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Enteroendocrine cells in terminal ileal Crohn's disease☆

Gordon W. Moran, Joanne Pennock, John T. McLaughlin*

Inflammation Sciences Research Group and Manchester Academic Health Science Centre, University of Manchester, Manchester, M13 9PL, UK

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Abstract

Background and aims: Enteroendocrine cells sense gut luminal contents, and orchestrate digestive physiology whilst contributing to mucosal homeostasis and innate immunity. The terminal ileum is the key site of EEC expression but detailed assessment of their subtypes, lineage transcription factors and expression products has not been undertaken in terminal ileal Crohn's disease. Recent Crohn's disease gene wide association studies have linked the neuroendocrine transcription factor Phox2b; while autoantibodies to an enteroendocrine protein, ubiquitination protein 4a, have been identified as a disease behaviour biomarker.
Methods: Terminal ileal tissue from small or large bowel Crohn's disease and normal controls was analysed for enteroendocrine marker expression by immunohistochemistry and quantitative polymerase chain reaction. Inflammation was graded by endoscopic, clinical, histological and biochemical scoring.
Results: In small bowel disease, glucagon-like peptide 1 and chromogranin A cells were increased 2.5-fold ($p=0.049$) and 2-fold ($p=0.031$) respectively. Polypeptide YY cells were unchanged. Ileal enteroendocrine cell expression was unaffected in the presence of Crohn's colitis. Phox2b was co-localised to enteroendocrine cells and showed a 1.5-fold increase in ileal disease. Significant mRNA increases were noted for chromogranin A (3.3-fold; $p=0.009$), glucagon-like peptide 1

Abbreviations AR, Antigen retrieval; BSA, Bovine serum albumin; CgA, Chromogranin A; CCK, Cholecystokinin; CD, Crohn's disease; CDAI, CD activity index; Cy5, Cyanine 5; DAPI, 4′,6-diamidino-2-phenylindole; EDTA, Ethylenediaminetetraacetic acid; EEC, Enteroendocrine cells; FITC, Fluorescein isothiocyanate; GAPDH, Glyceraldehyde 3 phosphate dehydrogenase; GLP-1, Glucagon-like peptide 1; GWAS, Gene wide association studies; HSK, House-keeping gene; hpf, High power field; HPRT, Hypoxanthine phosphoribosyl transferase 1; IMS, Industrial methylated spirit; IBD, Inflammatory bowel disease; LB-CD, Large bowel Crohn's disease; Ngn3, Neurogenin 3; PYY, Polypeptide YY; QPCR, Quantitative polymerase chain reaction; RT, Room temperature; SB-CD, Small bowel Crohn's disease; TBS, Tris-Buffered Solution; YWAHZ, Tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation protein, zeta polypeptide; VCU, Villus crypt unit

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* Corresponding author at: Inflammation Sciences Research Group, School of Translational Medicine, Clinical Sciences Building, University of Manchester, Salford Royal NHS Foundation Trust, Salford, Manchester, M6 8HD. Tel.: +44 161 206 4362/3; fax: +44 161 206 1495.
E-mail addresses: gwmoran@ucalgary.ca (G.W. Moran), Joanne.Pennock@manchester.ac.uk (J. Pennock), John.Mclaughlin@manchester.ac.uk (J.T. McLaughlin).

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(3.1-fold; p=0.007) and ubiquitination protein 4a (2.2-fold; p=0.02). Neurogenin 3, an enteroendocrine transcription factor showed ~2 fold-upregulation (p=0.048).
Conclusions: Enhanced enteroendocrine cell activity is present in small bowel disease, and observed in restricted cell lineages. This may impact on the epithelial immune response, cellular homeostasis and nutrient handling and influence appetite via increased satiety signalling in the gut-brain axis.

1. Introduction

In health, enteroendocrine cells (EEC) play multiple roles in the gastrointestinal response to food, coordinating secretory and motor events to maximise the efficient digestion and absorption of food. They also play a key function in maintaining epithelial integrity, and contribute to the mucosal innate immune system. An important further role is the short-term control of food intake. Despite these pivotal functions, little is known about their status and activity in inflammatory bowel disease.

