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## Antimicrobial peptides and the gut microbiome in inflammatory bowel disease

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### Abstract

Antimicrobial peptides (AMP) are highly diverse and dynamic molecules that are expressed by specific intestinal epithelial cells, Paneth cells, as well as immune cells in the gastrointestinal (GI) tract. They play critical roles in maintaining tolerance to gut microbiota and protecting against enteric infections. Given that disruptions in tolerance to commensal microbiota and loss of barrier function play major roles in the pathogenesis of inflammatory bowel disease (IBD) and converge on the function of AMP, the significance of AMP as potential biomarkers and novel therapeutic targets in IBD have been increasingly recognized in recent years. In this frontier article, we discuss the function and mechanisms of AMP in the GI tract, examine the interaction of AMP with the gut microbiome, explore the role of AMP in the pathogenesis of IBD, and review translational applications of AMP in patients with IBD.

**Key Words:** Antimicrobial peptides; Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Gut microbiome; Biomarkers

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infection while maintaining intestinal homeostasis to support commensalism with the gut microbiome. AMPs have broad spectrum antimicrobial activity with diverse mechanisms of action and regulate gut microbiome composition. Defects in endogenous AMP expression and function have been linked with animal models of inflammatory bowel disease (IBD). Exogenous delivery of AMPs such as defensins, cathelicidin, and elafin attenuates intestinal inflammation in murine models of IBD. AMPs such as calprotectin and lactoferrin are useful biomarkers for patients with IBD. Challenges with AMP stability, bioavailability, and selectivity are major barriers to their application as potential therapies.

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## INTRODUCTION

The gastrointestinal (GI) tract is a highly complex and dynamic ecosystem consisting of a protective epithelial barrier in constant exposure to commensal microorganisms that are collectively known as the gut microbiome[1]. An intricate balance between tolerance to commensal microorganisms and protection against enteric pathogens is required to maintain intestinal homeostasis. A breakdown in this balance has been recognized to play a role in the pathogenesis of inflammatory disorders of the GI tract such as inflammatory bowel disease (IBD)[2]. Antimicrobial peptides (AMPs) are diverse and bioactive compounds that play critical roles in host defense and maintaining tolerance to commensal microorganisms[3,4]. Here we provide a comprehensive review of the significant AMP functions in the GI tract and the gut microbiome, potential roles of AMPs in the pathogenesis and treatment of IBD based on preclinical animal models, and translational applications of AMPs in patients with IBD.

## ANTIMICROBIAL PEPTIDES IN THE GASTROINTESTINAL TRACT

### Human defensins

**Table 1** summarizes the major classes of AMPs in the GI tract. Defensins, which consist of small cationic peptides, protect against bacterial infections by directly disrupting bacterial membranes. The two major classes of defensins include  $\alpha$ -defensins and  $\beta$ -defensins which differ structurally in their cysteine pairings[5]. Human  $\alpha$ -defensins are also known as human neutrophil peptides (hNP). Human defensin 5 and 6 (HD5 and HD6) are the only  $\alpha$ -defensins produced in the GI tract by Paneth cells, highly specialized secretory epithelial cells with antimicrobial function[6]. Known functions of HD5 include conferring resistance to oral challenge with enteric pathogens[7] and regulating the intestinal microbiota by reducing levels of segmented filamentous bacteria[8]. HD6 has been shown to restrict infection by limiting intestinal epithelial cell invasion[9].  $\beta$ -defensins are expressed by enterocytes of the small and large intestine. The most relevant intestinal  $\beta$ -defensins include human  $\beta$ -defensins 1-4 (hBD-1, hBD-2, hBD-3, and hBD-4). hBD-2 and hBD-3 expression increases in response to infectious stimuli, whereas hBD-1 is constitutively expressed by the GI tract[10].  $\beta$ -defensins hBD-2-4 have antimicrobial activity against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*, whereas hBD-1 only has activity against gram positive commensals[11-13].

### Cathelicidin

Cathelicidin is another class of cationic peptides that mediates its bactericidal effects through direct disruption and lysis of bacterial membranes. Cathelicidin, also known as LL-37 or hCAP18, is an 18 kDa antimicrobial peptide involved in innate immune defenses and is encoded by the CAMP gene in humans[14]. Cathelicidin has a broad-



**Table 1** Antimicrobial peptides in the gastrointestinal tract

Antimicrobial peptide class	Gene	Specific antimicrobial peptides	Tissue expression	Biologic function
$\alpha$ -defensins (human neutrophil peptides)[5-9]	DEFA	Human defensin 5 and 6 (HD5 and HD6)	Paneth cells	Confers resistance to oral challenge with enteric pathogens, regulates the intestinal microbiota by reducing levels of segmented filamentous bacteria, restricts infection by limiting intestinal epithelial cell invasion
$\beta$ -defensins[5,10-13]	DEFB	Human $\beta$ -defensins 1-4 (hBD-1, hBD-2, hBD-3, and hBD-4)	Intestinal epithelial cells	Antimicrobial activity (hBD-2-4) against bacterial pathogens including <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , and <i>Streptococcus pyogenes</i> , antimicrobial activity (hBD-1) against gram-positive commensals
Cathelicidin[14-22]	CAMP	Cathelicidin (LL-37/hCAP18)	Colonic epithelial cells, neutrophils, monocytes, macrophages, mast cells	Cationic peptide that directly disrupts bacterial cell membranes, deficiency increases susceptibility to infection with enterohemorrhagic <i>E. coli</i> (EHEC)
Regenerating (Reg) protein [23-29]	REG	RegIII; Hepatocarcinoma-intestine pancreas (HIP)/pancreatitis-associated protein (PAP)	Paneth cells, intestinal epithelial cells	Regulates intestinal homeostasis by maintaining a physical separation between epithelial cells and the microbiota, selective for gram-positive bacteria through interaction with cell wall peptidoglycan
Lactoferrin[30]	LTF	Lactoferrin	Epithelial cells	Secreted iron binding protein, sequesters free iron required for bacterial growth
Lipocalin[31,129]	LCN2	Lipocalin-2 (neutrophil gelatinase-associated lipocalin, GAL)	Neutrophils, granulocytes, macrophages, epithelial cells	Binds to bacterial siderophore enterobactin and inhibits bacterial growth by sequestering iron
Calprotectin[32]	S100A8, S100A9	Calprotectin	Intestinal epithelial cells, neutrophils	Chelates and sequesters metal co-factors (manganese, zinc, iron) during infection and inhibits bacterial growth
Hepcidin[33]	HAMP, LEAP1	Hepcidin antimicrobial peptide	Intestinal epithelial cells	Regulates iron absorption and homeostasis, inhibits bacterial growth by limiting iron availability
Galectin[34,35]	LGALS	Galectin-3, Galectin-4, Galectin-8	Intestinal epithelial cells	Galectins has bactericidal activity against bacteria expressing blood group antigen, Gal-8 targets damaged vesicles for autophagy during bacteria invasion
Lysozyme[36]	LYZ	Lysozyme	Paneth cells	Enzymatic degradation of bacterial membranes, preference towards Gram-positive pathogens
Elafin[37]	PI3	Elafin (peptidase inhibitor 3)	Intestinal epithelial cells	Binds to bacterial lipopolysaccharide (LPS) and modulates innate immunity
Secretory Leukocyte Protease Inhibitor (SLPI)[38,39]	SLPI	SLPI	Intestinal epithelial cells, paneth cells, neutrophils, macrophages	Protease inhibitor binds to bacterial mRNA and DNA, dose-dependent bactericidal properties of SLPI against both Gram-positive and Gram-negative bacteria, has fungicidal properties

spectrum activity against bacteria, enveloped viruses, and fungi[15]. It is expressed by differentiated colonic epithelial cells as well as resident immune cells in the GI tract including neutrophils, monocytes, and macrophages, and mast cells[16,17]. Cathelicidin expression has been reported to be increased in inflamed and noninflamed mucosa in ulcerative colitis patients[18]. Butyrate[18] and vitamin D[19, 20] are known inducers of cathelicidin expression on colonic epithelial cells and immune cells. Cathelicidin deficiency increases susceptibility to infection with enterohemorrhagic *E. coli* (EHEC)[21]. Vitamin D induction of cathelicidin in human colonic epithelial cells has been shown to inhibit *in vitro* *E. coli* growth[21]. Likewise, cathelicidin protects against colonization with epithelial adherent bacterial pathogens [22].

### Regenerating protein

Another class of antimicrobial peptides expressed in the GI tract include the soluble lectins belonging to the regenerating (Reg) Protein family. RegIII $\gamma$  and its human counterpart RegIII $\alpha$ , also known as Hepatocarcinoma-Intestine Pancreas/Pancreatitis-Associated Protein (HIP/PAP), are expressed by enterocytes and Paneth cells in response to microbial and inflammatory stimuli[23,24]. RegIII $\alpha$  selectively binds to cell wall peptidoglycan in gram-positive bacteria to induce pore formation[25]. RegIII $\beta$  interacts with surface Lipid A structures to target gram-negative bacteria[26]. In mice, RegIII $\gamma$  maintains physical separation between the gut microbiota and the intestinal epithelial surface and regulates bacterial colonization and intestinal immune responses by the microbiota[27]. In mice, RegIII is strongly induced in gut epithelial cells following bacterial reconstitution and colitis[28]. In human studies, Reg I $\alpha$ , Reg I $\beta$ , and Reg IV are overexpressed in colon mucosa with ulcerative colitis, whereas Reg IV is overexpressed in Crohn's disease[29].

### Metal sequestering antimicrobial peptides

Some antimicrobial peptides function by sequestering metal micronutrients which are required as co-factors for microbial growth. Lactoferrin is a secreted iron binding protein that is expressed by intestinal epithelial cells. Lactoferrin mediates its antimicrobial activity by sequestering free iron required for bacteria growth[30]. Lipocalin-2 (neutrophil gelatinase-associated lipocalin, GAL) is expressed by intestinal epithelial cells after stimulation by proinflammatory cytokines IL-17 and IL-22. Lipocalin-2 sequesters the siderophore enterobactin which then prevents bacteria cells from binding iron[31]. Calprotectin, a heterodimer consisting of S100A8 and S100A9, is produced by intestinal epithelial cells and neutrophils. Calprotectin inhibits bacterial growth by sequestering zinc and manganese during infection[32]. The cationic peptide hepcidin plays a key role in regulating iron homeostasis through its binding to the iron exporter ferroportin. During infection and inflammation, hepcidin is upregulated and subsequently limits iron availability to bacterial pathogens. Hepcidin has antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa*, and group A *Streptococcus*[33].

### Antimicrobial peptides with different mechanisms of action

Other AMPs of various mechanisms of action have also been characterized. Galectins are  $\beta$ -galactoside-binding lectins that can bind to galactose-containing glycans on glycoproteins and glycolipids. They are highly expressed by intestinal epithelial cells and innate immune cells. Galectin-3, -4, and -8 recognize human blood group B antigen-like determinants on the surface of *E. coli* O86 and have bactericidal activity. Galectin-3 can bind to lipopolysaccharide (LPS) on gram-negative bacteria. Galectin-8 targets damaged vesicles for autophagy during bacteria invasion[34,35]. Another mechanism involves enzymatic degradation of bacterial membranes. Lysozyme which is secreted by Paneth cells preferentially binds to gram-positive bacteria and degrades bacterial membranes by hydrolyzing peptidoglycan linkages[36]. AMPs also function as protease inhibitors such as elafin and secretory leukocyte protease inhibitor (SLPI). Elafin is produced by epithelial cells of mucosal surfaces including the GI tract. Elafin mediates its antimicrobial activity by binding to LPS from gram-negative bacteria and modulating macrophages[37]. SLPI is a major serine proteinase inhibitor that is expressed and apically secreted by human intestinal epithelium as well as Paneth cells, neutrophils, and macrophages. SLPI has antimicrobial activity against the enteric pathogen *Salmonella typhimurium* as well as gram-positive and gram-negative bacteria and fungi[38,39].

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## ANTIMICROBIAL PEPTIDES AND THE GUT MICROBIOME

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The appropriate maintenance of the gut microbiome is critical for health. In addition to offering competitive protection against pathogen growth, the microbiome regulates gut development[40] modulates digestion[41] and provides nutrients[42]. Thus, the microbiome must be carefully cultivated, without being permitted to proliferate excessively. However, the rapid renewal of epithelial layers, particularly in the gut where renewal rates are amongst the most rapid[43,44], poses a unique challenge for maintaining microbial composition and distribution. AMPs are a critical mechanism for regulating the microbiome, and act as part of a complex interplay between the gut microbiome, the innate immune system, and epithelium renewal. Reduced AMP production is associated with disorders such as IBD[45], which will be discussed in more depth in section III. In wounds or acute infections, multiple classes of AMPs are



rapidly upregulated, frequently through PAMP-dependent induction. Above threshold doses, they achieve rapid bacterial killing by synergistically targeting diverse yet critical microbial functions[46]. In contrast, direct interactions between AMPs and the gut microbiome occur at sub-lethal doses[47], though AMPs also act indirectly on the gut microbiome through the local modulation of immune response [48].

