# Metabolic Drivers of Immunity

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# The intestinal immunoendocrine axis: novel cross-talk between enteroendocrine cells and the immune system during infection and inflammatory disease

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## Abstract

**The intestinal epithelium represents one of our most important interfaces with the external environment. It must remain tightly balanced to allow nutrient absorption, but maintain barrier function and immune homoeostasis, a failure of which results in chronic infection or debilitating inflammatory bowel disease (IBD). The intestinal epithelium mainly consists of absorptive enterocytes and secretory goblet and Paneth cells and has recently come to light as being an essential modulator of immunity as opposed to a simple passive barrier. Each epithelial sub-type can produce specific immune modulating factors, driving innate immunity to pathogens as well as preventing autoimmunity. The enteroendocrine cells comprise just 1% of this epithelium, but collectively form the bodies' largest endocrine system. The mechanisms of enteroendocrine cell peptide secretion during feeding, metabolism and nutrient absorption are well studied; but their potential interactions with the enriched numbers of surrounding immune cells remain largely unexplored. This review focuses on alterations in enteroendocrine cell number and peptide secretion during inflammation and disease, highlighting the few in depth studies which have attempted to dissect the immune driven mechanisms that drive these phenomena. Moreover, the emerging potential of enteroendocrine cells acting as innate sensors of intestinal perturbation and secreting peptides to directly orchestrate immune cell function will be proposed. In summary, the data generated from these studies have begun to unravel a complex cross-talk between immune and enteroendocrine cells, highlighting the emerging immunoendocrine axis as a potential target for therapeutic strategies for infections and inflammatory disorders of the intestine.**

## Introduction

Dispersed throughout the intestinal epithelium are the enteroendocrine cells which, despite only comprising 1% of the epithelium, collectively form the largest endocrine system in humans. Enteroendocrine cells respond to luminal nutrients by secreting >20 peptide hormones, including cholecystokinin (CCK), glucagon-like peptide 1 and 2 (GLP-1, GLP-2), glucose-dependent insulinotropic peptide (GIP), peptide YY (PYY), somatostatin and ghrelin; as well as bioactive amines such as serotonin (5-HT). The historical dogma of differentiated enteroendocrine cellular sub-types secreting distinct hormone peptides has been superseded via the use of transgenic reporter mice, to the recognition that enteroendocrine cells can secrete a comprehensive array of peptide hormones altering based on their location within the gut [1]. These secreted peptide hormones act on distant organs such as pancreatic islets or locally on neighbouring cells such as enterocytes and vagal nerve endings. Enteroendocrine cells have classically been studied for their roles in enabling efficient postprandial assimilation of

**Key words:** cholecystokinin, glucagon-like peptide-1, gut, inflammation, mucosal immunology, serotonin.

**Abbreviations:** CXCL, chemokine (C-X-C motif) ligand; CCK, cholecystokinin; CD, Crohn's disease; DC, dendritic cell; DSS, dextran sulfate sodium; GLP, glucagon-like peptide; GPR, G protein-coupled receptor, IBD, inflammatory bowel disease; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; PYY, peptide YY; SCID, severe combined immunodeficiency; 5-HT, serotonin; Th, T-helper; TLR, toll-like receptor; TNBS, 2,4,6-trinitrobenzenesulfonic acid; UC, ulcerative colitis.

