, in a cohort of patients with

typical symptoms of PI-IBS these microscopic changes were still persistent at 1 year. A similar but more striking response was seen by immunohistochemical staining using the universal EEC marker, synaptophysin. Synaptophysin-positive cell expression was 5-fold above normal in 85% at two weeks. Similarly, persistent high results were present at 1 year. At three months, T lymphocyte and EEC counts both remained significantly elevated. There was also a change in chemical coding of the CgA positive cells, reversing from predominantly PYY to serotonin. In keeping with ongoing low grade inflammation, there is also increased gut permeability as measured by an increased lactulose/mannitol flux [Spiller *et al.* 2000; Spiller *et al.* 2003, Dunlop *et al.* 2003].

Further supportive evidence of serotonergic dysfunction in IBS lies in the detailed observations that plasma and platelet 5-HT concentrations are significantly elevated [Houghton *et al.* 2003]. Therefore, it is likely that the chronic inflammatory state is driving this form of EEC hyperplasia or hyperfunctionality, perhaps by similar (but more subtle) mechanisms to the more overt inflammatory processes described in the previous section. What perpetuates the inflammation in a minority of individuals experiencing infection is unclear. Clinically, the functional increase in EEC might be directly driving symptoms, perhaps contributing to the looser and more frequent motions that persist, or encoding increased rectal mechanosensitivity. The increased expression of EEC with altered 5-HT or SERT expression [Wheatcroft *et al.* 2005] might also partly explain the therapeutic benefit of serotonin receptor modulating drugs in a subset of IBS patients.

Loss of function in EEC physiology

Endocrine cell dysgenesis has recently been reported, in three infants presenting with intestinal failure of unknown cause. The patients presented with a life-threatening malabsorptive congenital diarrhea syndrome effecting all nutrients from birth apart from water. Both small and large bowel are affected, with a histological picture characterized by a near complete absence of endocrine cells in the mucosa (anendocrinosis). This defect was discovered to be secondary to point mutations in *ngn3* resulting in arrest in endocrine cell development [Cortina *et al.* 2007]. Might more subtle functional variants

cause relative changes in EEC activity, basally or under inflammatory perturbation?

There have also been limited case reports of abnormal EEC in autoimmune enteropathy (AIE), autoimmune polyglandular syndrome type 1 and IPEX syndrome (Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome) [Al Khalidi *et al.* 2006; Hogenauer *et al.* 2001]. Most striking was a case report of a patient with profound malabsorption and autoimmune polyglandular syndrome type 1, in whom CCK in plasma and intestinal biopsies was undetectable concurrently with episodes of hypocalcaemia [Hogenauer *et al.* 2001]. This raises the intriguing possibility that autoimmune disorders of the EEC might more broadly exist: they clearly do for all other endocrine systems and cells outside the gut, and indeed are relatively common, so this possibility is almost a certainty. But a possibility that has never been rigorously sought. It is also interesting to speculate that ion channelopathies, a growing area of interest in other neuroendocrine systems, may also pertain to the EEC system.

Finally, it is broadly overlooked that patients with untreated celiac disease, presenting with intra-epithelial lymphocytosis and villus atrophy, also show decreased CCK mRNA, CCK mucosal content and blunted postprandial CCK rise. The decreased CCK levels observed in celiac patients are not strictly related to the mucosal atrophy but rather to the lymphocytic mucosal infiltrate [Deprez *et al.* 2002]. An association between coeliac disease and chronic pancreatitis has already been described [Sood *et al.* 2007], but in isolated coeliac disease functional hypopancreatism also comes into play due to blunted gut-pancreas signaling responses. It is probable that the reduced expression of the CCK gene related to suppressive immunomodulatory factors induced by the inflammatory infiltrate, and would therefore merit comparison to the processes described above which lead to gain-of-function in the enteroendocrine system. Certainly these data continue to corroborate a potential role of autoimmunity in EEC dysfunction.

Enter the enteroendocrinologist?

It is clear that EEC play important roles in both health and disease, but up to now have been relegated to a rather neglected role in the GI clinical and scientific literature. More basic

and translational research work is now urgently required in enteroendocrinology, as it seems highly likely that these fascinating but neglected cells are therapeutically tractable players in a broad range of GI diseases.

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