### **Evolutionary analysis of AMPs offers insights into AMP function**

Across a wide array of species, the regional control of which AMP classes are expressed acts in concert with local environmental conditions to fine-tune both the microbiome's spatial heterogeneities as well as bacterial phenotype[49]. The requirements for broad-spectrum pathogen resistance, coupled with carefully tuned microbiome maintenance, lead to fascinating AMP evolutionary behavior. While genes associated with immune defense are associated with rapid evolution, AMP amino acid sequences evolve more slowly than the genome average. Indeed, they can be highly conserved across multiple species[50]. The relatively slow evolution rate of AMP amino acid sequences therefore suggests that pathogen control is likely a result of a complex AMP mixture, and that any individual AMP exerts minimal co-evolutionary pressure[51,52].

Given the importance of microbiome composition for health, and in light of the highly conserved AMP amino acid sequences, one might expect strict control over AMP copy number and regulation. Unexpectedly, this is not what has been observed. While AMP coding sequences are highly conserved within a species, there is substantial variability in both copy number and regulatory sequences, as reviewed in [53]. This is particularly intriguing given that there is a high evolutionary cost associated with AMPs; when model organisms are propagated in germ-free environments, AMPs are rapidly lost[54]. Together, these data strongly suggest that the regulatory variability that is observed within humans may be a function of geography, specifically long-term local diets, local pathogens, and/or candidate microbiome components.

### **Dynamics of AMP-microbiome interactions**

AMP serve as key regulators of host-gut microbiota interactions in a bi-directional and highly dynamic process[55]. AMP can shape the composition of the gut microbiome. For example, sublethal doses of AMPs could prime *E. coli* to develop tolerance and increase persistence by production of curli or colonic acid[56]. Prior studies have demonstrated that species-specific AMP profiles in animals maintains species-specific bacterial communities. Loss-of-function experiments have also shown that antimicrobial peptide composition is a predictor of bacterial colonization[57]. Furthermore, AMP resistance patterns maintains the resilience of prominent gut commensals during perturbations such as inflammation[58]. Conversely, the gut microbiome produces a complex array of metabolites[59] that directly regulate AMP production and function [60,61]. For example, the microbiota metabolite short chain fatty acid promoted the production of the AMP RegIII $\gamma$  and  $\beta$ -defensins by intestinal epithelial cells[62].

Manipulation of gut microbiome composition has been shown to control AMP production and function. Cazorla *et al*[63] demonstrated that oral administration of probiotics in mice increased Paneth cell and intestinal antimicrobial activity. In addition, treatment of mice with VSL #3, a common probiotic used in patients with IBD, was associated with restoration of AMP gene expression in the small intestine and increased abundance of bacterial commensals in the gut[64]. Some probiotic strains produce AMP and has been proposed as a strategy to improve immune responses in immunocompromised patients[65]. Finally, fecal microbial transplant also modulates AMP expression in the GI tract. Teng *et al*[66] demonstrated that fecal microbial transplant of piglets resulted in increased expression in porcine  $\beta$ -defensins in the jejunum and subsequent increased gut *Firmicutes* and decreased *Bacteroides*.

### **Gut microbiome effects of different antimicrobial peptides**

Different locations and cellular origins of AMP production are superimposed along the GI tract. Defensins, the most abundant AMPs in the gut, are notable for their multiple disulfide bridges which confer substantial structural resistance to bacterial-derived peptidases[67]. Defensins exert antimicrobial activity through forming pores in target bacterial membranes. Above sufficient thresholds, this results in cell death. Although the effect of sub-lethal concentrations is still undergoing characterization in humans, it is notable that a similar strategy is used by plants[50]. Here, pore-forming AMPs are used to facilitate the release of endosymbiotic microbe-derived nutrients.

Local immune cell populations such as macrophages, T cells, and B cells[68] secrete both classes of defensins. The highly spatially restricted secretion of  $\alpha$ -defensins, in comparison to the ubiquitous secretion of  $\beta$ -defensins, strongly suggests that their role is likely to prevent bacterial overgrowth[61]. Indeed, Paneth cells are positioned just beneath the actively proliferating epithelial stem cells which are critical for epithelium renewal. Single-crypt studies show that Paneth cell degranulation of  $\alpha$ -defensins is induced by both gram-negative and gram-positive bacteria, regardless of whether they are alive or dead, as well as bacterial components such as lipopolysaccharide, lipoteichoic acid, lipid A, and muramyl dipeptide[69]. Furthermore, the antimicrobial products of Paneth cells are protective against *in vitro* microbial challenges many orders of magnitude ( $> 10^6$ ) higher than those encountered *in vivo*. Notably, degranulation is not induced by eukaryotic pathogens, including live fungi and protozoa[69]. While  $\alpha$ -defensin deficiencies in mouse models do not affect total bacterial load, they do result in reduced *Bacteroides* abundance and increased *Firmicutes* abundance[70].

$\beta$ -defensins act in the gut as a two-layered, ubiquitous defense system.  $\beta$ -defensin-1 is constitutively expressed at low levels, even in the gut of germ-free models[71].  $\beta$ -defensin-2 and  $\beta$ -defensin-3 can be further induced by the local microbiome, and additionally act as potent chemo-attractants for neutrophils and memory T cells[72]. In contrast to  $\alpha$ -defensins, cell culture models suggest that gut  $\beta$ -defensin induction may rely on live bacteria; pre-incubation of Caco-2 epithelial cells with *Enterococcus faecium* reduced *Salmonella typhimurium* uptake, while pre-incubation with heat-killed *E. faecium* did not[72]. Unlike  $\alpha$ -defensins, at least one ( $\beta$ -defensin-3) has anti-fungal activity[72].

Cathelicidins (in humans: LL-37) have broad anti-microbial and immunomodulatory function, and act to maintain epithelial barrier integrity[73,74]. Cathelicidins also have a two-tiered anti-microbial activity. While their primary mechanism of activity at high concentrations is to disrupt bacterial membranes, their immunomodulatory functions occur at substantially lower concentrations. Epithelial barrier integrity maintenance is accomplished primarily through increasing tight junction protein expression, as well as post-translational effects including the redistribution of tight junctions[75]. Together, this suggests that cathelicidins are primarily used when the epithelial barrier becomes compromised. Furthermore, LL-37 has also been shown to alter the composition of the gut microbiome in mice. Cathelicidin knockout mice had significantly more OTUs belonging to the phylum *Verrucomicrobia* and had lower amount of OTUs belonging to phylum *Proteobacteria* and the genus *Lactobacillus* than the other genotypes[76].

Reg III AMPs, primarily secreted by Paneth cells and epithelial cells[28,61], are soluble lectins that appear to primarily govern spatial relationships between the microbiome host tissues *via* the mucosa. In mice, Reg III $\beta/\gamma$  are co-regulated; Reg III $\alpha$  is the human ortholog[27,77]. Thinning of the mucosa driven by dietary restrictions in microbiota-accessible carbohydrates resulted in increased Reg III $\beta$ [78], as did increased mucosal inflammation[28]. Reg III $\gamma$ -/- mice exhibited increased mucosal bacterial burden and impaired spatial relationships between bacteria and their host tissues[27].

## FUNCTION AND MECHANISMS OF ANTIMICROBIAL PEPTIDES IN THE PATHOGENESIS OF IBD

### **Alpha defensins: HNP-1**

Several prior studies have linked defects or alterations in GI tract AMPs with the pathogenesis of IBD. Table 2 summarizes studies exploring the function and mechanisms of AMPs in IBD. HNPs and their role in IBD continues to be investigated. Maeda *et al*[79] found that mild transgenic overexpression of HNP-1 reduces the susceptibility to murine dextran sulfate sodium (DSS) induced colitis. Not only did the colon of HNP-1 transgenic mice show less tissue damage, but mice also had significantly lower disease activity index (DAI) scores when compared to wild type mice. Additionally, the authors found intraperitoneal injection of low dose HNP-1 mitigates DSS-induced colitis and results in reduced expression of pro-inflammatory cytokines in the colon of mice. This improvement of colitis from low-dose HNP-1 could be from its antimicrobial activity[79].

Furthermore, Hashimoto *et al*[80] found that intraperitoneal injection of high concentrations of HNP-1 exacerbate DSS-induced colitis in pathogen free (BALB/c) mice and severe combined immunodeficient (SCID) mice. Clinically, HNP-1 treated BALB/c mice had significantly decreased weight and colon length as well as significantly increased DAI score, histologic score and myeloperoxidase (MPO)

Table 2 Antimicrobial peptides in preclinical models of inflammatory bowel disease

Ref.	Antimicrobial peptides (expression location)	Antimicrobial peptide delivery	Preclinical models (animal, human cell culture)	Key findings
Maeda <i>et al</i> [79]	Alpha defensins: Human neutrophil peptide-1 (HNP-1) murine colon	Genetic overexpression, intraperitoneal	Murine dextran sulfate sodium (DSS) colitis	Mild transgenic overexpression of HNP-1 reduces the susceptibility to DSS-induced colitis; Intraperitoneal injection of low-dose HNP-1 ameliorates DSS-induced colitis; The amelioration of colitis by low-dose HNP-1 may be explained by its indirect antimicrobial activity
Hashimoto <i>et al</i> [80]	Alpha defensins: Human neutrophil peptide-1 (HNP-1); Murine colon, human colon cells	Intraperitoneal	Murine dextran sulfate sodium (DSS) colitis, SCID mice, human colon cell cultures	Body weight and colon length significantly decreased, and the disease activity index score, histologic score, and myeloperoxidase activity significantly increased in HNP-1-treated mice compared with PBS-treated mice. High concentrations of HNP-1 aggravate DSS-induced colitis, including upregulated expression of such macrophage-derived cytokines as IL-1 $\beta$
Han <i>et al</i> [82]	Porcine $\beta$ -defensin (pBD)2: Murine colon	Intrarectal	Murine dextran sulfate sodium (DSS) colitis, human colon cell cultures	Administration of pBD2 effectively attenuated colonic inflammation in mice with DSS induced colitis. pBD2 reduced the increased serum and colon levels of TNF- $\alpha$ , IL-6 and IL-8 all caused by DSS. The effects of pBD2 appeared to be through upregulation of the expression of genes associated with tight junctions and mucins
Koeninger <i>et al</i> [81]	Beta defensins: human beta defensin 2 (HBD-2): Murine colon	Subcutaneous	Murine dextran sulfate sodium (DSS) colitis, 2,4,6-trinitrobenzenesulfonic acid (TNBS) colitis, T cell transfer colitis model	Treatment improved disease activity index and hindered colitis-induced body weight loss on par with anti-TNF- $\alpha$ and steroids. Mechanistically, hBD2 engaged with CCR2 on its DC target cell to decrease NF- $\kappa$ B, and increase CREB phosphorylation, hence curbing inflammation
Koon <i>et al</i> [73]	Cathelicidin (LL-37): Murine colon	Genetic knockouts	Murine dextran sulfate sodium (DSS) colitis	Increased expression of cathelicidin in the colon of DSS-exposed mice; Compared with WT mice, cathelicidin KO mice developed a more severe form of DSS-induced colitis; Cathelicidin protects against induction of colitis in mice; Increased expression of cathelicidin in monocytes and experimental models of colitis involves activation of TLR9-ERK signaling by bacterial DNA
Fabisiak <i>et al</i> [83]	Cathelicidin (LL-37) KR-12 (active fragment of LL-37): Murine colon	Intraperitoneal	Murine dextran sulfate sodium (DSS) colitis, 2,4,6-trinitrobenzenesulfonic acid (TNBS) colitis, T cell transfer colitis model	LL-37 and KR-12 (1 mg/kg, ip, twice daily) showed a decrease in macroscopic and ulcer scores in the acute TNBS-induced model of colitis. KR-12 (5 mg/kg, ip, twice daily) reduced microscopic and ulcer scores in the semi-chronic and chronic TNBS-induced models of colitis compared with inflamed mice
Yoo <i>et al</i> [84]	Cathelicidin (LL-37): Murine colon	Intracolonic, intravenous	2,4,6-trinitrobenzenesulfonic acid (TNBS) Colitis,	Intracolonic cathelicidin (mCRAMP peptide) administration or intravenous delivery of lentivirus-overexpressing cathelicidin gene significantly reduced colonic col1a2 mRNA expression in TNBS-exposed mice compared with vehicle administration. Cathelicidin can reverse intestinal fibrosis by directly inhibiting collagen synthesis in colonic fibroblasts
Tai <i>et al</i> [85]	Cathelicidin (LL-37): Murine colon	Genetic knockouts, intrarectal	Murine dextran sulfate sodium (DSS) colitis	Cathelicidin knockout mice had more severe symptoms and mucosal disruption than the wild-type mice in response to DSS colitis. Intrarectal administration of plasmids encoding cathelicidin reversed colitis in cathelicidin knockout mice
Gubatan <i>et al</i> [21]	Cathelicidin (LL-37): Murine colon, human colon cells	Intrarectal	Murine dextran sulfate sodium (DSS) colitis, human colon cell cultures	Vitamin D-induced cathelicidin in human colonic epithelial cells suppressed <i>Escherichia coli</i> growth. Intrarectal cathelicidin reduced the severity of DSS colitis but did not mitigate the impact of colitis on microbial composition
Motta <i>et al</i> [91]	Elafin: Murine colon	Transgenic expression, adenoviral delivery	Murine dextran sulfate sodium (DSS) colitis, 2,4,6-trinitrobenzenesulfonic acid (TNBS) colitis	In mice given TNBS or DSS, transgenic expression of elafin protected against the development of colitis. Similarly, adenoviral delivery of Elafin significantly inhibited inflammatory parameters. Elafin modulated a variety of inflammatory mediators <i>in vitro</i> and <i>in vivo</i> and strengthened intestinal epithelial barrier
Ogawa <i>et al</i> [28]	RegIII (HIP/PAP): Murine colon	Endogenous expression	Murine dextran sulfate sodium (DSS) colitis	Epithelial expression of Reg III or HIP/PAP was induced under mucosal inflammation initiated by exposure to commensal bacteria or DSS as well as inflamed IBD colon