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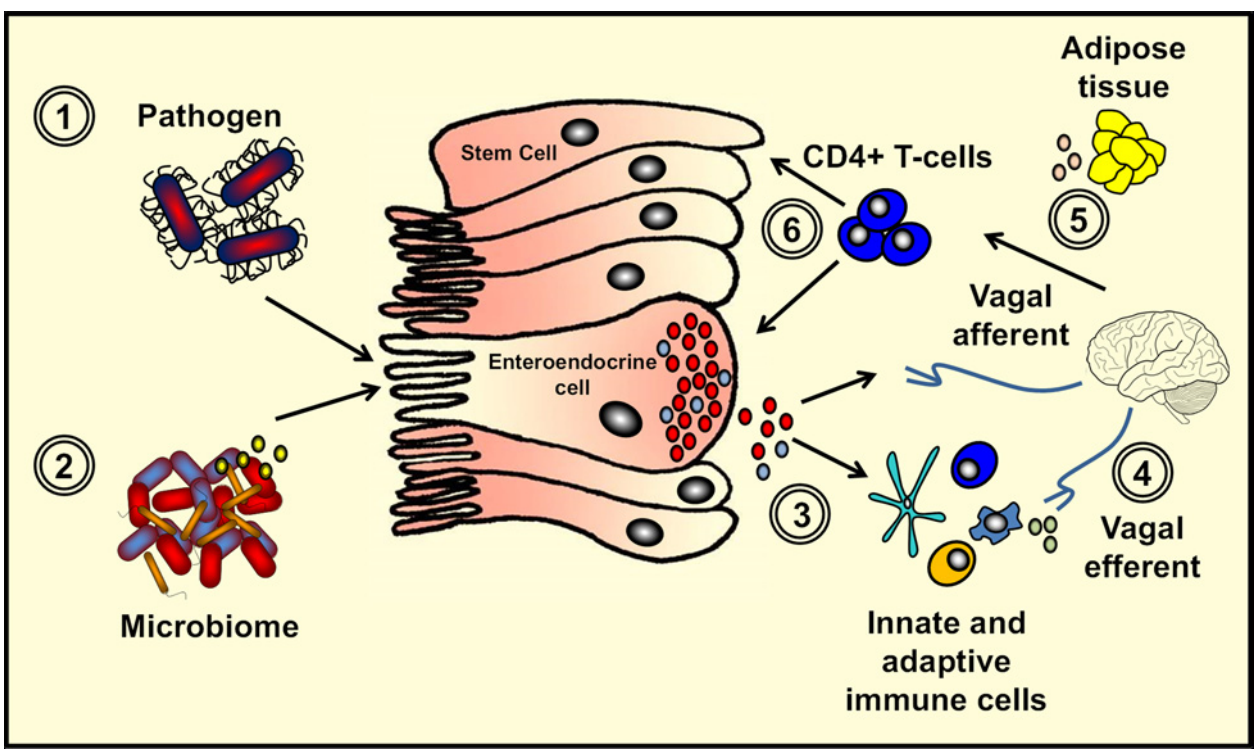
**Figure 1 | Enteroendocrine cells: key orchestrators of intestinal immunity**

Enteroendocrine cells make up 1 % of the intestinal epithelium and, beyond their classical role of detecting luminal nutrients, they also detect and respond to (**1**) pathogens via the expression of TLRs and (**2**) the intestinal microbiome via the expression of specific receptors for the metabolites commensal bacteria produce. (**3**) In response to pathogens and microbial metabolites, enteroendocrine cells secrete peptide hormones and classical cytokines to the surrounding immune cell rich milieu. In addition to classical cytokine receptors, immune cells express a vast array of receptors for peptide hormones which have direct immunomodulatory effects. (**4**) Enteroendocrine-secreted hormone peptides also signal to vagal afferents triggering an anti-inflammatory vagal reflex. The resulting acetylcholine released from vagal efferents inhibits inflammatory responses from the surrounding immune cells. (**5**) Vagal afferent signalling also modulates classical feeding pathways resulting in altered fat deposits. This, in turn, modifies the levels of fat secreted adipokines, such as leptin, influencing immune cell function. (**6**) CD4+ T-cells directly influence the function of peptide hormones via increased secretion and hyperplasia of enteroendocrine cells via direct enteroendocrine and indirect stem cell signalling.




nutrients via endocrine and paracrine induced alterations in gastrointestinal secretion, motility, pancreatic insulin release and satiety [2].

