Published in final edited form as:
Curr Pharm Des. 2013 ; 19(1): 40–47.

Antimicrobial Peptides and Colitis

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Abstract

Antimicrobial peptides (AMPs) are important components of innate immunity. They are often expressed in response to colonic inflammation and infection. Over the last several years, the roles of several antimicrobial peptides have been explored. Gene expression of many AMPs (beta defensin HBD2-4 and cathelicidin) is induced in response to invasion of gut microbes into the mucosal barrier. Some AMPs are expressed in a constitutive manner (alpha defensin HD 5-6 and beta defensin HBD1), while others (defensin and bactericidal/permeability increasing protein BPI) are particularly associated with Inflammatory Bowel Disease (IBD) due to altered defensin expression or development of autoantibodies against Bactericidal/permeability increasing protein (BPI). Various AMPs have different spectrum and strength of antimicrobial effects. Some may play important roles in modulating the colitis (cathelicidin) while others (lactoferrin, hepcidin) may represent biomarkers of disease activity. The use of AMPs for therapeutic purposes is still at an early stage of development. A few natural AMPs were shown to be able to modulate colitis when delivered intravenously or intracolonically (cathelicidin, elafin and SLPI) in mouse colitis models. New AMPs (synthetic or artificial non-human peptides) are being developed and may represent new therapeutic approaches against colitis. This review discusses the latest research developments in the AMP field with emphasis in innate immunity and pathophysiology of colitis.

Keywords

Antimicrobial peptides; colitis; infection; microflora; protein; Crohn’s disease; ulcerative colitis

INTRODUCTION

Antimicrobial peptides (AMPs) are endogenous antibiotics with antimicrobial activities. They are generally expressed in the intestinal lining in close contact with the gut microflora. AMPs are expressed in a constitutive or inducible manner in intestinal epithelial cells, and Paneth and immune cells, respectively. Over the last few years, many endogenous AMPs have been studied for their expression during intestinal inflammation in Crohn’s disease (CD) or Ulcerative colitis (UC) as well as during infections such as *E. Coli., C. difficile* or Amoeba.

Many AMPs, in addition to their antimicrobial effects, can also modulate immune responses. One AMP, hepcidin, can act as a hormone and regulate iron metabolism. Many AMPs are

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CONFLICT OF INTEREST
The authors confirm that this article content has no conflicts of interest.

expressed during inflammatory processes and can serve as disease biomarkers, as in the case of Inflammatory Bowel Disease (IBD). Since the pathophysiology of colitis involves interactions between the gut microflora and the host mucosa, recent reports suggested that AMP induction is associated with dysfunctional gut barrier and involvement of bacterial components.

This review article summarizes recent findings of several major antimicrobial peptides found in the ileum and colon and discusses their role in the pathophysiology of intestinal inflammation of different etiologies.

DEFENSIN FAMILY

Defensin is a large group of 10 peptides in humans. Defensins represent an important part of the gut's innate immune response and they are secreted from Paneth cells, epithelial cells, as well as immune cells. Defensins are classified as alpha defensin and beta-defensin based on their molecular distribution of cysteine amino acids and the resulting disulfide bonds [1]. Defensins are further classified as constitutive (expression remains unchanged during inflammation or infection) and inducible (increased expression during inflammation or infection) [1, 2].

Human Alpha Defensin (HNP1-4)