Jiang <i>et al</i> [93]	Donkey milk lysozyme (DML): Murine colon	Oral	Murine dextran sulfate sodium (DSS) colitis	DML ameliorated weight loss, colon damage and mucosal inflammation in DSS colitis mice. DML improved mechanical barrier function and increased gut microbiota composition diversity, promoting growth of probiotics and inhibiting pernicious bacteria
Reardon <i>et al</i> [92]	Secretory leukocyte peptidase inhibitor (SLPI): Murine colon	Genetic SLPI deficiency, oral	Murine dextran sulfate sodium (DSS) colitis, T cell transfer colitis model	Tslp <sup>-/-</sup> mice lead to endogenous SLPI deficiency which exacerbated DSS colitis. Treatment with recombinant SLPI (rSLPI) reduced DSS-induced mortality in Tslp <sup>-/-</sup> mice
Togawa <i>et al</i> [95]	Lactoferrin: Rat colon	Oral	Rat dextran sulfate sodium (DSS) colitis	DSS-induced colitis was attenuated by oral administration of lactoferrin in a dose-dependent manner. Reduced inflammation in response to lactoferrin was correlated with the significant induction of the anti-inflammatory cytokines and with significant reductions in the pro-inflammatory cytokines
Shanmugam <i>et al</i> [96]	Hepcidin: Murine colon	Endogenous expression	Murine dextran sulfate sodium (DSS) colitis, T cell transfer Colitis model	TNF $\alpha$ inhibits hepcidin expression in two distinct types of innate colitis, with down-regulation of Smad1 protein playing an important role in this process

activity when compared to control mice. Furthermore, inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$  were significantly higher in colon of HNP-1 treated mice. In both murine models, an increased recruitment of F4/80-positive macrophages in the inflamed colonic mucosa after HNP-1 injection has been observed. This enhanced disease activity is thought to be due in part to HNP-1 induced cytokine production in macrophages.

### **Beta defensins: Porcine B-defensin and hBD-2**

Beta defensins are epithelial cell derived AMPs that have immunomodulating properties. Koeninger *et al*[81] found that subcutaneous recombinant hBD-2 reduced intestinal inflammation in three distinct animal models of IBD: chemically induced mucosal injury (DSS), loss of mucosal tolerance (TNBS), and T cell transfer into immunodeficient recipient mice. Mice treated with hBD-2 had less weight loss, better stool score and improved DAI scores in comparison to the T cell colitis control group. Additionally, mice given hBD-2 had less mucosal damage and inflammation as they maintained crypt anatomy and had reduced colon weight.

In addition to the protective effects of hBD-2, Han *et al*[82] found that intrarectal administration of porcine beta-defensin 2 (pBD2) ameliorated colonic inflammation in mice during the induction of DSS-induced colitis. Mice in the pBD2 plus DSS group had less symptoms, including less weight loss, firmer and less bloody stools compared to the DSS-treated group. Mice treated with pBD2 plus DSS also had less evidence of macroscopic and histological colitis in addition to reduced production of TNF- $\alpha$ , IL-6 and IL-8 when compared to the DSS-treated group. Through colon cell culture, the effects of pBD2 seemed to occur *via* an upregulation of genes associated with tight junctions and mucins. This may explain how pBD2 can improve DSS-induced changes in the mucosa and paracellular permeability through possible activation of the NF- $\kappa$ B signaling.

### **Cathelicidin (LL-37)**

Koon *et al*[73] demonstrated that genetic knockout of LL-37 in mice had more severe forms of DSS-induced colitis and that inflamed colon in wild type mice in DSS colitis

models had increased cathelicidin expression. The authors suggested that this upregulation of cathelicidin involves activation of TLR9-ERK signaling from bacterial DNA, which may play a role in the development of colitis. In addition to its protection against the induction of colitis, Fabisiak *et al*[83] showed that intraperitoneal injection of LL-37, and its shortest active metabolite, KR-12, decreases ulcer and macroscopic scores in DSS-induced and TNBS-induced models of colitis. The study showed that intraperitoneal injection of KR-12 altered the microbiomes of TNBS-induced colitis mice by reducing total and *E. coli* group bacteria.

In addition to the protective and antimicrobial properties of LL-37, Yoo *et al*[84] found that intracolonic cathelicidin or intravenous delivery of lentivirus-overexpressing cathelicidin gene significantly reduced colonic collagen deposition TNBS-induced colitis mice when compared to TNBS-induced mice not receiving LL-37. These results suggest that cathelicidin reverses fibrosis in the intestines *via* inhibition of collagen synthesis in colonic fibroblasts.

Another unique property of LL-37 was investigated by Tai *et al*[85], who describe that intrarectal administration of plasmids containing cathelicidin to DSS-induced colitis mice reestablished colonic mucus thickness *via* increased expression of mucin genes and reduced severe symptoms compared to cathelicidin knockout mice with DSS-induced colitis. This increase in mucin genes protected against mucosal damage and was linked to the activation of MAP kinase.

Gubatan *et al*[21] found that cathelicidin is a key mediator of the protective role of vitamin D in ulcerative colitis (UC). The authors found higher levels of 25(OH)D correlate with increased levels of both serum and colonic LL-37 in UC patients, and these higher levels are associated with decreased histologic inflammation and probability of clinical relapse. Intrarectal LL-37 reduced the severity of DSS-induced colitis in mice, but did not alter the intestinal microbial imbalance, whereas 25(OH)D-induced cathelicidin in human colonic epithelial cells suppressed *E.coli* growth. The study demonstrated that 25(OH)D is an independent predictor of cathelicidin in UC patients in remission and may protect against microbial associated gut inflammation.

Arachidonic acid and its metabolism also play a role in the regulation of antimicrobial peptides in inflammatory bowel disease. Arachidonic metabolites such as leukotrienes and are elevated in both animal models of colitis and patients with IBD [86]. Leukotrienes have been shown to trigger release of human cathelicidin from neutrophils[87], whereas prostaglandins suppress cathelicidin in human macrophages [88]. In addition, cyclooxygenase-2 (COX-2), an enzyme that metabolizes arachidonic acid, is also induced in colonic epithelial cells in IBD[89]. Cox-2 selective inhibitors have been shown to inhibit production of human beta defensins but not cathelicidin [90].

### **Elafin**

Motta *et al*[91] showed that in TNBS or DSS-induced mouse models of colitis, transgenic expression of elafin or disruption of enzymes that elafin inhibits protected against development of colitis. Transgenic mice expressing elafin had reduced inflammation as measured by a reduction in macroscopic tissue damage and myeloperoxidase (MPO) activity when compared to TNBS or DSS-induced mice that were not expressing elafin. Authors showed that adenoviral delivered elafin inhibited inflammatory parameters. The authors demonstrated that elafin is involved in inflammatory mediators and its protective effect could in part be from a bolstering of epithelial and mucosal barriers.

### **SLPI**

Reardon *et al*[92] reported that thymic stromal lymphopoietin-deficient (TSLP-/-) mice led to endogenous SLPI deficiency, which prevented recovery from DSS-induced colitis and resulted in death. The authors demonstrated that the mechanism by which the absence of SLPI prevents healing of the colon is from increased neutrophil elastase (NE) activity in TSLP-/- mice. When TSLP-/- mice were treated with oral recombinant SLPI (rSLPI) there was reduced DSS-induced mortality.

### **Reg III (HIP/PAP)**

Ogawa *et al*[28] aimed to identify genes that were modulated by bacterial flora to better understand mucosal inflammation in IBD patients. The authors found that expression of Reg III (HIP/PAP) was increased in DSS-induced colitis. Furthermore, the upregulation of Reg III may be due to an increase in the acute phase reactant IL-6 that occurs during gut inflammation.



**Donkey milk lysozyme**

Donkey milk contains high lysozyme levels and was studied by Jiang *et al*[93] due to its antimicrobial properties. Authors found that mice given donkey milk lysozyme (DML) orally in a DSS-induced colitis model had improved symptoms of colitis measured by a reduction in weight loss, loose stools, rectal bleeding and mucosal inflammation. The authors showed that 50% DML treatment brought cytokines, TNF- $\alpha$  and IL-13, a pleiotropic cytokine that has proinflammatory effects on intestinal epithelial cells resulting in apoptosis and epithelial barrier dysfunction in intestinal inflammation[94] back to basal levels similar to control mice. They hypothesized that DML improves the intestinal barrier by increasing expression of tight junction proteins in the colon. They also presume that DML increases gut microbiota diversity and reduces detrimental bacteria thereby restoring the gut microflora.

**Lactoferrin**

Lactoferrin, a known immunomodulator, was studied by Togawa *et al*[95] and was found to reduce DSS-induced colitis in a dose-dependent manner after oral administration to rats. The DAI, shortening of colon length, histological/macroscopic damage score, tissue levels of MPO activity, WBC, and reduction in hemoglobin were decreased when DSS-induced colitis rats were treated with lactoferrin. The authors postulate that the protective properties of lactoferrin were tied to its modulation of the immune system by reducing pro-inflammatory cytokines TNF- $\alpha$ , IL-1B and IL-6 as well as the augmented levels of anti-inflammatory cytokines IL-4 and IL-10 in colonic tissue of DSS-induced colitis rats given lactoferrin.

**Hepcidin**

Hepcidin is regulator of iron metabolism and is upregulated during the inflammation in IBD, often resulting in anemia. Shanmugam *et al*[96] investigated the mechanisms that control hepcidin during periods of inflammation. They showed that the pro-inflammatory cytokine TNF- $\alpha$  inhibits hepcidin in both a DSS-induced colitis and T cell transfer colitis model in mice with downregulation of Smad1 protein mediating this effect.

## TRANSLATIONAL APPLICATIONS OF ANTIMICROBIAL PEPTIDES AS BIOMARKERS IN PATIENTS WITH IBD

The diagnosis and long-term monitoring of IBD commonly involve invasive and costly endoscopy combined with histologic screening. Consequently, a biomarker that reflects the ongoing severity of disease is attractive as a non-invasive, cost-effective, and convenient alternative for diagnosing new IBD cases and identifying flares of disease. Given their involvement in disease pathophysiology, AMPs represent such potential markers, and several have been studied to determine their utility in differentiating CD and UC from other conditions, such as celiac disease and IBS, as well as active from quiescent disease states. In addition to reflecting ongoing severity of inflammation, several AMPs have shown promise as predictors of relapse, complication risk, and treatment response in the setting of IBD. Table 3 summarizes the application of AMPs as biomarkers in IBD.

**Calprotectin**

Among all known AMPs, calprotectin is the one most frequently used in the clinical diagnosis and monitoring of IBD. It has been known for decades that fecal calprotectin (FC) concentrations are markedly increased in the setting of both CD and UC[97-100]. Elevated FC is a highly sensitive marker and is thus a particularly useful tool in the initial diagnosis and discrimination of IBD from non-inflammatory causes of abdominal discomfort and bowel dysfunction like IBS[97-103]. Based on this diagnostic utility, current practice guidelines from the World Gastroenterology Organization support measuring FC in the initial work-up of suspected IBD in both adult and pediatric patients[101,102]. Recent research has supported using FC measurements for the early diagnosis of IBD in at-risk populations, such as patients with ankylosing spondylitis[104].