The key feature of enteroendocrine cells is to sense luminal nutrients and bring about the ideal absorption conditions for the particular nutrients detected. Classical examples of this fine tuning in nutrient detection are the enteroendocrine I-cells of the duodenum. In response to sensing long-chain fatty acids via activation of G protein-coupled receptors (GPRs), I-cells undergo $Ca^{2+}$ flux and membrane depolarization, culminating in secretion of the hormone CCK. CCK acts through the CCK receptor to cause gall bladder contraction and pancreatic enzyme secretion allowing efficient assimilation of the long-chain fatty acids detected [3]. Further to mediating digestion and metabolism, secreted hormones can also terminate meal size by vagally triggering satiation in feeding centres of the brain [2]. Therefore, clinical trials are focusing on the use of enteroendocrine peptide receptor agonists for the therapeutic treatment of obesity and metabolic diseases [2]. Of particular note is the use of GLP-1 receptor agonists for the treatment of diabetes, following the key observation that the incretin GLP-1 is anti-apoptotic for pancreatic $\beta$-cells [4].

Intriguingly, both murine and human studies have demonstrated alterations in enteroendocrine cell number and secretion during inflammation [5,6] and the vast immune system that serves the gut has been shown to express an array of enteroendocrine cell peptide receptors [7]. Furthermore, *in vitro/in vivo* studies have demonstrated that enteroendocrine cells possess functional toll-like receptors (TLRs) [8] and can directly respond to metabolites produced from commensal bacteria [9]. These observations indicate that enteroendocrine cells may have direct and critical roles in orchestrating intestinal immune responses to both pathogens and commensal bacteria (Figure 1) and despite the ongoing therapeutic trials and use of enteroendocrine cell peptide

receptor agonists, few studies have examined the potential importance of this immunoendocrine axis.

This review will focus on alterations in enteroendocrine number and peptide secretion during inflammation and disease, highlighting in-depth mechanistic mouse model studies. Furthermore, the emerging potential of enteroendocrine cells acting as innate sensors of pathogens and perturbations in the intestinal microbiome will be discussed, identifying enteroendocrine cells as key orchestrators of intestinal immunity.

## Enteroendocrine cells and inflammatory bowel disease

Given that reduced feeding, anorexia and altered intestinal motility often accompany intestinal inflammation, it is surprising that enteroendocrine cells, as key instigators of these changes during homoeostasis, have been neglected as possible orchestrators of these pathologies during disease. However, genome-wide association studies for Crohn's disease (CD) have identified a single nuclear polymorphism in the enteroendocrine associated homeodomain transcription factor paired-like homeobox 2B (Phox2B) [10]. This, coupled with the detection of autoantibodies for the ubiquitination factor E4A, specifically in enteroendocrine cells during Crohn's [11], has brought some focus upon the possible role of enteroendocrine cells in the pathogenesis of inflammatory bowel disease (IBD). Indeed, alterations in enteroendocrine cell numbers and secretion have been noted during IBD with increased PYY and 5-HT cells in lymphatic colitis, reduced colonic PYY cells in both CD and ulcerative colitis (UC), increases in GLP-1 and PYY cell number in terminal ileal CD and increases in GLP-2 in both CD and UC [5]. GLP-2 is a well-known epithelial growth factor with additional anti-inflammatory properties, including aiding secretion of antibacterial peptides from Paneth cells [12] and is therefore the most simplistic example of enteroendocrine function influencing intestinal disease pathology. Indeed, GLP-2 has been shown to be protective in animal models of IBD [13] and long acting analogues of GLP-2 are currently on trial for the treatment of CD [14]. Despite this beneficial change in enteroendocrine function during IBD, the reduced appetite, anorexia and nausea associated with IBD is also likely to be driven by altered enteroendocrine function. Although, increases in GLP-1 in UC are not thought to be responsible for any changes in feeding patterns, due to unaltered gastric emptying; small bowel Crohn's-associated feeding decreases and nausea do correlate with increased PYY levels [5]. Furthermore, increased enteroendocrine numbers in long-standing UC have been suggested to act as promoters for the neoplasia associated with IBD [15], whereas recent data has demonstrated enteroendocrine cells as being key producers of the pro-inflammatory cytokine interleukin (IL)-17C during CD and UC, possibly playing a key role in disease progression [16]. Taken together, this suggests that enteroendocrine cells play an essential and varied role in the pathology of IBD and are strong candidates for therapeutic intervention.