Human alpha defensins 1-4 (HNP1-4), also called human neutrophil peptides, are primarily secreted from neutrophils [3]. They appear to contribute to innate immunity at the systemic level as neutrophils can circulate around the whole body with a broad spectrum of antibacterial activity against many pathogens [4]. HNP-1 had been shown to block LPS induced IL-1β release from monocytes, suggesting anti-inflammatory effects against this endotoxin [5]. But another study showed that intraperitoneal administration of HNP-1 to mice with DSS-induced colitis leads to more severe colitis with higher colonic cytokine levels compared to controls, suggesting a potential pro-inflammatory role for HNP-1 in colitis [6]. On the other hand, HNP-1 and HNP-3 had been shown to inhibit cytotoxicity and Rho glucosylation in Caco-2 cells caused by *C. difficile* toxin B, but not toxin, A while beta defensin had no such protective effect [7]. Interestingly, HNP1-3 protein is expressed in active IBD mucosa and this response may be associated with increased neutrophil infiltration into IBD tissues [8]. Plasma concentrations of HNP1-3 are also significantly increased in IBD patients, but not normal subjects, possibly due to increased HNP expression from circulating neutrophils [9, 10]. Up to now, there is no report indicating a role for HNP-4 in IBD or any other form of colitis, although its anti-bacterial effects are stronger compared to HNP1-3 [11].

Human Alpha Defensin (HD5 and HD6)

Another group of human alpha defensin (HD5 and HD6) is expressed only in Paneth cells of the human duodenum, jejunum and ileum [3]. HD-5 and HD-6 are not expressed in normal adult colon, possibly due to the lack of Paneth cells [12]. But in ileal CD patients, expression of HD-5 and HD-6 is reduced, compared to ileum of control subjects [13, 14]. Although one study suggested that NOD2 mutations may be associated with reduced expression of alpha defensin (HD-5 and HD-6) [15], another study did not find a mechanistic link between NOD2 and Paneth cell alpha defensin expression [16]. Interestingly, HD-5 is also expressed in metaplastic Paneth cells in the colons of IBD patients [17, 18], which may represent a self-defense response to bacterial challenges during colitis.

Paneth cell alpha defensin has multiple roles in infection and inflammation. Mature HD-5 shows bactericidal activities to all bacterial strains. It also induces IL-8 expression in intestinal epithelial cells and injection of exogenous HD-5 reduces mortality in mice with

DSS colitis [19]. Transgenic mice overexpressing HD-5 are highly resistant to enteric *Salmonella* infection [20]. HD-5 can also inhibit *C. difficile* toxin B cytotoxicity in intestinal epithelial Caco2 cell monolayers by inhibiting toxin B-catalyzed Rho glucosylation [7].

Mouse Alpha Defensin (cryptdin)

Mouse cryptdins (Crps), like HD-5 and HD-6, are expressed in Paneth cells. The precursor forms of Crps are localized in Paneth cell granules and processed into their microbicidal form by interacting with matrix metalloproteinase-7 (MMP-7) [21, 22]. Thus, Crps preparations from MMP7 $^{-/-}$ mice have decreased antimicrobial activity 21, while MMP-7 deficient mice are more susceptible to colitis [5]. Crps, like other alpha defensins, are resistant to proteolysis and may remain functional in the ileum to colon [23, 24]. There are 6 isoforms of cryptdin with cryptdin-4 being the most bactericidal against many gut bacteria species [25, 26]. Although *Salmonella* infection inhibit Cryptdin expression in gut Paneth cells [27], cryptdin-2 is effective in treating *Salmonella Typhimurium* infection in mice [28].

Human beta-defensin 1 (HBD1)

Human beta defensin 1 (HBD-1) is constitutively expressed in colonic and ileal epithelium of humans regardless of colitis [29, 30]. Even challenges with IL-1α and/or *E.coli* still fail to alter the mRNA expression of hBD-1 in human colonic epithelial Caco-2 and HT-29 cells. A study showed that Peroxisome proliferator-activated receptor gamma (PPARγ) directly regulates DEFB1 expression in human colonic Caco-2. PPARγ deficient mice express much less beta-defensin mDefB10 in the colonic mucosa, with defective killing of *Candida albicans*, *Bacteroides fragilis*, *Enterococcus faecalis* and *E. coli* [31], suggesting that this defensin may play a role in colonic infection and inflammation.