FC is also particularly useful in the evaluation of IBD severity and the early identification of disease flares[104-106]. Data suggest that FC concentrations positively correlate with histologic inflammation in IBD, and assays can be used to accurately classify inactive, mild, moderate, and severe disease[102,103]. Cut-off values of fecal



**Table 3 Biomarker applications of antimicrobial peptides in patients with inflammatory bowel disease**

Ref.	Antimicrobial peptides	Type of IBD	Biomarker application	Key findings
Holgersen <i>et al</i> [110]	Alpha defensins 5 and 6 (DEFA5/DEFA6)	UC	IBD diagnosis	Marked upregulation of DEFA5 and DEFA6 in terminal ileal biopsies of inflamed ulcerative colitis relative to normal controls
Wehkamp <i>et al</i> [111]	Alpha defensin (HD -5/6)	UC/CD	IBD diagnosis	HD-5/6 both decreased in ileal Crohn's, and this correlated with a decrease in transcription factor Tcf-4, a known regulator of Paneth cell differentiation. Normal levels were observed in UC and colonic Crohn's
Yamaguchi <i>et al</i> [112]	Alpha defensin (HNP1-3), beta-defensin (HBD-2)	UC/CD	Disease activity	HNP-1-3 all elevated in IBD patients, while HBD-2 levels normal; serum HNP1-3 levels correlated with disease severity for Crohn's
Kanmura <i>et al</i> [113]	Alpha defensin (HNP)	UC/CD	Disease activity	Fecal-HNP levels were markedly elevated in both UC and Crohn's, but slightly more so in Crohn's; F-HNP was significantly higher during flares of UC than remission. For UC, HNP levels correlated with Mayo endoscopic score
Cunliffe <i>et al</i> [114]	Alpha defensin (HNP 1-3)	UC/CD	Disease activity	Surface epithelial cells strongly immunoreactive for neutrophil defensins and lysozyme were seen in active ulcerative colitis and Crohn's disease (but not normal or inactive IBD) mucosal samples. Many of these cells coexpressed both antimicrobial proteins.
Tran <i>et al</i> [116]	Cathelicidin	UC/CD	Disease activity	Cathelicidin levels were significantly increased in IBD patients and were inversely correlated with CD activity. In moderate to severe IBD, higher cathelicidin levels before treatment correlated with better prognosis.
Krawiec <i>et al</i> [115]	Cathelicidin	UC/CD	IBD diagnosis	Cathelicidin was significantly increased in patients with ulcerative colitis ( $1073.39 \pm 214.52$ ng/mL) and Crohn's disease ( $1057.63 \pm 176.03$ ng/mL) patients compared to controls ( $890.56 \pm 129.37$ ng/mL) ( $P = 0.0003$ )
Gubatan <i>et al</i> [21]	Cathelicidin	UC	Disease activity, clinical relapse	In ulcerative colitis patients, serum 25(OH)D positively correlated with serum and colonic cathelicidin. Higher serum cathelicidin is associated with decreased risk of histologic inflammation and clinical relapse but not independent of 25(OH)D or baseline inflammation
Borkowska <i>et al</i> [118]	Lactoferrin	UC/CD	IBD diagnosis, disease activity	Fecal concentration of lactoferrin in children with IBD was significantly higher than in the controls. The sensitivity and specificity were 80.7% and 92.7%, respectively, and its positive and negative prognostic values were 96.8% and 63.3%, respectively
Sugi <i>et al</i> [119]	Lactoferrin, lysozyme	UC/CD	Disease activity	Lactoferrin and lysozyme were significantly increased in the active phases of CD and UC relative to inactive. They both correlated with fecal Hb concentration in UC, and with alpha 1-AT concentration in CD
Sidhu <i>et al</i> [120]	Lactoferrin	UC/CD	IBD diagnosis, disease activity	Lactoferrin levels were significantly higher in IBD patients compared with IBS/healthy controls ( $P < 0.001$ ). The sensitivity, specificity, positive and negative predictive values of lactoferrin in distinguishing active IBD from IBS/healthy controls were 67% and 96%, 87% and 86.8% respectively
Wang <i>et al</i> [121]	Lactoferrin	UC/CD	IBD diagnosis	FL test has a high sensitivity (82%) and specificity (95%) for the discrimination of patients with IBD against non-IBD patients
Kane <i>et al</i> [122]	Lactoferrin	UC/CD	Disease activity	Fecal lactoferrin was 90% specific for identifying inflammation in patients with active IBD. Elevated fecal lactoferrin was 100% specific in ruling out IBS
Turner <i>et al</i> [123]	Lactoferrin	UC	IBD diagnosis	Lactoferrin levels significantly were elevated in pediatric UC patients, but were not responsive to change or predictive of response to corticosteroids
Wang <i>et al</i> [132]	Elafin	CD	Disease activity, intestinal strictures	High serum elafin levels were associated with a significantly elevated risk of intestinal stricture in CD patients. Serum elafin levels had weak positive correlations with clinical disease activity but not endoscopic disease activity
Zhang <i>et al</i> [133]	Elafin	UC/CD	Disease activity	The expression of elafin mRNA in peripheral blood in active IBD patients is decreased, which may be correlated with the activity of IBD, and negatively correlated with corresponding disease activity score
Motta <i>et al</i> [130]	Elafin	UC	Disease activity	Study identified a previously unrevealed production of elastase 2A (ELA2A) by colonic epithelial cells, which was enhanced in IBD patients.

				Study demonstrated that ELA2A hyperactivity is sufficient to lead to a leaky epithelial barrier and modified the cytokine gene expression profile with an increase of pro-inflammatory cytokine transcript
Schmid <i>et al</i> [134]	Elafin and SLPI	UC/CD	Disease activity	Levels of mRNA and immunostaining of the antiproteases elafin and SLPI were enhanced strongly in inflamed versus noninflamed UC
Frol'ová <i>et al</i> [124]	Galectin-3	UC/CD	Disease activity	Serum concentrations were significantly increased in specimen of patients with active and remission-stage ulcerative colitis and Crohn's disease (relative to healthy controls)
Yu <i>et al</i> [125]	Galectin-1, -3	UC/CD	IBD diagnosis	Serum level of galectin-1 and -3, but not galectins-2, -4, -7 and -8, were significantly higher in IBD patients than in healthy people. None of the galectins however were able to distinguish active disease from remission in UC or CD
Tibble <i>et al</i> [97]	Calprotectin	CD	IBD diagnosis	The cross-sectional study showed a sensitivity of 96% for calprotectin in discriminating between normal subjects and those with Crohn's disease. With a cutoff point of 30 mg/L fecal calprotectin has 100% sensitivity and 97% specificity in discriminating between active CD and irritable bowel syndrome
Moniuszko <i>et al</i> [100]	Calprotectin	UC/CD	Disease activity, progression	Rapid bedside FC test reliably detected disease flares in patients with both UC and CD. FC levels increased even with early signs of inflammations; values were lower in isolated small bowel disease for CD patients
Pous-Serrano <i>et al</i> [101]	Calprotectin	CD	Disease activity	FC was the only inflammatory marker significantly associated with the degree of histologic inflammation in surgical specimens
Scheopfer <i>et al</i> [102]	Calprotectin	CD	Disease activity	FC correlates more closely with endoscopic disease activity than CRP, blood leukocytes, and CDAI. It was the only marker that reliably discriminated inactive from mild, moderate, and highly active disease, underscoring its value in disease monitoring
Ferreiro-Iglesias <i>et al</i> [103]	Calprotectin	UC/CD	Relapse	In IBD patients under Infliximab maintenance therapy, high FC levels allow predicting relapse within the following 2 mo. Long-term remission is associated with low calprotectin levels
Klingberg <i>et al</i> [104]	Calprotectin	CD	IBD diagnosis, treatment monitoring	FC was a useful predictor of the development of CD in patients with ankylosing spondylitis; NSAIDs increase FC levels; FC levels drop following TNF-blocker treatments
Godny <i>et al</i> [109]	Calprotectin	CD	Treatment monitoring	FC decreases following successful diet-based treatment of active CD
Karaskova <i>et al</i> [126]	Hepcidin	UC/CD	IBD diagnosis	Serum hepcidin concentration was significantly decreased in IBD children compared with controls; levels did not differ significantly between patients with CD and UC
Martinelli <i>et al</i> [128]	Hepcidin	UC/CD	IBD diagnosis, iron deficiency Monitoring	Serum hepcidin was significantly higher in IBD patients with active disease versus healthy and celiac patients. Hepcidin levels corresponded with iron malabsorption and other inflammatory biomarkers like ESR
Aksan <i>et al</i> [129]	Hepcidin	UC/CD	Response to iron supplementation	Higher hepcidin and other inflammatory markers correlated with decreased iron absorption follow supplementation
Zollner <i>et al</i> [127]	Lipocalin	CD	Clinical and endoscopic activity	Fecal lipocalin-2 levels of 78.4 and 0.56 µg/g in Crohn's disease patients for clinical and endoscopic activity, respectively, corresponded well with fecal calprotectin levels in UC patients ( $R = 0.87$ , $P < 0.001$ )

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease.

calprotectin to differentiate active disease *vs* remission in patients with IBD have been previously evaluated[107]: A cutoff value of 50 mg/g had a pooled sensitivity of 0.92 and specificity of 0.60 (0.52–0.67), a cutoff value of 100 mg/g had a pooled sensitivity of 0.84 and specificity of 0.66, a cutoff value of 250 mg/g had a pooled sensitivity of 0.80 (0.76–0.84) and specificity of 0.82 (0.77–0.86). Decreased levels of FC after therapy are associated with clinical, endoscopic and histological improvement with a normal-

ization of FC (< 50 mg/g) signifying deeper remission[108].

Notably, FC has been found to correlate more strongly with IBD activity than other markers of inflammation, including C-reactive protein and blood leukocytes[104,105]. FC elevations are more pronounced in patients with pan-colonic CD than in those with isolated small bowel disease, indicating that concentrations may reflect disease location[105]. Rapid bedside and at-home FC assays are currently available as tools for monitoring IBD activity, with elevated concentrations detectable early in disease flares [104,109]. FC can be used to predict the risk of relapse for patients with quiescent CD and UC[105]. FC monitoring also plays a role in the treatment of IBD, as levels decrease following effective medical and diet-based management of disease[107,110].

Despite its clear clinical utility, FC remains an imperfect biomarker for the diagnosis and monitoring of IBD. Like many other inflammatory biomarkers, FC is not 100% specific for IBD. Other factors, including the use of NSAIDs, can also result in elevated FC, thereby introducing potential inaccuracy when using the biomarker to evaluate IBD[104,105].

### **Defensins**

Previous studies have revealed increased defensin concentration at the intestinal surface epithelium in the setting of IBD, and dysregulation of defensin gene expression has been proposed as one pathogenic mechanism of disease[110,111]. Thus, defensins have been explored as potential biomarkers of IBD[112,113]. Among the 10 known human defensins, the alpha defensins HNP-1, HNP-2, and HNP-3 have been found to be significantly elevated in the sera of both UC and CD patients[114,115]. In CD, serum HNP-1-3 Levels have been shown to correlate with disease severity, as measured by Crohn's disease Activity Index (CDAI)[114]. In UC, these levels are significantly greater in active disease than in inactive disease, and serum HNP-1-3 Levels decrease following successful treatment with corticosteroids[113]. Notably, serum HNP-1-3 Levels do not decrease following corticosteroid administration in non-responders, signifying the potential use of defensins in the monitoring of treatment efficacy[114]. Fecal HNP-1-3 Levels are also significantly elevated in both CD and UC as well, with greater elevations measured during UC flares than in remission[113]. In the same study, fecal HNP-1-3 Levels correlated more closely with endoscopic severity than calprotectin. Results involving the ability to differentiate between UC and CD using defensin levels remain mixed[110-113].

### **Cathelicidin**

Significantly elevated levels of serum LL-37 have been detected in both adult and pediatric IBD cohorts[115,116]. Multiple studies have indicated that cathelicidin can be used to reliably differentiate both CD and UC from healthy controls, reflecting the AMP's potential diagnostic utility[115,116]. While cathelicidin levels are increased in both active and remission-stage IBD patients relative to controls, these levels seem to inversely correlate with disease activity, histologic inflammation, and risk of clinical relapse[21,116,117]. In moderate to severe IBD, higher serum cathelicidin prior to treatment is associated with better prognosis and may therefore serve as a predictor of treatment response[21]. Cathelicidin may also be a useful indicator of complication risk, as reduced serum levels correlate with significantly increased risk of intestinal stricture in CD[117]. Serum levels positively correlate with 25(OH)D levels, and the apparent protective effect of elevated cathelicidin is likely at least partially dependent on this increase in vitamin D[21].

### **Lactoferrin**

Lactoferrin is among the most thoroughly explored AMPs in the diagnosis and clinical evaluation of IBD. Fecal concentrations of lactoferrin are consistently elevated among both children and adults with IBD relative to healthy controls[118-123]. While estimates of fecal lactoferrin sensitivity in identifying CD and UC vary, several studies have confirmed the AMP's utility as a highly specific marker of IBD-related inflammation[120-122]. This specificity makes lactoferrin a particularly valuable biomarker for differentiating IBD from IBS, with studies indicating that lactoferrin levels can discriminate between the two conditions with a specificity at or near 100%[119-121]. Lactoferrin levels positively correlate with disease activity, with significantly higher fecal concentrations found in those with moderate to severe IBD relative to those with mild or inactive disease[122]. Unlike some of the other AMPs, lactoferrin has not been shown to predict responsiveness to corticosteroid treatment, and only insignificant concentration changes have been detected following both effective and ineffective treatment regimens[123].

### Galectin

Many members of the galectin family of proteins have been studied as potential biomarkers of IBD. Though several galectins are known to be expressed by intestinal epithelial cells, only galectin-1 and -3 have been shown to be significantly elevated in the serum of IBD patients[124,125]. Unlike those of galectin-1 and -3, serum levels of galectins-2, -4, -7, and -8 have not been shown to differentiate IBD patients from healthy controls[125]. Of note, galectin-1 and -3 Levels cannot reliably distinguish active from remission-stage CD or UC, nor can they distinguish CD and UC from each other[125,126]. Evidence also suggests that galectin-1 is a slightly more sensitive marker of IBD than galectin-3[125]. Nevertheless, galectins-1 and -3 may have use as biomarkers either alone or when combined with other molecules, and their upregulation in the intestinal cells of IBD patients may indicate their potential as therapeutic targets[124,125].

### Hepcidin

Data regarding the utility of hepcidin as a diagnostic biomarker remain mixed[126-129]. However, given hepcidin's crucial role in regulating iron absorption, the AMP may be useful in the monitoring of iron deficiency and related anemia, which are two common comorbidities seen in IBD patients[126,127]. These comorbidities are most frequently seen in pediatric IBD patients[126,127]. Consequently, multiple studies have aimed to elucidate the relationship between hepcidin expression and these comorbidities in pediatric IBD cohorts. In pediatric patients with IBD, elevated hepcidin levels negatively correlate with iron absorption and serum iron levels[125, 126]. Elevated hepcidin corresponds with decreased response to iron supplementation in these patients, suggesting that the biomarker may serve a role in predicting response to oral iron supplementation in the setting of IBD[129].