## Enteroendocrine cells in mouse models of IBD

Further mechanistic study of the pathways involved in enteroendocrine cell pathology during IBD has been made possible via the use of animal models of intestinal inflammation. Colitis can be induced chemically via the administration of dextran sulfate sodium (DSS) or 2,4,6-trinitrobenzenesulfonic acid (TNBS) and both models are well associated with reduced feeding and weight loss. Interestingly, it has been reported that the feeding alterations seen in TNBS-induced colitis are probably due to alterations in enteroendocrine satiety as opposed to simple malaise, due to changes in gastric emptying [17]. Guinea pigs with TNBS-induced colitis have been shown to have hyperplasia of 5-HT and GLP-2 enteroendocrine cells. Through the use of Bromodeoxyuridine (BrdU) labelling of proliferative cells it has been demonstrated that, although a small capacity of 5-HT producing enterochromaffin cells retain proliferative capacity, the majority of hyperplasia is due to alterations in the stem cell niche [18]. As all epithelial cells arise from the same pluripotent stem cell [6], this is suggestive that alterations in enteroendocrine number occur at the stem cell level and due to the high turnover of intestinal epithelial cells can quickly influence the inflammatory state. These chemical-induced colitis models have been particularly useful in establishing the role of enteroendocrine cells in the pathogenesis of mouse models of disease. Further elucidations have been made utilizing infection-based models of intestinal inflammation, which have demonstrated a key role for enteroendocrine cells during infection, as well as offering translational lessons for IBD.

## Enteroendocrine cells as mediators of intestinal infection

There are numerous reports of alterations in enteroendocrine cell number and secretion during a variety of infectious agents in a diverse range of animals. For example decreased somatostatin-positive cells are seen during schistomiasis in mice [19], whereas increases in CCK-positive cells occur in giardia-infected humans [6] and myxozoa-infected fish [20]. Many studies within the livestock industry have associated changes in enteroendocrine function with weight loss during intestinal infection. Infection with the intestinal parasites *Ascaris suum* in pigs and *Trichostrongylus colubriformis* in lambs results in hypophagia that is coupled with an increase in CCK [6], whereas increased 5-HT and CCK enteroendocrine cells significantly correlate with the cachexia seen in *Enteromyxum scophthalmi* infected Turbot [20]. Animal models have been particularly useful for dissecting the mechanisms responsible for the hyperplasia of enteroendocrine cells during inflammation with studies suggesting an immune-driven alteration. There is a close physical association of

immune cells with enteroendocrine cells [21] and the 5-HT hyperplasia observed during *Citrobacter rodentium* infection is absent from severe combined immunodeficiency (SCID) mice [22] which lack adaptive immunity.

We have carried out in-depth studies with the helminth *Trichinella spiralis* which causes a well-characterized transient enteritis and weight loss in mice, with parasite expulsion dependent on T-helper (Th) 2 cytokines and mastocytosis [23]. Utilizing a variety of transgenic mice, we have dissected the molecular mechanisms and actual function of the hypophagia seen during this parasitic infection. Intriguingly both CCK + cell hyperplasia [23] and CCK hypersecretion [24] are observed during *T. spiralis* infection and this correlates with the period of hypophagia seen during enteritis. Furthermore, the absence of CD4 + T-cells or the CCK signalling pathway results in a complete lack of hypophagia during enteritis [23,24], whereas the adoptive transfer of CD4 + T-cells to infected SCID mice restores the otherwise absent hypophagia [23]. Collectively, this indicates that the adaptive immune system hijacks classical feeding pathways to reduce food intake during infection. We further pursued the possible benefit of such a mechanism, beyond a simple innate device to prevent continued feeding at an infected site, by examining if reduced feeding was in any way beneficial to the host in coping with the parasitic burden. The period of immune-mediated CCK-induced hypophagia during infection resulted in a significant reduction in weight and visible reduction in visceral fat pads, a rich source of immune manipulating adipokines, most notably leptin [25]. We therefore postulated that the immune driven reductions in leptin, a strong Th1-inducing adipokine [25], could be beneficial in allowing the helminth expelling Th2 immune response to develop, allowing parasite expulsion. To investigate such an effect, we restored basal leptin levels throughout infection-induced hypophagia via the injection of recombinant leptin and saw a significant reduction in CD4 + T-cell Th2 cytokine production and mastocytosis, culminating in a significant reduction in parasite expulsion. Hence, we have identified immune-driven alterations in enteroendocrine feeding pathways as a novel mechanism in helminth expulsion [23].