INDUCIBLE human beta-defensin (HBD-2, -3, -4)

Human beta-defensin 2 (HBD-2) is barely expressed in normal colon but its expression is significantly increased in inflamed colonic epithelium of IBD patients [30]. Despite this increase, however, plasma levels of HBD-2 in IBD patients remain unchanged [9]. Unlike constitutive HBD-1, exposure of human colonic epithelial Caco-2 and HT-29 cells to proinflammatory IL-1α and/or enteroin-vasive *E.coli (O29:NM)* significantly increase HBD-2 expression, suggesting an important role for HBD-2 in the pathophysiology of colitis and colitis-associated microflora. Fahlgren *et al* also found that HBD-3 and HBD-4 expression are similarly increased in colonic crypts of UC, but not CD patients [32]. Mouse beta defensin-3 (analogue of HBD-2 in humans) is strongly up-regulated in the colonic epithelium of mice with chronic experimental, DSS-induced colitis [33]. However, unlike HD-5, beta defensin has no protective effect against cytotoxicity of *C. difficile* toxin B [7].

Theta Defensin (Not Existing Naturally in Humans)

Theta defensin protein is not expressed in humans because of a stop codon at the human theta defensin DNA [34]. But synthetically modified theta defensin (retrocyclin) possesses remarkable antibacterial activity and excellent antiviral activity against HIV [35]. There is no report showing a therapeutic role for theta defensin in colitis *in vivo*. However, compared to alpha defensin HNP-1, another modified theta defensin (RC-1) exerts an even greater inhibition of intracellular growth of the gut pathogen *Listeria monocytogenes* within macrophages [36].

CATHELICIDIN

Cathelicidins are a family of peptides with established antibacterial, anti-viral and anti-fungal effects [37, 38]. LL-37 and mCRAMP are the only forms of cathelicidin in humans and mice, respectively. Cathelicidin is located on chromosome 3 and contains 4 exons [39, 40]. Although the exact antimicrobial mechanism of cathelicidin is not fully understood, it is established that LL-37 is able to form transmembrane pores which can penetrate bacteria [41]. Cathelicidin permeabilizes the bacterial cell membrane and inhibits cell wall biogenesis leading to inhibition of bacterial cell growth, as in the case of *E. coli* [42].

Cathelicidin peptides are secreted from surfaces exposed to the external environment, including the gut. In addition, cathelicidins are present in amniotic fluid and breast milk. Western blot analysis of human milk showed that the mature form of LL-37 is present in human milk. LL-37 also possesses antimicrobial activity against *Staphylococcus aureus*, group A *Streptococcus* and enteroinvasive *E. coli* O29, common pediatric pathogens [43]. This indicates that LL-37 can confer immune protection to fetuses and newborns before full autonomous immunity is established.

In a total 89 normal and IBD patients, colonic cathelicidin *Camp* mRNA expression is significantly increased, but only in UC, not CD patients [44]. There is also no significant difference of cathelicidin expression levels among different NOD2 gene polymorphism status or severity of inflammation in CD patients [44]. In the colonic mucosa, cathelicidin is typically expressed at the top of colonic crypts but not in deeper crypts and this expression is similar among normal and IBD patients. However, such cathelicidin induction is unrelated to the presence of pro-inflammatory cytokines in IBD patients, since TNFα, IFNγ, LPS, IL-12, IL-4 and IL-13 are not able to induce cathelicidin expression in human colonic epithelial HT-29 cells [44].

There is some evidence showing that the mechanism of cathelicidin expression involves stimulation by bacterial components. For example, the short-chain fatty acid butyrate, a bacteria metabolite, is a well established inducer of cathelicidin. Sodium butyrate belongs to HDAC inhibitors family and therefore is not surprising that another HDAC inhibitor, Trichostatin A induces expression of cathelicidin [45]. In addition, the transcription factor PU.1 of the Ets family binds to *Camp* promoter segments and triggers *Camp* gene expression in HT-29 cells [46]. Upon cell stimulation by vitamin D, butyrate, or the secondary bile acid lithocholic acid, both Vitamin D receptor and PU.1 are recruited to the Camp promoter thereby enhancing cathelicidin gene transcription [46]. Another study showed that vitamin D, but not lithocholic acid induces cathelicidin mRNA and protein expression in human colonic Caco-2 cells [47], although another group failed to reproduce this response [48].