### Elafin

Elafin is known to be markedly upregulated in the intestinal mucosa of UC patients [130,131]. Intestinal expression seems to correlate closely with disease progression, as elevated concentrations are detectable in the right colon of patients with pan-colonic disease, but not those with exclusively left-sided disease[130]. This finding is further supported by enhanced colonic mRNA immunostaining in inflamed relative to non-inflamed UC samples[131]. While serum elafin levels are increased in UC patients relative to healthy controls, some evidence suggests an inverse correlation between serum elafin and disease severity within UC cohorts[131,132]. Among UC patients, significantly elevated serum elafin tends to correlate with decreased disease activity scores, with the highest elafin levels measured during disease remission[133,134]. Data involving elafin as a biomarker in CD remain mixed, with most results indicating only weak correlations between elafin and CD activity[132-134]. However, serum elafin measurements may play a role in the evaluation of complication risk in CD, as elevations are significantly associated with increased risk of intestinal stricture[132].

## CONCLUSION

AMPs produced by innate immune cells of the GI tract and cells that support barrier function such intestinal epithelial cells and Paneth cells play critical roles in protecting against enteric pathogens while maintaining tolerance to support a complex ecosystem of commensal gut microbiota. These highly dynamic molecules have broad spectrum antimicrobial activity against bacteria, fungi, and enveloped viruses and mediate their protective effects through diverse mechanisms of action from disrupting cell membranes, binding microbial components such as LPS, and sequestering metal co-factors to limit microbial growth. AMPs also play major roles in regulating gut microbiome composition and spatial relationships between the microbiota and intestinal barrier.

Defects in endogenous AMP expression and function have been linked with intestinal inflammation in mice. Conversely, exogenous delivery of AMPs such as defensins, cathelicidin, and elafin have been shown to attenuate intestinal inflammation in murine models of IBD. AMPs such as calprotectin and lactoferrin have found clinical applications as biomarkers of intestinal inflammation in patients with IBD. Other AMPs including alpha- and beta-defensins, cathelicidin, and elafin may be useful biomarkers for disease activity and predicting clinical outcomes in patients with IBD. Although the protective effects of AMPs have been demonstrated in murine models of IBD, there are currently no AMP-based therapies approved or in clinical

trials for IBD. Future studies should focus on translation of AMPs as potential therapies in patients with IBD. Several challenges with AMPs including limited stability due to enzymatic degradation by endogenous proteases[135,136] and cross-reactivity of AMPs with host cells leading to cytotoxicity[137] pose major barriers to their application as therapies. Biochemical modifications to enhance AMP stability, selectivity, and delivery are being explored[46,137].

## REFERENCES

- 1 **Bengmark S.** Ecological control of the gastrointestinal tract. The role of probiotic flora. *Gut* 1998; **42**: 2-7 [PMID: 9505873 DOI: 10.1136/gut.42.1.2]
- 2 **Chang JT.** Pathophysiology of Inflammatory Bowel Diseases. *N Engl J Med* 2020; **383**: 2652-2664 [PMID: 33382932 DOI: 10.1056/NEJMra2002697]
- 3 **Cunliffe RN, Mahida YR.** Expression and regulation of antimicrobial peptides in the gastrointestinal tract. *J Leukoc Biol* 2004; **75**: 49-58 [PMID: 14525966 DOI: 10.1189/jlb.0503249]
- 4 **Chung LK, Raffatellu M.** G.I. pros: Antimicrobial defense in the gastrointestinal tract. *Semin Cell Dev Biol* 2019; **88**: 129-137 [PMID: 29432952 DOI: 10.1016/j.semcdb.2018.02.001]
- 5 **Bauer F, Schweimer K, Klüber E, Conejo-Garcia JR, Forssmann WG, Rösch P, Adermann K, Sticht H.** Structure determination of human and murine beta-defensins reveals structural conservation in the absence of significant sequence similarity. *Protein Sci* 2001; **10**: 2470-2479 [PMID: 11714914 DOI: 10.1110/ps.24401]
- 6 **Wehkamp J, Chu H, Shen B, Feathers RW, Kays RJ, Lee SK, Bevins CL.** Paneth cell antimicrobial peptides: topographical distribution and quantification in human gastrointestinal tissues. *FEBS Lett* 2006; **580**: 5344-5350 [PMID: 16989824 DOI: 10.1016/j.febslet.2006.08.083]
- 7 **Ogushi K, Wada A, Niidome T, Mori N, Oishi K, Nagatake T, Takahashi A, Asakura H, Makino S, Hojo H, Nakahara Y, Ohsaki M, Hatakeyama T, Aoyagi H, Kurazono H, Moss J, Hirayama T.** Salmonella enteritidis FliC (flagella filament protein) induces human beta-defensin-2 mRNA production by Caco-2 cells. *J Biol Chem* 2001; **276**: 30521-30526 [PMID: 11387317 DOI: 10.1074/jbc.M011618200]
- 8 **Salzman NH, Hung K, Haribhai D, Chu H, Karlsson-Sjöberg J, Amir E, Teggatz P, Barman M, Hayward M, Eastwood D, Stoel M, Zhou Y, Sodergren E, Weinstock GM, Bevins CL, Williams CB, Bos NA.** Enteric defensins are essential regulators of intestinal microbial ecology. *Nat Immunol* 2010; **11**: 76-83 [PMID: 19855381 DOI: 10.1038/ni.1825]
- 9 **Chu H, Pazgier M, Jung G, Nuccio SP, Castillo PA, de Jong MF, Winter MG, Winter SE, Wehkamp J, Shen B, Salzman NH, Underwood MA, Tsois RM, Young GM, Lu W, Lehrer RI, Bäuml AJ, Bevins CL.** Human  $\alpha$ -defensin 6 promotes mucosal innate immunity through self-assembled peptide nanonets. *Science* 2012; **337**: 477-481 [PMID: 22722251 DOI: 10.1126/science.1218831]
- 10 **Kagnoff MF.** The intestinal epithelium is an integral component of a communications network. *J Clin Invest* 2014; **124**: 2841-2843 [PMID: 24983425 DOI: 10.1172/JCI75225]
- 11 **García JR, Krause A, Schulz S, Rodríguez-Jiménez FJ, Klüber E, Adermann K, Forssmann U, Frimpong-Boateng A, Bals R, Forssmann WG.** Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. *FASEB J* 2001; **15**: 1819-1821 [PMID: 11481241]
- 12 **Hoover DM, Wu Z, Tucker K, Lu W, Lubkowski J.** Antimicrobial characterization of human beta-defensin 3 derivatives. *Antimicrob Agents Chemother* 2003; **47**: 2804-2809 [PMID: 12936977 DOI: 10.1128/aac.47.9.2804-2809.2003]
- 13 **Sass V, Schneider T, Wilmes M, Körner C, Tossi A, Novikova N, Shamova O, Sahl HG.** Human beta-defensin 3 inhibits cell wall biosynthesis in Staphylococci. *Infect Immun* 2010; **78**: 2793-2800 [PMID: 20385753 DOI: 10.1128/IAI.00688-09]
- 14 **Elloumi HZ, Holland SM.** Complex regulation of human cathelicidin gene expression: novel splice variants and 5'UTR negative regulatory element. *Mol Immunol* 2008; **45**: 204-217 [PMID: 17709140 DOI: 10.1016/j.molimm.2007.04.023]
- 15 **Gennaro R, Zanetti M.** Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. *Biopolymers* 2000; **55**: 31-49 [PMID: 10931440 DOI: 10.1002/1097-0282(2000)55:1<31::AID-BIP40>3.0.CO;2-9]
- 16 **Hase K, Eckmann L, Leopard JD, Varki N, Kagnoff MF.** Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. *Infect Immun* 2002; **70**: 953-963 [PMID: 11796631 DOI: 10.1128/iai.70.2.953-963.2002]
- 17 **Yoshioka M, Fukuishi N, Kubo Y, Yamanobe H, Ohsaki K, Kawasoe Y, Murata M, Ishizumi A, Nishii Y, Matsui N, Akagi M.** Human cathelicidin CAP18/LL-37 changes mast cell function toward innate immunity. *Biol Pharm Bull* 2008; **31**: 212-216 [PMID: 18239275 DOI: 10.1248/bpb.31.212]
- 18 **Schauber J, Rieger D, Weiler F, Wehkamp J, Eck M, Fellermann K, Scheppach W, Gallo RL, Stange EF.** Heterogeneous expression of human cathelicidin hCAP18/LL-37 in inflammatory bowel diseases. *Eur J Gastroenterol Hepatol* 2006; **18**: 615-621 [PMID: 16702850 DOI: 10.1097/00042737-200606000-00007]
- 19 **Schwab M, Reynders V, Shastri Y, Loitsch S, Stein J, Schröder O.** Role of nuclear hormone receptors in butyrate-mediated up-regulation of the antimicrobial peptide cathelicidin in epithelial



- colorectal cells. *Mol Immunol* 2007; **44**: 2107-2114 [PMID: [17055059](#) DOI: [10.1016/j.molimm.2006.09.016](#)]
- 20 **White JH**. Vitamin D as an inducer of cathelicidin antimicrobial peptide expression: past, present and future. *J Steroid Biochem Mol Biol* 2010; **121**: 234-238 [PMID: [20302931](#) DOI: [10.1016/j.jsbmb.2010.03.034](#)]
  - 21 **Gubatan J**, Mehigan GA, Villegas F, Mitsuhashi S, Longhi MS, Malvar G, Cszimadia E, Robson S, Moss AC. Cathelicidin Mediates a Protective Role of Vitamin D in Ulcerative Colitis and Human Colonic Epithelial Cells. *Inflamm Bowel Dis* 2020; **26**: 885-897 [PMID: [31955203](#) DOI: [10.1093/ibd/izz330](#)]
  - 22 **Zhang L**, Yu J, Wong CC, Ling TK, Li ZJ, Chan KM, Ren SX, Shen J, Chan RL, Lee CC, Li MS, Cheng AS, To KF, Gallo RL, Sung JJ, Wu WK, Cho CH. Cathelicidin protects against *Helicobacter pylori* colonization and the associated gastritis in mice. *Gene Ther* 2013; **20**: 751-760 [PMID: [23254369](#) DOI: [10.1038/gt.2012.92](#)]
  - 23 **Cash HL**, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006; **313**: 1126-1130 [PMID: [16931762](#) DOI: [10.1126/science.1127119](#)]
  - 24 **Christa L**, Carnot F, Simon MT, Levavasseur F, Stinnakre MG, Lasserre C, Thepot D, Clement B, Devinoy E, Brechot C. HIP/PAP is an adhesive protein expressed in hepatocarcinoma, normal Paneth, and pancreatic cells. *Am J Physiol* 1996; **271**: G993-1002 [PMID: [8997243](#) DOI: [10.1152/ajpgi.1996.271.6.G993](#)]
  - 25 **Mukherjee S**, Zheng H, Derebe MG, Callenberg KM, Partch CL, Rollins D, Propheter DC, Rizo J, Grabe M, Jiang QX, Hooper LV. Antibacterial membrane attack by a pore-forming intestinal C-type lectin. *Nature* 2014; **505**: 103-107 [PMID: [24256734](#) DOI: [10.1038/nature12729](#)]
  - 26 **Miki T**, Holst O, Hardt WD. The bactericidal activity of the C-type lectin RegIII $\beta$  against Gram-negative bacteria involves binding to lipid A. *J Biol Chem* 2012; **287**: 34844-34855 [PMID: [22896700](#) DOI: [10.1074/jbc.M112.399998](#)]
  - 27 **Vaishnava S**, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, Ley R, Wakeland EK, Hooper LV. The antibacterial lectin RegIII $\gamma$  promotes the spatial segregation of microbiota and host in the intestine. *Science* 2011; **334**: 255-258 [PMID: [21998396](#) DOI: [10.1126/science.1209791](#)]
  - 28 **Ogawa H**, Fukushima K, Naito H, Funayama Y, Unno M, Takahashi K, Kitayama T, Matsuno S, Ohtani H, Takasawa S, Okamoto H, Sasaki I. Increased expression of HIP/PAP and regenerating gene III in human inflammatory bowel disease and a murine bacterial reconstitution model. *Inflamm Bowel Dis* 2003; **9**: 162-170 [PMID: [12792221](#) DOI: [10.1097/00054725-200305000-00003](#)]
  - 29 **Tsuchida C**, Sakuramoto-Tsuchida S, Taked M, Itaya-Hironaka A, Yamauchi A, Misu M, Shobatake R, Uchiyama T, Makino M, Pujol-Autonell I, Vives-Pi M, Ohbayashi C, Takasawa S. Expression of *REG* family genes in human inflammatory bowel diseases and its regulation. *Biochem Biophys Res* 2017; **12**: 198-205 [PMID: [29090282](#) DOI: [10.1016/j.bbrep.2017.10.003](#)]
  - 30 **Farnaud S**, Evans RW. Lactoferrin--a multifunctional protein with antimicrobial properties. *Mol Immunol* 2003; **40**: 395-405 [PMID: [14568385](#) DOI: [10.1016/s0161-5890\(03\)00152-4](#)]
  - 31 **Flo TH**, Smith KD, Sato S, Rodríguez DJ, Holmes MA, Strong RK, Akira S, Aderem A. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature* 2004; **432**: 917-921 [PMID: [15531878](#) DOI: [10.1038/nature03104](#)]
  - 32 **Kehl-Fie TE**, Chitayat S, Hood MI, Damo S, Restrepo N, Garcia C, Munro KA, Chazin WJ, Skaar EP. Nutrient metal sequestration by calprotectin inhibits bacterial superoxide defense, enhancing neutrophil killing of *Staphylococcus aureus*. *Cell Host Microbe* 2011; **10**: 158-164 [PMID: [21843872](#) DOI: [10.1016/j.chom.2011.07.004](#)]
  - 33 **Maisetta G**, Vitali A, Scorciapino MA, Rinaldi AC, Petruzzelli R, Brancatisano FL, Esin S, Stringaro A, Colone M, Luzi C, Bozzi A, Campa M, Batoni G. pH-dependent disruption of *Escherichia coli* ATCC 25922 and model membranes by the human antimicrobial peptides hepcidin 20 and 25. *FEBS J* 2013; **280**: 2842-2854 [PMID: [23587102](#) DOI: [10.1111/febs.12288](#)]
  - 34 **Chen HY**, Weng IC, Hong MH, Liu FT. Galectins as bacterial sensors in the host innate response. *Curr Opin Microbiol* 2014; **17**: 75-81 [PMID: [24581696](#) DOI: [10.1016/j.mib.2013.11.006](#)]
  - 35 **Thurston TL**, Wandel MP, von Muhlinen N, Foeglein A, Randow F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature* 2012; **482**: 414-418 [PMID: [22246324](#) DOI: [10.1038/nature10744](#)]
  - 36 **Ragland SA**, Criss AK. From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS Pathog* 2017; **13**: e1006512 [PMID: [28934357](#) DOI: [10.1371/journal.ppat.1006512](#)]
  - 37 **McMichael JW**, Roghanian A, Jiang L, Ramage R, Sallenave JM. The antimicrobial antiprotease elafin binds to lipopolysaccharide and modulates macrophage responses. *Am J Respir Cell Mol Biol* 2005; **32**: 443-452 [PMID: [15668324](#) DOI: [10.1165/rcmb.2004-0250OC](#)]
  - 38 **Nugteren S**, Samsom JN. Secretory Leukocyte Protease Inhibitor (SLPI) in mucosal tissues: Protects against inflammation, but promotes cancer. *Cytokine Growth Factor Rev* 2021; **59**: 22-35 [PMID: [33602652](#) DOI: [10.1016/j.cytogr.2021.01.005](#)]
  - 39 **Doumas S**, Kolokotronis A, Stefanopoulos P. Anti-inflammatory and antimicrobial roles of secretory leukocyte protease inhibitor. *Infect Immun* 2005; **73**: 1271-1274 [PMID: [15731023](#) DOI: [10.1128/IAI.73.3.1271-1274.2005](#)]
  - 40 **Fontaine SS**, Kohl KD. Optimal integration between host physiology and functions of the gut