Parallel studies have demonstrated CD4 + T-cell control of 5-HT producing enterochromaffin cells during a large intestinal helminth infection, which is thought to be driven at the enterochromaffin cell level via the expression of IL-13R$\alpha$1 expression [26]. Indeed, CD4 + Th2 cytokines are essential for these alterations, as a chronic dose of the same helminth, resulting in a Th1 immune response does not drive the enterochromaffin hyperplasia [27]. Although, the precise function of these changes has not been defined, 5-HT has many possible immune-modulating abilities [28] and could therefore again be an adaptively driven mechanism of parasite expulsion. The possibility IL-13 is responsible for the alterations in CCK seen during *T. spiralis* infection is less likely, given the ample natural killer (NK) cell-derived, IL-13-induced goblet cell hyperplasia observed in infected SCID mice, but lack of accompanying I-cell hyperplasia [23]. This uncoupling of enteroendocrine differentiation during inflammation holds promising therapeutic potential, given the diverse potential functional roles of individual enteroendocrine peptide hormones.

## Direct immunomodulatory roles of enteroendocrine cells

Intriguingly, immune cells express a vast array of receptors for enteroendocrine secreted hormone peptides [7], suggesting an exciting potential of bi-directional signalling in the immunoendocrine axis. The production of the amine 5-HT from enterochromaffin endocrine cells is well established as a direct immunomodulatory factor, with the seven receptor isoforms expressed on mast cells, monocytes, dendritic cells (DCs), eosinophils, T- and B-cells and neutrophils [28]. Immune cells can also produce 5-HT independently of endocrine cells and the effect on immune cells is varied from cellular recruitment, activation, phagocytosis, antigen presentation and cytokine secretion [28]. Recent and ongoing studies are dissecting the potential for peptide hormones to influence immunity in a similar manner to the well-studied actions of 5-HT. Indeed, carboxypeptidase E-null mice, an enteroendocrine-associated exopeptidase essential for processing and packaging endocrine peptides, demonstrate increased IL-6 and chemokine (C-X-C motif) ligand (CXCL) 1 and exacerbated DSS-induced colitis [29].

CCK octapeptide has been shown to inhibit TLR9 stimulation of plasmacytoid DCs via tumour necrosis factor receptor-associated factor 6 signalling [30], whereas it can promote IL-12 production from DCs and reduce IL-6 and IL-23 production offering protection during collagen-induced arthritis [31]. CCK octapeptide can also directly affect T- and B-cells and has been shown to promote a Th2 and regulatory T-cell phenotype *in vitro* [32], promote IL-2 production in the Jurkat T-cell line [33], stimulate B-cells to produce acetylcholine [34] and reduce B-cell lipopolysaccharide (LPS)-induced activation [35]. Strikingly, the huge atrophy in lymphoid tissue, including Peyer's patches, IgA production and total cellularity, seen during parenteral feeding can be rescued via the infusion of CCK alone [7], functionally rescuing immune responses to infectious bacteria [36].