EEC act as sensors of luminal content via G-protein coupled 'taste' receptors (T1R2, T1R3 and T2R), nutrient (e.g. fatty acid) receptors (GPR 40, 41, 43, 120)[1–7] Moreover, recent studies have demonstrated that EEC also express sensory receptors of the mucosal innate immune system so play a direct role in immune surveillance of luminal contents.[8–10] They function as transepithelial signal transduction conduits by releasing regulatory peptides and amines. Key biological mediators are cholecystokinin (CCK), glucagon-like peptide-1 and -2 (GLP-1/2) which are both tissue-specific products of their precursor proglucagon, and polypeptide YY (PYY). These exert their effects either in a classical endocrine fashion through the porto-systemic circulation or via paracrine pathways, principally the vagal afferents.[11–18]

EEC are also centrally implicated in epithelial homeostasis and repair, particularly via GLP-2.[19–28] Finally, recent genomic data have linked two EEC genes directly to Crohn's disease: ubiquitination protein 4a (UbE4A)[29] and Phox2B.[30] The functional role of EEC in inflammatory diseases of the gut therefore requires further attention.

A clear area of clinical relevance may be that increased EEC function may play a role in the hypophagic state observed in intestinal inflammation. Poor appetite and reduced food intake are important components of the nutritional health issues of patients with active inflammatory disease. In patients with tropical sprue, an increase in postprandial gut hormones[31–33] has been noted. In *Giardia* enteritis, high plasma CCK levels were correlated with patient symptoms upon feeding. Treatment of the infection led to normalisation of the CCK plasma levels and abolished symptoms.[33]

To assess whether a relationship between EEC and inflammation is causal in hypophagia we recently utilised a mouse model of the nematode *Trichinella spiralis*. This induced proximal enteritis and a concurrent increase in CCK secreting I-cell numbers and circulating levels[34] while food intake reduced by half. Hypophagic effects were attenuated by CCK_1 receptor inhibition and all were abolished by CD4 immunoneutralisation.

Two areas of the gut express the most abundant EEC. These are the duodenum (CCK) and terminal ileum (PYY and GLP-1/2). In the current study we therefore sought to explore the impact of inflammation on EEC in the segment of the gut most commonly affected by CD. The duodenum is rarely affected by the disease, but terminal ileal involvement is very common. However, knowledge of the status of EEC populations in the affected terminal ileum is very limited. An increase in total endocrine cells defined as chromogranin A (CgA) immunoreactive has been described in both actively inflamed and non-inflamed ileal CD. The only specific subpopulation of EEC cells reported to show a significant increase in number are the 5-HT secreting EC cells.[35] Further data on other cell types and new genomic markers are lacking.

The purpose of the current study was therefore to undertake a systematic assessment of the key peptide secreting EEC subtypes and also to assess the expression of newly described EEC markers in CD, and to assess whether the changes observed relate to the site of disease activity.

2. Materials and methods

2.1. Basic protocol and patient recruitment

All CD tissue was obtained fresh from patients with active disease affecting either the terminal ileum or colon. Normal tissue was obtained from otherwise healthy individuals undergoing a screening procedure to exclude colonic cancer. Only patients with normal colonoscopies were included in the study as controls. Four extra biopsies from the terminal ileum were taken in this study, from both CD patients and controls. Ethical approval for this project was obtained as indicated in detail below. All CD patients and normal controls gave their informed consent prior to the endoscopic investigation. Four groups were recruited: active small bowel disease (SB-CD), active large bowel disease (LB-CD), inactive small bowel CD and controls. Tissue was fixed in 10% formalin for 24–48 h and later embedded and mounted in paraffin. The number of EEC peptide positive cells was counted per high power field (×40). Data are expressed as positive cells per crypt per high power field (hpf) as previously shown.[36] This method of normalisation was undertaken to take into account changes in morphology such as oedema and crypt destruction due to the inflammation in active disease. Nine hpf counts were taken per section. For quantitative polymerase chain reaction (qPCR), tissue samples were immediately placed in 'RNA later' (Qiagen, Crawley, UK) and stored at −80 °C until further processing. At the time of the study,