Toll-like receptors (TLRs) are sensors of pathogen-associated molecular patterns. Several TLR ligands stimulate cathelicidin in several different cell types, including macrophages [49]. Koon *et al* recently found that cathelicidin deficient *Camp*$^{-/-}$ mice develop worse experimental acute colitis than wild-type mice [50]. Administration of bacterial DNA induces colonic cathelicidin expression in normal mice as well as in mice with DSS-induced colitis. Bacterial DNA, a known TLR9 ligand, stimulates LL-37 expression in primary human monocytes via an ERK1/2-dependent mechanism. Further, bone marrow transplantation experiments demonstrated that expression of cathelicidin from bone-marrow derived immune cells plays an important role in the development of DSS-induced colitis in mice [50].

Therapeutic Effects of Cathelicidin

As shown by Koon *et al*, endogenous cathelicidin plays an anti-inflammatory role in the development of DSS colitis in mice [50]. Tai *et al* used intracolonic administration of the cathelicidin mCRAMP to treat mouse colitis in the same model [51]. Intrarectal administration of mCRAMP to mice with DSS colitis significantly ameliorated several colonic inflammatory parameters, restored colonic mucus thickness through increased expression of mucin genes (MUC1-4), and suppressed colonic apoptosis with negligible effects on mucosal healing. Importantly, mCRAMP administration reduced the total population of fecal microflora, suggesting significant antimicrobial effects. *In vitro* experiments showed that LL-37 has no effects on cell proliferation, but exerts anti-apoptotic and wound healing effects in human intestinal epithelial HT-29 and Caco-2 cells [52]. One of the putative LL-37 receptors, P2X7 is expressed in primary intestinal epithelial cells and Caco-2 cells but not HT-29 cells [52]. LL-37 induces mucin gene expression via P2X7-dependent pathway [52].

Induction of endogenous cathelicidin may also produce similar therapeutic effects in models of infection. Oral administration of butyrate or phenylbutyrate to a rabbit model of shigellosis significantly increased cathelicidin mRNA and protein expression in colonic and rectal mucosa, a response associated with reduced clinical signs of infection [53, 54]. Despite these positive findings, however, both human and mouse cathelicidin failed to kill *Entamoeba histolytica* and did not ameliorate colitis in response to this pathogen in a mouse model. *Entamoeba histolytica* releases a cysteine protease that cleaves cathelicidin, leading to degradation of this antimicrobial peptide and resistance to killing [55].

In conclusion, cathelicidin may be a potential therapeutic solution for colitis, at least during acute colitis states, while its role in chronic colitis remains to be evaluated. Pro-angiogenic effects, alterations of the gut microflora, and participation of the putative cathelicidins receptors FPRL1 or P2X in the development of colitis remain to be evaluated.

PROTEASE INHIBITORS: ELAFIN AND SECRETORY LEUKOCYTE PEPTIDASE INHIBITOR (SLPI)

During colitis, a delicate balance between proteases and anti-protease responses determines in part the development of inflammation [56]. In inflammatory states, proteases damage tissues while protease inhibitors stabilize tissue damage and facilitate healing. Elafin is a protease inhibitor with anti-bacterial effects [57] that modulates inflammation via its anti-protease activity [58]. In a microarray study of human colonic biopsies, UC patients expressed approximately 30 times more elafin mRNA compared to the healthy controls [59]. Such elevation of elafin occurs in rectal epithelium, including goblet cells and enterocytes in patients with total colon UC [59]. Another report also showed similar results as elafin was expressed in active, but not inactive inflamed colonic mucosa of UC patients [60]. Surprisingly, increased elafin expression is not evident in CD patients [60]. Expression of another anti-protease equivalent, SLPI, was also elevated in inflamed UC mucosa, but not in non-inflamed UC mucosa or colon of CD patients [60]. It is possible that low expression of the anti-protease elafin and SLPI match with high expression of MMPs in CD which leads to high risk of fistula. In UC patients, elafin and SLPI levels are high and may tend to act as a self-protective mechanism against colitis [60]. SLPI is expressed in human jejunum and colonic biopsies as well as human colonic epithelial Caco-2-BBE, T84, and HT29-Cl.19A cells [61]. Although SLPI exerts direct antimicrobial effects including against *Salmonella typhimurium*, it does not affect epithelial barrier integrity [61].

