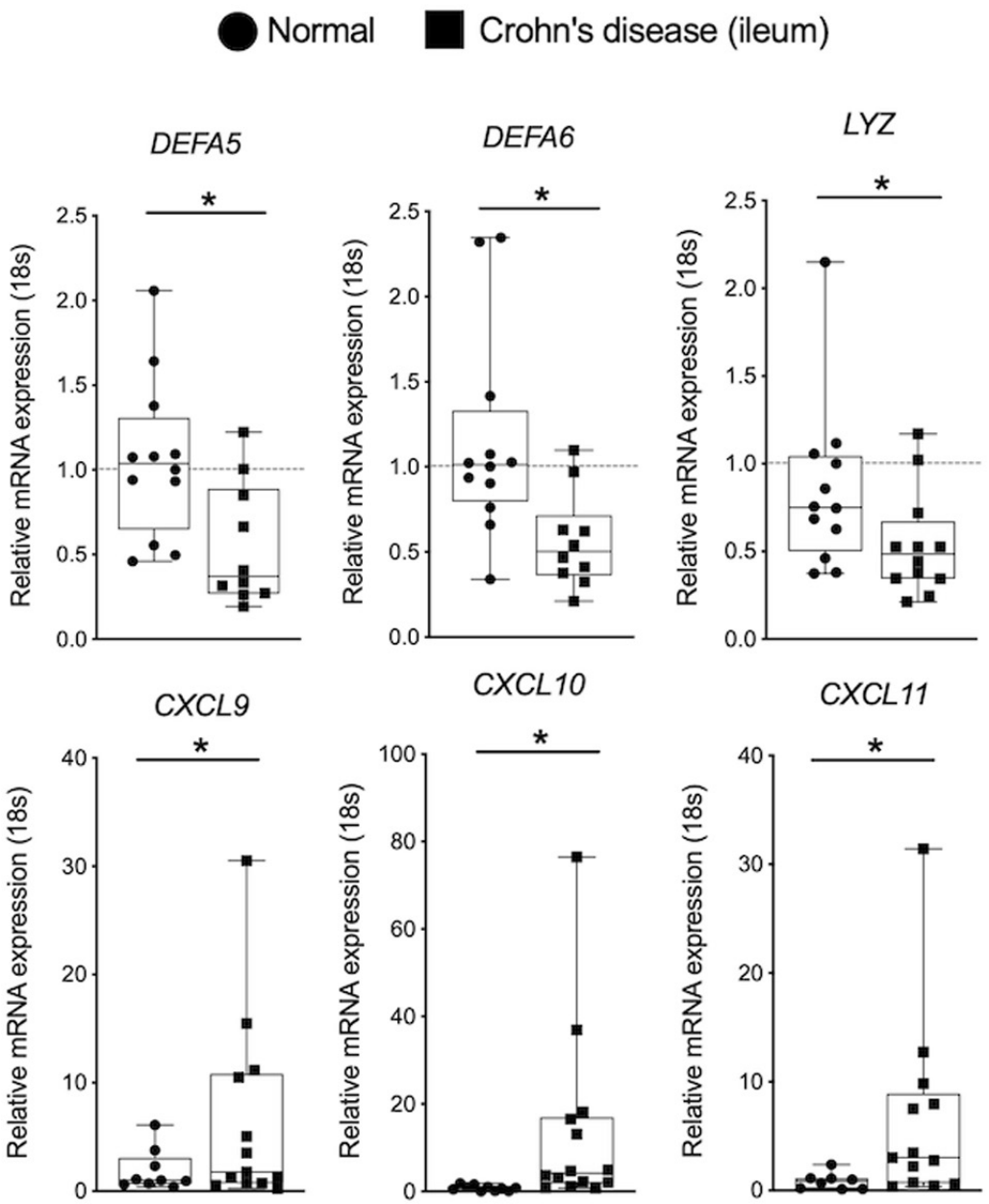

Methods, 13 CD patients and 16 healthy controls attending for routine endoscopic evaluation were prospectively recruited at St.Vincent's University Hospital, Dublin and ileal biopsies were collected in media. RNA was isolated from homogenised ileal biopsies (Quiagen kit), DNAse treated using DNAse I (Invitrogen) and reverse transcribed using M-MLV reverse transcriptase (Promega). Target cDNAs were quantified using an Applied BiosystemsTM QuantStudioTM 7 Flex Real-Time PCR System.

Results: We identified an increase in the relative mRNA expression of CXCR3 associated chemokines, with significantly higher levels of CXCL9, CXCL10 and CXCL11 in ileal CD patients compared to healthy controls. This coincides with a reduction in paneth cell-derived antimicrobial peptides with significantly lower levels of alpha defensins (DEFA5, DEFA6) and lysozyme in CD patients compared to healthy controls.

Conclusion: This study supports data from animal models and provides a hypothesis for the loss of paneth cells in patients with ileal CD. The reduction in paneth cell derived anti-microbial peptides may help to explain the altered microbiome and provide an explanation for the loss of mucosal barrier integrity in Inflammatory Bowel Disease (IBD). A greater understanding of the role of chemokines in the development of mucosal immunity and inflammation in IBD may allow the targeting of chemokines in novel therapeutic strategies.