- microbiome. *Philos Trans R Soc Lond B Biol Sci* 2020; **375**: 20190594 [PMID: [32772673](#) DOI: [10.1098/rstb.2019.0594](#)]
- 41 **Martinez-Guryn K**, Hubert N, Frazier K, Urlass S, Musch MW, Ojeda P, Pierre JF, Miyoshi J, Sontag TJ, Cham CM, Reardon CA, Leone V, Chang EB. Small Intestine Microbiota Regulate Host Digestive and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host Microbe* 2018; **23**: 458-469.e5 [PMID: [29649441](#) DOI: [10.1016/j.chom.2018.03.011](#)]
  - 42 **Jumpertz R**, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr* 2011; **94**: 58-65 [PMID: [21543530](#) DOI: [10.3945/ajcn.110.010132](#)]
  - 43 **Lee KZ**, Lestrade M, Socha C, Schirmeier S, Schmitz A, Spénlé C, Lefebvre O, Keime C, Yamba WM, Bou Aoun R, Liegeois S, Schwab Y, Simon-Assmann P, Dalle F, Ferrandon D. Enterocyte Purge and Rapid Recovery Is a Resilience Reaction of the Gut Epithelium to Pore-Forming Toxin Attack. *Cell Host Microbe* 2016; **20**: 716-730 [PMID: [27889464](#) DOI: [10.1016/j.chom.2016.10.010](#)]
  - 44 **Flanagan DJ**, Phesse TJ, Barker N, Schwab RH, Amin N, Malaterre J, Stange DE, Nowell CJ, Currie SA, Saw JT, Beuchert E, Ramsay RG, Sansom OJ, Ernst M, Clevers H, Vincan E. Frizzled7 functions as a Wnt receptor in intestinal epithelial Lgr5(+) stem cells. *Stem Cell Reports* 2015; **4**: 759-767 [PMID: [25892522](#) DOI: [10.1016/j.stemcr.2015.03.003](#)]
  - 45 **Yao X**, Zhang C, Xing Y, Xue G, Zhang Q, Pan F, Wu G, Hu Y, Guo Q, Lu A, Zhang X, Zhou R, Tian Z, Zeng B, Wei H, Strober W, Zhao L, Meng G. Remodelling of the gut microbiota by hyperactive NLRP3 induces regulatory T cells to maintain homeostasis. *Nat Commun* 2017; **8**: 1896 [PMID: [29196621](#) DOI: [10.1038/s41467-017-01917-2](#)]
  - 46 **Chongsiriwatana NP**, Lin JS, Kapoor R, Wetzler M, Rea JAC, Didwania MK, Contag CH, Barron AE. Intracellular biomass flocculation as a key mechanism of rapid bacterial killing by cationic, amphipathic antimicrobial peptides and peptoids. *Sci Rep* 2017; **7**: 16718 [PMID: [29196622](#) DOI: [10.1038/s41598-017-16180-0](#)]
  - 47 **Forbes S**, Dobson CB, Humphreys GJ, McBain AJ. Transient and sustained bacterial adaptation following repeated sublethal exposure to microbicides and a novel human antimicrobial peptide. *Antimicrob Agents Chemother* 2014; **58**: 5809-5817 [PMID: [25049246](#) DOI: [10.1128/AAC.03364-14](#)]
  - 48 **Findlay EG**, Currie AJ, Zhang A, Ovcariakova J, Young L, Stevens H, McHugh BJ, Canel M, Gray M, Milling SWF, Campbell JDM, Savill J, Serrels A, Davidson DJ. Exposure to the antimicrobial peptide LL-37 produces dendritic cells optimized for immunotherapy. *Oncoimmunology* 2019; **8**: 1608106 [PMID: [31413918](#) DOI: [10.1080/2162402X.2019.1608106](#)]
  - 49 **Lourenço M**, Chaffringeon L, Lamy-Besnier Q, Pédrón T, Campagne P, Eberl C, Bérard M, Stecher B, Debarbieux L, De Sordi L. The Spatial Heterogeneity of the Gut Limits Predation and Fosters Coexistence of Bacteria and Bacteriophages. *Cell Host Microbe* 2020; **28**: 390-401.e5 [PMID: [32615090](#) DOI: [10.1016/j.chom.2020.06.002](#)]
  - 50 **Simmaco M**, Mignogna G, Barra D. Antimicrobial peptides from amphibian skin: what do they tell us? *Biopolymers* 1998; **47**: 435-450 [PMID: [10333736](#)]
  - 51 **Unckless RL**, Howick VM, Lazzaro BP. Convergent Balancing Selection on an Antimicrobial Peptide in *Drosophila*. *Curr Biol* 2016; **26**: 257-262 [PMID: [26776733](#) DOI: [10.1016/j.cub.2015.11.063](#)]
  - 52 **Unckless RL**, Lazzaro BP. The potential for adaptive maintenance of diversity in insect antimicrobial peptides. *Philos Trans R Soc Lond B Biol Sci* 2016; **371** [PMID: [27160594](#) DOI: [10.1098/rstb.2015.0291](#)]
  - 53 **Tennessen JA**. Molecular evolution of animal antimicrobial peptides: widespread moderate positive selection. *J Evol Biol* 2005; **18**: 1387-1394 [PMID: [16313451](#) DOI: [10.1111/j.1420-9101.2005.00925.x](#)]
  - 54 **Hanson MA**, Lemaitre B, Unckless RL. Dynamic Evolution of Antimicrobial Peptides Underscores Trade-Offs Between Immunity and Ecological Fitness. *Front Immunol* 2019; **10**: 2620 [PMID: [31781114](#) DOI: [10.3389/fimmu.2019.02620](#)]
  - 55 **DeLuca JA**, Allred KF, Menon R, Riordan R, Weeks BR, Jayaraman A, Allred CD. Bisphenol-A alters microbiota metabolites derived from aromatic amino acids and worsens disease activity during colitis. *Exp Biol Med (Maywood)* 2018; **243**: 864-875 [PMID: [29874946](#) DOI: [10.1177/1535370218782139](#)]
  - 56 **Rodríguez-Rojas A**, Baeder DY, Johnston P, Regoes RR, Rolff J. Bacteria primed by antimicrobial peptides develop tolerance and persist. *PLoS Pathog* 2021; **17**: e1009443 [PMID: [33788905](#) DOI: [10.1371/journal.ppat.1009443](#)]
  - 57 **Franzenburg S**, Walter J, Künzel S, Wang J, Baines JF, Bosch TC, Fraune S. Distinct antimicrobial peptide expression determines host species-specific bacterial associations. *Proc Natl Acad Sci U S A* 2013; **110**: E3730-E3738 [PMID: [24003149](#) DOI: [10.1073/pnas.1304960110](#)]
  - 58 **Becattini S**, Taur Y, Pamer EG. Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. *Trends Mol Med* 2016; **22**: 458-478 [PMID: [27178527](#) DOI: [10.1016/j.molmed.2016.04.003](#)]
  - 59 **Franzosa EA**, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, Vatanen T, Hall AB, Mallick H, McIver LJ, Sauk JS, Wilson RG, Stevens BW, Scott JM, Pierce K, Deik AA, Bullock K, Imhann F, Porter JA, Zhernakova A, Fu J, Weersma RK, Wijmenga C, Clish CB, Vlamakis H, Huttenhower C, Xavier RJ. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* 2019; **4**: 293-305 [PMID: [30531976](#) DOI: [10.1038/s41564-019-0564-4](#)]