Other promising immunomodulatory enteroendocrine hormone peptides include the orexigenic peptide ghrelin. Ghrelin increases T-cell proliferation via phosphoinositide 3-kinase, extracellular-signal-regulated kinases and protein kinase C [37] and has been shown to have an anti-inflammatory effect in DSS-induced colitis [38]. Interestingly ghrelin actually has direct anti-parasitic [39] and anti-bacterial effects [40]. Similarly to 5-HT, T-cells themselves can produce ghrelin that is involved in anti-inflammatory responses in terms of reducing Th1 and Th17 responses [41]. Moreover, somatostatin is inhibitory to T-cell proliferation [42], GLP-1 also has anti-inflammatory effects on T-cells via decreased mitogen-activated protein kinase (MAPK) activation [43] and

may modulate regulatory T-cells [44]. Indeed, intraepithelial lymphocytes respond to GLP-1 to influence the response to DSS-induced colitis [45].

Enteroendocrine cells are also direct sources of cytokines, being key producers of the pro-inflammatory cytokine IL-17C during CD and UC [16]. Enteroendocrine cells have been shown to express functional TLRs, *in vitro* and *in vivo* studies have shown that CCK-secreting cells express TLR 1, 2 and 4, with stimulation resulting in increased nuclear factor kappa light chain enhancer of activated B cells (NF$\kappa\beta$), MAPK signalling, as well as Ca flux culminating in tumour necrosis factor-$\alpha$, transforming growth factor-$\beta$ and macrophage inhibitory protein 2, as well as CCK release [8]. Indeed, enteroendocrine cells appear to be able to modulate their response between pathogenic and nutrient sensing, secreting CXCL1/3 and IL-32 in response to flagellin and LPS, but not to fatty acids *in vitro* [46]. Taken together, this indicates that enteroendocrine cells can act as front-line pathogen detectors releasing either classical cytokines or peptide hormones that can directly orchestrate adaptive and innate immunity.

## Vagally-mediated immunomodulatory roles of enteroendocrine released peptide hormones

As well as being able to directly influence immune cells, enteroendocrine-secreted products can indirectly influence immune responses via the triggering of vagal afferents. This anti-inflammatory pathway was first examined during haemorrhagic shock. Nutritional stimulation of CCK via a high-fat diet protected via a vagal reflex releasing acetylcholine which inhibited pro-inflammatory cytokine secretion from macrophages [47]. Others have demonstrated similar vagal-macrophage regulation in a variety of inflammatory settings [48], with the pathway also regulating other innate immune cells [48]. However, these results should be considered in parallel with other data demonstrating direct effects of CCK on macrophages, CCK inhibits inducible nitric oxide synthase (iNOS) production by macrophages [49], as well as studies demonstrating direct alteration of acetylcholine production by B-cells in response to CCK [34]. This anti-inflammatory role of the vagus nerve and, therefore, enteroendocrine peptide hormone stimulation is an exciting and growing area of research.

## Enteroendocrine cells as sensors of the intestinal microbiome

Finally, the current explosion in studies into the intestinal microbiome has not failed in linking both enteroendocrine cells and vagal signalling to the billions of bacteria which inhabit our intestines. Historic studies have demonstrated germ-free mice have drastically altered enteroendocrine cell numbers [50]; whereas, recently it has been shown that enteroendocrine cells have specific receptors which can respond to bacterial products. In particular GLP-1-secreting cells have receptors for many microbiome metabolites such as GPR41 and 43 for short-chain fatty acids, read GPR 131 for bile acids and GPR119 for *N*-oleoylethanolamide and 2-oleoylglycerol and can secrete GLP-1, GLP-2 and PYY in response to stimulation [9]. It is therefore highly likely that our intestinal microbiome is able to influence not only obesity, but also our entire immune system via regulating the production of immunomodulatory enteroendocrine hormone peptides.

## Summary

In summary, emerging data have begun to demonstrate a huge interaction between enteroendocrine cells and the immune system. Enteroendocrine cells can secrete classical cytokines as well as hormonal peptides that have the ability to directly and indirectly influence the entire breadth of our intestinal immune system. Due to the scarcity of these cells and lack of specific markers for purification, this immunoendocrine axis has until recently remained neglected. The transgenic reporter models now available have led to a huge potential to fully investigate this exciting cross-talk between our intestinal endocrine and immune systems, opening up new therapeutic targets and the possibility to utilize current drugs used for metabolic syndromes in wider immune inflammatory settings.

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