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The involvement of extracellular Nicotinamide Phosphoribosyltransferase (eNAMPT) and Nicotinate Phosphoribosyltransferase (eNAPRT) in inflammatory bowel disease

G. Colombo*[1], D.G. Ribaldone[2], G.P. Caviglia[3], A. Genazzani[1], C. Travelli[4]

[1]*Università del Piemonte Orientale, Dipartimento di Scienze del Farmaco, Novara, Italy,* [2]*Università degli Studi di Torino, Department of Surgical Sciences, Torino, Italy,* [3]*Università degli Studi di Torino, Division of Gastroenterology- Department of Medical Sciences, Torino, Italy,* [4]*Università degli Studi di Pavia, Department of Pharmaceutical Sciences, Pavia, Italy*

Background: Nicotinamide phosphoribosyltrasferase (NAMPT) is a pleiotropic enzyme which catalyses the first and rate-limiting step in the biosynthesis of NAD. It is present in two different forms: an intracellular form, called iNAMPT, (Chiarugi et al., 2012), and an extracellular form, eNAMPT. eNAMPT is considered an important factor for granulocyte-colony stimulating factor-(G-CSF)-induced myeloid differentiation, with paracrine and autocrine effects on different cell types (*i.e.* immune and cancer cells), binding TLR4. NAMPT is structurally and functionally related to the enzyme nicotinate phosphoribosyltransferase (iNAPRT), which is rate-limiting in the NAD salvage pathway that starts from nicotinic acid. The NAD biosynthetic pathways controlled by NAMPT and NAPRT are closely interconnected and can compensate for each other. Also, NAPRT is identified as an extracellular ligand (eNAMPRT) for TLR4 and a mediator of inflammation (Managò et al., 2020).
Importantly, iNAMPT and eNAMPT levels are increased in several pathologies, included inflammatory bowel disease (IBD). It has been reported that serum eNAMPT levels correlate with the stage of the pathology: in an active state of the disease the levels of NAMPT are very high, however its levels are partially reduced in a remission stage (Moschen et al., 2007).
Methods: First, we investigated the role of eNAMPT and eNAPRT in murine IBD models (especially in DNBS and DSS model). We took into account phenotypic effect as weight loss and colon shortening, but also the reduction of mRNA of inflammatory genes with RT-PCR, tissue damage with H&E and IHC analysis and systemic and local production through colon explant. Secondly, we determined serum eNAMPT and eNAPRT levels in a cohort of adult IBD patients.
Results: Both eNAMPT and eNAPRT have been found elevated in 180 IBD patients, as proinflammatory marker of the pathologies. These levels are also elevated in serum and colonic explant of DSS and DNBS preclinical models, associated to an active state of the disease, as a pro-inflammatory response developed locally and systemically.
Moreover, we performed ELISA analysis on sera of 100 IBD patients, eligible for anti-TNF treatment, both pediatric and adults. Serum eNAMPT levels are increased before the treatment, responsive patients verified a reduction of these levels, while no-responsive ones verified higher levels.
Conclusion: eNAMPT and eNAPRT could be considered pro-inflammatory markers of IBD and possible druggable targets.

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An increased autophagy and decreased apoptosis is detected in intestinal fibroblasts from Crohn's Disease patients

L. Gisbert-Ferrandiz[1], M. Queralt[1], J. Cosín-Roger*[2], S. Coll[1], C. Bauset[1], D. Ortiz-Masia[3], J. Llau[1], F. Navarro-Vicente[4], S. Calatayud[1], M.D. Barrachina[1]
[1]*Universitat de València, Pharmacology, Valencia, Spain,* [2]*Unidad Mixta Facultad Medicina - Hospital Dr Peset- FISABIO, Pharmacology, Valencia, Spain,* [3]*CIBERehd-Univ. de València, Medicine, Valencia, Spain,* [4]*Hospital Manises, Surgery, Valencia, Spain*

Background: Fibrosis is a complication commonly present in Crohn's disease (CD) patients with a structuring (B2) or penetrating (B3) phenotype, with no effective treatment. This process is characterized by a disequilibrium between the production and degradation of the extracellular matrix (ECM), mainly regulated by myofibroblasts. We aim to analyse here, the expression of markers of autophagy, apoptosis and proliferation in intestinal fibroblasts from CD patients.
Methods: Fibroblasts were isolated from the damaged intestinal mucosa of CD patients with a penetrating and stenotic behaviour. Control cells were obtained from the non-damaged intestine of patients with colorectal cancer. Protein levels of markers of autophagy and apoptosis were determined by Western Blot in isolated fibroblasts. The proliferation marker Ki67 was analysed by immunohistochemistry (IHC) in 5 µm slides of intestinal tissue from control or CD patients. Statistical significance was measured by t-test.
Results: In fibroblasts from CD patients, we detected a significant decrease in the ratio phospho-mTOR / mTOR (Fig. A) in parallel with a non-significant increment in the LC3 II / LC3 I protein ratio (174% ± 46.5), and a decrease in p62 protein levels (84.8% ± 5.5). When compared between CD behaviours, a significant decreased in the phospho-mTOR / mTOR protein ratio was detected in fibroblasts from B2- compared to that obtained in cells from B3-CD patients (Fig. B). The analysis of the expression of an apoptosis marker, Caspase 3, revealed a decreased of cleaved caspase 3 protein levels in CD fibroblasts compared to levels detected in control cells (Fig C). Finally, we observed in the lamina propria of the intestine from CD patients an increased number of Ki67 positive cells, compared to that detected in control tissue.

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