- 10.1038/s41564-018-0306-4]
- 60 **Collado MC**, González A, González R, Hernández M, Ferrús MA, Sanz Y. Antimicrobial peptides are among the antagonistic metabolites produced by *Bifidobacterium* against *Helicobacter pylori*. *Int J Antimicrob Agents* 2005; **25**: 385-391 [PMID: 15848292 DOI: 10.1016/j.ijantimicag.2005.01.017]
  - 61 **Nuding S**, Frasc T, Schaller M, Stange EF, Zabel LT. Synergistic effects of antimicrobial peptides and antibiotics against *Clostridium difficile*. *Antimicrob Agents Chemother* 2014; **58**: 5719-5725 [PMID: 25022581 DOI: 10.1128/AAC.02542-14]
  - 62 **Zhao Y**, Chen F, Wu W, Sun M, Bilotta AJ, Yao S, Xiao Y, Huang X, Eaves-Pyles TD, Golovko G, Fofanov Y, D'Souza W, Zhao Q, Liu Z, Cong Y. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunol* 2018; **11**: 752-762 [PMID: 29411774 DOI: 10.1038/mi.2017.118]
  - 63 **Cazorla SI**, Maldonado-Galdeano C, Weill R, De Paula J, Perdigón GDV. Oral Administration of Probiotics Increases Paneth Cells and Intestinal Antimicrobial Activity. *Front Microbiol* 2018; **9**: 736 [PMID: 29713315 DOI: 10.3389/fmicb.2018.00736]
  - 64 **Kumar M**, Kisson-Singh V, Coria AL, Moreau F, Chadee K. Probiotic mixture VSL#3 reduces colonic inflammation and improves intestinal barrier function in Muc2 mucin-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 2017; **312**: G34-G45 [PMID: 27856417 DOI: 10.1152/ajpgi.00298.2016]
  - 65 **Mandal SM**, Silva ON, Franco OL. Recombinant probiotics with antimicrobial peptides: a dual strategy to improve immune response in immunocompromised patients. *Drug Discov Today* 2014; **19**: 1045-1050 [PMID: 24881782 DOI: 10.1016/j.drudis.2014.05.019]
  - 66 **Teng T**, Gao F, He W, Fu H, Guo J, Bai G, Shi B. An Early Fecal Microbiota Transfer Improves the Intestinal Conditions on Microflora and Immunoglobulin and Antimicrobial Peptides in Piglets. *J Agric Food Chem* 2020; **68**: 4830-4843 [PMID: 32252520 DOI: 10.1021/acs.jafc.0c00545]
  - 67 **Liu L**, Zhao C, Heng HH, Ganz T. The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. *Genomics* 1997; **43**: 316-320 [PMID: 9268634 DOI: 10.1006/geno.1997.4801]
  - 68 **Grigat J**, Soruri A, Forssmann U, Riggert J, Zwirner J. Chemoattraction of macrophages, T lymphocytes, and mast cells is evolutionarily conserved within the human alpha-defensin family. *J Immunol* 2007; **179**: 3958-3965 [PMID: 17785833 DOI: 10.4049/jimmunol.179.6.3958]
  - 69 **Ayabe T**, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ. Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat Immunol* 2000; **1**: 113-118 [PMID: 11248802 DOI: 10.1038/77783]
  - 70 **Salzman NH**. Paneth cell defensins and the regulation of the microbiome: détente at mucosal surfaces. *Gut Microbes* 2010; **1**: 401-406 [PMID: 21468224 DOI: 10.4161/gmic.1.6.14076]
  - 71 **Putsep K**, Axelsson LG, Boman A, Midtvedt T, Normark S, Boman HG, Andersson M. Germ-free and colonized mice generate the same products from enteric prodefensins. *J Biol Chem* 2000; **275**: 40478-40482 [PMID: 11010975 DOI: 10.1074/jbc.M007816200]
  - 72 **Fusco A**, Savio V, Cammarota M, Alfano A, Schiraldi C, Donnarumma G. Beta-Defensin-2 and Beta-Defensin-3 Reduce Intestinal Damage Caused by *Salmonella typhimurium* Modulating the Expression of Cytokines and Enhancing the Probiotic Activity of *Enterococcus faecium*. *J Immunol Res* 2017; **2017**: 6976935 [PMID: 29250559 DOI: 10.1155/2017/6976935]
  - 73 **Koon HW**, Shih DQ, Chen J, Bakirtzi K, Hing TC, Law I, Ho S, Ichikawa R, Zhao D, Xu H, Gallo R, Dempsey P, Cheng G, Targan SR, Pothoulakis C. Cathelicidin signaling via the Toll-like receptor protects against colitis in mice. *Gastroenterology* 2011; **141**: 1852-63.e1 [PMID: 21762664 DOI: 10.1053/j.gastro.2011.06.079]
  - 74 **Otte JM**, Zdebek AE, Brand S, Chromik AM, Strauss S, Schmitz F, Steinstraesser L, Schmidt WE. Effects of the cathelicidin LL-37 on intestinal epithelial barrier integrity. *Regul Pept* 2009; **156**: 104-117 [PMID: 19328825 DOI: 10.1016/j.regpep.2009.03.009]
  - 75 **Marin M**, Holani R, Blyth GAD, Drouin D, Odeón A, Cobo ER. Human cathelicidin improves colonic epithelial defenses against *Salmonella typhimurium* by modulating bacterial invasion, TLR4 and pro-inflammatory cytokines. *Cell Tissue Res* 2019; **376**: 433-442 [PMID: 30788579 DOI: 10.1007/s00441-018-02984-7]
  - 76 **Rouches MV**. The Cathelicidin Anti-Microbial Peptide Gene Alters the Mouse Gut Microbiome. Oregon State University, 2018. Available from: [https://ir.library.oregonstate.edu/concern/honors\\_college\\_theses/dz010w278](https://ir.library.oregonstate.edu/concern/honors_college_theses/dz010w278)
  - 77 **Cash HL**. The Ligand and Function of the RegIII Family of Bactericidal C-Type Lectins. UT Southwestern Graduate School of Biomedical Sciences, 2006. Available from: <https://utswmed-ir.tdl.org/handle/2152.5/235>
  - 78 **Loonen LM**, Stolte EH, Jaklofsky MT, Meijerink M, Dekker J, van Baarlen P, Wells JM. REG3γ-deficient mice have altered mucus distribution and increased mucosal inflammatory responses to the microbiota and enteric pathogens in the ileum. *Mucosal Immunol* 2014; **7**: 939-947 [PMID: 24345802 DOI: 10.1038/mi.2013.109]
  - 79 **Maeda T**, Sakiyama T, Kanmura S, Hashimoto S, Ibusuki K, Tanoue S, Komaki Y, Arima S, Nasu Y, Sasaki F, Taguchi H, Numata M, Uto H, Tsubouchi H, Ido A. Low concentrations of human neutrophil peptide ameliorate experimental murine colitis. *Int J Mol Med* 2016; **38**: 1777-1785 [PMID: 27840892 DOI: 10.3892/ijmm.2016.2795]
  - 80 **Hashimoto S**, Uto H, Kanmura S, Sakiyama T, Oku M, Iwashita Y, Ibusuki R, Sasaki F, Ibusuki K, Takami Y, Moriuchi A, Oketani M, Ido A, Tsubouchi H. Human neutrophil peptide-1 aggravates

- dextran sulfate sodium-induced colitis. *Inflamm Bowel Dis* 2012; **18**: 667-675 [PMID: [21928371](#) DOI: [10.1002/ibd.21855](#)]
- 81 **Koeninger L**, Armbruster NS, Brinch KS, Kjaerulf S, Andersen B, Langnau C, Autenrieth SE, Schneidawind D, Stange EF, Malek NP, Nordkild P, Jensen BAH, Wehkamp J. Human  $\beta$ -Defensin 2 Mediated Immune Modulation as Treatment for Experimental Colitis. *Front Immunol* 2020; **11**: 93 [PMID: [32076420](#) DOI: [10.3389/fimmu.2020.00093](#)]
  - 82 **Han F**, Zhang H, Xia X, Xiong H, Song D, Zong X, Wang Y. Porcine  $\beta$ -defensin 2 attenuates inflammation and mucosal lesions in dextran sodium sulfate-induced colitis. *J Immunol* 2015; **194**: 1882-1893 [PMID: [25601921](#) DOI: [10.4049/jimmunol.1402300](#)]
  - 83 **Fabisiak N**, Fabisiak A, Chmielowiec-Korzeniowska A, Tymczyńska L, Kamysz W, Kordek R, Bauer M, Kamysz E, Fichna J. Anti-inflammatory and antibacterial effects of human cathelicidin active fragment KR-12 in the mouse models of colitis: a novel potential therapy of inflammatory bowel diseases. *Pharmacol Rep* 2021; **73**: 163-171 [PMID: [33219923](#) DOI: [10.1007/s43440-020-00190-3](#)]
  - 84 **Yoo JH**, Ho S, Tran DH, Cheng M, Bakirtzi K, Kukota Y, Ichikawa R, Su B, Hing TC, Chang I, Shih DQ, Issacson RE, Gallo RL, Fiocchi C, Pothoulakis C, Koon HW. Anti-fibrogenic effects of the anti-microbial peptide cathelicidin in murine colitis-associated fibrosis. *Cell Mol Gastroenterol Hepatol* 2015; **1**: 55-74.e1 [PMID: [25729764](#) DOI: [10.1016/j.jcmgh.2014.08.001](#)]
  - 85 **Tai EK**, Wong HP, Lam EK, Wu WK, Yu L, Koo MW, Cho CH. Cathelicidin stimulates colonic mucus synthesis by up-regulating MUC1 and MUC2 expression through a mitogen-activated protein kinase pathway. *J Cell Biochem* 2008; **104**: 251-258 [PMID: [18059019](#) DOI: [10.1002/jcb.21615](#)]
  - 86 **Sharon P**, Stenson WF. Metabolism of arachidonic acid in acetic acid colitis in rats. Similarity to human inflammatory bowel disease. *Gastroenterology* 1985; **88**: 55-63 [PMID: [3917261](#) DOI: [10.1016/s0016-5085\(85\)80132-3](#)]
  - 87 **Wan M**, Sabirsh A, Wetterholm A, Agerberth B, Haeggström JZ. Leukotriene B4 triggers release of the cathelicidin LL-37 from human neutrophils: novel lipid-peptide interactions in innate immune responses. *FASEB J* 2007; **21**: 2897-2905 [PMID: [17446260](#) DOI: [10.1096/fj.06-7974com](#)]
  - 88 **Wan M**, Tang X, Rekha RS, Muvva SSVJR, Brighenti S, Agerberth B, Haeggström JZ. Prostaglandin E<sub>2</sub> suppresses hCAP18/LL-37 expression in human macrophages via EP2/EP4: implications for treatment of Mycobacterium tuberculosis infection. *FASEB J* 2018; **32**: 2827-2840 [PMID: [29401596](#) DOI: [10.1096/fj.201701308](#)]
  - 89 **Wang D**, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* 2010; **29**: 781-788 [PMID: [19946329](#) DOI: [10.1038/onc.2009.421](#)]
  - 90 **De UK**, Mukherjee R. Activity of cyclooxygenase-2 and nitric oxide in milk leucocytes following intramammary inoculation of a bio-response modifier during bovine Staphylococcus aureus subclinical mastitis. *Vet Res Commun* 2014; **38**: 201-207 [PMID: [24844929](#) DOI: [10.1007/s11259-014-9604-3](#)]
  - 91 **Motta JP**, Magne L, Descamps D, Rolland C, Squarzone-Dale C, Rousset P, Martin L, Cenac N, Balloy V, Huerre M, Fröhlich LF, Jenne D, Wartelle J, Belaouaj A, Mas E, Vinel JP, Alric L, Chignard M, Vergnolle N, Sallenave JM. Modifying the protease, antiprotease pattern by elafin overexpression protects mice from colitis. *Gastroenterology* 2011; **140**: 1272-1282 [PMID: [21199654](#) DOI: [10.1053/j.gastro.2010.12.050](#)]
  - 92 **Reardon C**, Lechmann M, Brüstle A, Gareau MG, Shuman N, Philpott D, Ziegler SF, Mak TW. Thymic stromal lymphopoietin-induced expression of the endogenous inhibitory enzyme SLPI mediates recovery from colonic inflammation. *Immunity* 2011; **35**: 223-235 [PMID: [21820333](#) DOI: [10.1016/j.immuni.2011.05.015](#)]
  - 93 **Jiang L**, Lv J, Liu J, Hao X, Ren F, Guo H. Donkey milk lysozyme ameliorates dextran sulfate sodium-induced colitis by improving intestinal barrier function and gut microbiota composition. *J Funct Foods* 2018; **48**: 144-152 [DOI: [10.1016/j.jff.2018.07.005](#)]
  - 94 **Heller F**, Fromm A, Gitter AH, Mankertz J, Schulzke JD. Epithelial apoptosis is a prominent feature of the epithelial barrier disturbance in intestinal inflammation: effect of pro-inflammatory interleukin-13 on epithelial cell function. *Mucosal Immunol* 2008; **1** Suppl 1: S58-S61 [PMID: [19079233](#) DOI: [10.1038/mi.2008.46](#)]
  - 95 **Togawa J**, Nagase H, Tanaka K, Inamori M, Nakajima A, Ueno N, Saito T, Sekihara H. Oral administration of lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. *J Gastroenterol Hepatol* 2002; **17**: 1291-1298 [PMID: [12423274](#) DOI: [10.1046/j.1440-1746.2002.02868.x](#)]
  - 96 **Shanmugam NK**, Ellenbogen S, Trebicka E, Wang L, Mukhopadhyay S, Lacy-Hulbert A, Gallini CA, Garrett WS, Cherayil BJ. Tumor necrosis factor  $\alpha$  inhibits expression of the iron regulating hormone hepcidin in murine models of innate colitis. *PLoS One* 2012; **7**: e38136 [PMID: [22675442](#) DOI: [10.1371/journal.pone.0038136](#)]
  - 97 **Tibble J**, Teahon K, Thjodleifsson B, Roseth A, Sigthorsson G, Bridger S, Foster R, Sherwood R, Fagerhol M, Bjarnason I. A simple method for assessing intestinal inflammation in Crohn's disease. *Gut* 2000; **47**: 506-513 [PMID: [10986210](#) DOI: [10.1136/gut.47.4.506](#)]
  - 98 **Bunn SK**, Bisset WM, Main MJ, Golden BE. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2001; **32**: 171-177 [PMID: [11321388](#) DOI: [10.1097/00005176-200102000-00015](#)]
  - 99 **Mármol I**, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodríguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. *Int J Mol Sci* 2017; **18** [PMID: [28106826](#) DOI: [10.3390/ijms18010197](#)]

- 100 **Moniuszko A**, Głuszek S, Rydzewska G. Rapid fecal calprotectin test for prediction of mucosal inflammation in ulcerative colitis and Crohn disease: a prospective cohort study. *Pol Arch Intern Med* 2017; **127**: 312-318 [PMID: [28442699](#) DOI: [10.20452/pamw.4009](#)]
- 101 **Pous-Serrano S**, Frasson M, Cerrillo E, Beltrán B, Iborra M, Hervás D, García-Granero E, Nos P. Correlation between fecal calprotectin and inflammation in the surgical specimen of Crohn's disease. *J Surg Res* 2017; **213**: 290-297 [PMID: [28601328](#) DOI: [10.1016/j.jss.2017.02.064](#)]
- 102 **Schoepfer AM**, Beglinger C, Straumann A, Trummel M, Vavricka SR, Bruegger LE, Seibold F. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. *Am J Gastroenterol* 2010; **105**: 162-169 [PMID: [19755969](#) DOI: [10.1038/ajg.2009.545](#)]
- 103 **Ferreiro-Iglesias R**, Barreiro-de Acosta M, Otero Santiago M, Lorenzo Gonzalez A, Alonso de la Peña C, Benitez Estevez AJ, Dominguez-Muñoz JE. Fecal Calprotectin as Predictor of Relapse in Patients With Inflammatory Bowel Disease Under Maintenance Infliximab Therapy. *J Clin Gastroenterol* 2016; **50**: 147-151 [PMID: [25811118](#) DOI: [10.1097/MCG.0000000000000312](#)]
- 104 **Klingberg E**, Strid H, Ståhl A, Deminger A, Carlsten H, Öhman L, Forsblad-d'Elia H. A longitudinal study of fecal calprotectin and the development of inflammatory bowel disease in ankylosing spondylitis. *Arthritis Res Ther* 2017; **19**: 21 [PMID: [28148281](#) DOI: [10.1186/s13075-017-1223-2](#)]
- 105 **Meling TR**, Aabakken L, Røseth A, Osnes M. Faecal calprotectin shedding after short-term treatment with non-steroidal anti-inflammatory drugs. *Scand J Gastroenterol* 1996; **31**: 339-344 [PMID: [8726300](#) DOI: [10.3109/00365529609006407](#)]
- 106 **Ikhtaire S**, Shajib MS, Reinisch W, Khan W. Fecal calprotectin: its scope and utility in the management of inflammatory bowel disease. *J Gastroenterol* 2016; **51**: 434-446 [PMID: [26897740](#) DOI: [10.1007/s00535-016-1182-4](#)]
- 107 **Bressler B**, Panaccione R, Fedorak RN, Seidman EG. Clinicians' guide to the use of fecal calprotectin to identify and monitor disease activity in inflammatory bowel disease. *Can J Gastroenterol Hepatol* 2015; **29**: 369-372 [PMID: [26125109](#) DOI: [10.1155/2015/852723](#)]
- 108 **Røseth AG**, Aadland E, Grzyb K. Normalization of faecal calprotectin: a predictor of mucosal healing in patients with inflammatory bowel disease. *Scand J Gastroenterol* 2004; **39**: 1017-1020 [PMID: [15513345](#) DOI: [10.1080/00365520410007971](#)]
- 109 **Godny L**, Reshef L, Pfeffer-Gik T, Goren I, Yanai H, Tulchinsky H, Gophna U, Dotan I. Adherence to the Mediterranean diet is associated with decreased fecal calprotectin in patients with ulcerative colitis after pouch surgery. *Eur J Nutr* 2020; **59**: 3183-3190 [PMID: [31813010](#) DOI: [10.1007/s00394-019-02158-3](#)]
- 110 **Holgersen K**, Kutlu B, Fox B, Serikawa K, Lord J, Hansen AK, Holm TL. High-resolution gene expression profiling using RNA sequencing in patients with inflammatory bowel disease and in mouse models of colitis. *J Crohns Colitis* 2015; **9**: 492-506 [PMID: [25795566](#) DOI: [10.1093/ecco-jcc/jjv050](#)]
- 111 **Wehkamp J**, Wang G, Kübler I, Nuding S, Gregorieff A, Schnabel A, Kays RJ, Fellermann K, Burk O, Schwab M, Clevers H, Bevins CL, Stange EF. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007; **179**: 3109-3118 [PMID: [17709525](#) DOI: [10.4049/jimmunol.179.5.3109](#)]
- 112 **Yamaguchi N**, Isomoto H, Mukae H, Ishimoto H, Ohnita K, Shikuwa S, Mizuta Y, Nakazato M, Kohno S. Concentrations of alpha- and beta-defensins in plasma of patients with inflammatory bowel disease. *Inflamm Res* 2009; **58**: 192-197 [PMID: [19184352](#) DOI: [10.1007/s00011-008-8120-8](#)]
- 113 **Kanmura S**, Hamamoto H, Morinaga Y, Oda K, Fujita T, Arima S, Nasu Y, Sasaki F, Hashimoto S, Taguchi H, Setoyama H, Ido A. Fecal Human Neutrophil Peptide Levels Correlate with Intestinal Inflammation in Ulcerative Colitis. *Digestion* 2016; **93**: 300-308 [PMID: [27220673](#) DOI: [10.1159/000446210](#)]
- 114 **Cunliffe RN**, Kamal M, Rose FR, James PD, Mahida YR. Expression of antimicrobial neutrophil defensins in epithelial cells of active inflammatory bowel disease mucosa. *J Clin Pathol* 2002; **55**: 298-304 [PMID: [11919217](#) DOI: [10.1136/jcp.55.4.298](#)]
- 115 **Krawiec P**, Pac-Kożuchowska E. Cathelicidin - A Novel Potential Marker of Pediatric Inflammatory Bowel Disease. *J Inflamm Res* 2021; **14**: 163-174 [PMID: [33519224](#) DOI: [10.2147/JIR.S288742](#)]
- 116 **Tran DH**, Wang J, Ha C, Ho W, Mattai SA, Oikonomopoulos A, Weiss G, Lacey P, Cheng M, Shieh C, Mussatto CC, Ho S, Hommes D, Koon HW. Circulating cathelicidin levels correlate with mucosal disease activity in ulcerative colitis, risk of intestinal stricture in Crohn's disease, and clinical prognosis in inflammatory bowel disease. *BMC Gastroenterol* 2017; **17**: 63 [PMID: [28494754](#) DOI: [10.1186/s12876-017-0619-4](#)]
- 117 **Fu Y**, Wang L, Xie C, Zou K, Tu L, Yan W, Hou X. Comparison of non-invasive biomarkers faecal BAFF, calprotectin and FOBT in discriminating IBS from IBD and evaluation of intestinal inflammation. *Sci Rep* 2017; **7**: 2669 [PMID: [28572616](#) DOI: [10.1038/s41598-017-02835-5](#)]
- 118 **Borkowska A**, Liberek A, Łuczak G, Jankowska A, Plata-Nazar K, Korzon M, Kamińska B. Fecal lactoferrin, a marker of intestinal inflammation in children with inflammatory bowel disease. *Acta Biochim Pol* 2015; **62**: 541-545 [PMID: [26339799](#) DOI: [10.18388/abp.2015\\_982](#)]
- 119 **Sugi K**, Saitoh O, Hirata I, Katsu K. Fecal lactoferrin as a marker for disease activity in inflammatory bowel disease: comparison with other neutrophil-derived proteins. *Am J Gastroenterol* 1996; **91**: 927-934 [PMID: [8633583](#)]



- 120 **Sidhu R**, Wilson P, Wright A, Yau CW, D'Cruz FA, Foye L, Morley S, Lobo AJ, McAlindon ME, Sanders DS. Faecal lactoferrin--a novel test to differentiate between the irritable and inflamed bowel? *Aliment Pharmacol Ther* 2010; **31**: 1365-1370 [PMID: [20331581](#) DOI: [10.1111/j.1365-2036.2010.04306.x](#)]
- 121 **Wang Y**, Pei F, Wang X, Sun Z, Hu C, Dou H. Diagnostic accuracy of fecal lactoferrin for inflammatory bowel disease: a meta-analysis. *Int J Clin Exp Pathol* 2015; **8**: 12319-12332 [PMID: [26722419](#)]
- 122 **Kane SV**, Sandborn WJ, Rufo PA, Zholudev A, Boone J, Lysterly D, Camilleri M, Hanauer SB. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am J Gastroenterol* 2003; **98**: 1309-1314 [PMID: [12818275](#) DOI: [10.1111/j.1572-0241.2003.07458.x](#)]
- 123 **Turner D**, Leach ST, Mack D, Uusoue K, McLernon R, Hyams J, Leleiko N, Walters TD, Crandall W, Markowitz J, Otley AR, Griffiths AM, Day AS. Faecal calprotectin, lactoferrin, M2-pyruvate kinase and S100A12 in severe ulcerative colitis: a prospective multicentre comparison of predicting outcomes and monitoring response. *Gut* 2010; **59**: 1207-1212 [PMID: [20801771](#) DOI: [10.1136/gut.2010.211755](#)]
- 124 **Frol'ová L**, Smetana K Jr, Borovská D, Kitanovicová A, Klimesová K, Janatková I, Malicková K, Lukás M, Drastich P, Benes Z, Tucková L, Manning JC, André S, Gabius HJ, Tlaskalová-Hogenová H. Detection of galectin-3 in patients with inflammatory bowel diseases: new serum marker of active forms of IBD? *Inflamm Res* 2009; **58**: 503-512 [PMID: [19271150](#) DOI: [10.1007/s00011-009-0016-8](#)]
- 125 **Yu TB**, Dodd S, Yu LG, Subramanian S. Serum galectins as potential biomarkers of inflammatory bowel diseases. *PLoS One* 2020; **15**: e0227306 [PMID: [31929564](#) DOI: [10.1371/journal.pone.0227306](#)]
- 126 **Karaskova E**, Volejnikova J, Holub D, Velganova-Veghova M, Sulovska L, Mihal V, Horvathova M, Pospisilova D. Hepcidin in newly diagnosed inflammatory bowel disease in children. *J Paediatr Child Health* 2018; **54**: 1362-1367 [PMID: [29923651](#) DOI: [10.1111/jpc.14093](#)]
- 127 **Zollner A**, Schmiderer A, Reider SJ, Oberhuber G, Pfister A, Texler B, Watschinger C, Koch R, Effenberger M, Raine T, Tilg H, Moschen AR. Faecal Biomarkers in Inflammatory Bowel Diseases: Calprotectin Versus Lipocalin-2-a Comparative Study. *J Crohns Colitis* 2021; **15**: 43-54 [PMID: [32556317](#) DOI: [10.1093/ecco-jcc/jjaa124](#)]
- 128 **Martinelli M**, Strisciuglio C, Alessandrella A, Rossi F, Auricchio R, Campostrini N, Girelli D, Nobili B, Staiano A, Perrotta S, Miele E. Serum Hepcidin and Iron Absorption in Paediatric Inflammatory Bowel Disease. *J Crohns Colitis* 2016; **10**: 566-574 [PMID: [26733407](#) DOI: [10.1093/ecco-jcc/jjv242](#)]
- 129 **Aksan A**, Wohlrath M, Iqbal TH, Farrag K, Dignass A, Stein J. Serum Hepcidin Levels Predict Intestinal Iron Absorption in Patients with Inflammatory Bowel Disease. *Clin Lab* 2019; **65** [PMID: [30868856](#) DOI: [10.7754/Clin.Lab.2019.190106](#)]
- 130 **Motta JP**, Rolland C, Edir A, Florence AC, Sagnat D, Bonnart C, Rousset P, Guiraud L, Quaranta-Nicaise M, Mas E, Bonnet D, Verdu EF, McKay DM, Buscail E, Alric L, Vergnolle N, Deraison C. Epithelial production of elastase is increased in inflammatory bowel disease and causes mucosal inflammation. *Mucosal Immunol* 2021; **14**: 667-678 [PMID: [33674762](#) DOI: [10.1038/s41385-021-00375-w](#)]
- 131 **Wędrychowicz A**, Tomasik P, Kowalska-Duplaga K, Pieczarkowski S, Fyderek K. Plasma elafin, cathelicidin, and  $\alpha$ -defensins are increased in paediatric inflammatory Crohn's disease and reflect disease location. *Arch Med Sci* 2021; **17**: 1114-1117 [PMID: [34336040](#) DOI: [10.5114/aoms/138349](#)]
- 132 **Wang J**, Ortiz C, Fontenot L, Xie Y, Ho W, Mattai SA, Shih DQ, Koon HW. High circulating elafin levels are associated with Crohn's disease-associated intestinal strictures. *PLoS One* 2020; **15**: e0231796 [PMID: [32287314](#) DOI: [10.1371/journal.pone.0231796](#)]
- 133 **Zhang W**, Teng GG, Tian Y, Wang HH. [Expression of elafin in peripheral blood in inflammatory bowel disease patients and its clinical significance]. *Zhonghua Yi Xue Za Zhi* 2016; **96**: 1120-1123 [PMID: [27095781](#) DOI: [10.3760/cma.j.issn.0376-2491.2016.14.012](#)]
- 134 **Schmid M**, Fellermann K, Fritz P, Wiedow O, Stange EF, Wehkamp J. Attenuated induction of epithelial and leukocyte serine antiproteases elafin and secretory leukocyte protease inhibitor in Crohn's disease. *J Leukoc Biol* 2007; **81**: 907-915 [PMID: [17200145](#) DOI: [10.1189/jlb.0906581](#)]
- 135 **Divyashree M**, Mani MK, Reddy D, Kumavath R, Ghosh P, Azevedo V, Barh D. Clinical Applications of Antimicrobial Peptides (AMPs): Where do we Stand Now? *Protein Pept Lett* 2020; **27**: 120-134 [PMID: [31553285](#) DOI: [10.2174/0929866526666190925152957](#)]
- 136 **Rubin SJS**, Qvit N. Engineering "Antimicrobial Peptides" and Other Peptides to Modulate Protein-Protein Interactions in Cancer. *Curr Top Med Chem* 2020; **20**: 2970-2983 [PMID: [33087030](#) DOI: [10.2174/1568026620666201021141401](#)]
- 137 **Mourtada R**, Herce HD, Yin DJ, Moroco JA, Wales TE, Engen JR, Walensky LD. Design of stapled antimicrobial peptides that are stable, nontoxic and kill antibiotic-resistant bacteria in mice. *Nat Biotechnol* 2019; **37**: 1186-1197 [PMID: [31427820](#) DOI: [10.1038/s41587-019-0222-z](#)]



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