In both the DSS and the TNBS colitis models, overexpression of elafin by adenovirus delivery significantly ameliorates colitis that is associated with reduced colonic proteolytic activity, and diminished cytokine levels and NF-κB activation [62]. Overexpression of elafin also significantly reduced TNFα mediated increased permeability of Caco-2 cells, suggesting a beneficial effect in epithelial barrier integrity [62]. Elafin can decrease IL-8 secretion and NF-κB luciferase activity induced by TNFα or LPS in HT-29 cells, indicating potent anti-inflammatory effects [62]. SLPI may also promote healing during colitis. Thymic stromal lymphopoetin (Tslp) deficient mice had reduced expression of SLPI [63].

Interestingly, these deficient mice developed colitis during the inflammatory stage to a similar degree to wild-type mice, but failed to recover from colitis, resulting in increased mortality [63]. Administration of recombinant SLPI to Tslp deficient mice reduced DSS colitis- associated mortality, indicating an important role for SLPI for mucosal healing following colitis.

BACTERICIDAL/PERMEABILITY INCREASING PROTEIN (BPI)

BPI can kill gram negative bacteria via binding to LPS and inhibit LPS-induced host cell toxic responses [64]. Its SNP (GLU216Lys) genotype is associated with CD but not UC [65], suggesting that it may be involved in impaired defense against gram-negative bacteria in CD patients. BPI expression is increased in the colonic mucosa of UC patients, compared to controls [66]. BPI is generally localized in PMN cells in the mucosa and stroma of colons and its concentration is correlated with disease activity in UC patients [66]. Several human intestinal epithelial cells (Caco-2, T84 and SW480) also express BPI mRNA, while over-expression of BPI in Caco-2 cells reduces *Salmonella* induced IL-8 secretion, suggesting anti-inflammatory effects [67].

Some IBD patients develop anti-neutrophil cytoplasmic auto-antibodies (ANCA) which target BPI [68]. Most IgG of UC and CD patients can neutralize BPI resulting in reduced antibiotic function of BPI [68]. BPI-targeting auto-antibodies in IBD patients is associated with greater mucosal damage and extent of disease [69] and thus may contribute to development of IBD.

HEPATOCARCINOMA-INTESTINE-PANCREAS (HIP) / PANCREATITIS-ASSOCIATED PROTEIN (PAP)

HIP/PAP belongs to the Rag family and RagIII subfamily. HIP/PAP is expresses in colorectal cancer [70]. In adults, HIP/PAP is expressed in Paneth cells at the base of intestinal pits and endocrine cells around the epithelial lining of the jejunum, ileum and ascending colon [71]. These cells coexpress the endocrine cell marker chromogranin A and synaptophysin [71]. HIP/PAP expression is significantly induced in colonic epithelial cells upon exposure to bacteria in germ free or colitic mice exposed to DSS [72]. HIP/PAP mRNA is also induced in the colonic epithelial cells of IBD patients [72]. HIP/PAP, as a C-type lectin, can kill bacteria by binding to peptidoglycan carbohydrate [73]. Although HIP/PAP possesses antibacterial activity [74], its role in the development of colitis is not fully understood.

LYSOZYME

Lysozyme is a common antimicrobial protein which damages bacterial cell wall by hydrolysis of peptidoglycans [75]. Lysozyme is secreted from polymorphonuclear (PMN) cells and exists in a wide range of host secreted products, such as mucus [76], tears [77] and milk [78]. Colonic epithelial cells of UC patients express significantly higher lysozyme mRNA than controls [18], but fecal lysozyme may not be the best indicator of UC, compared to other fecal IBD biomarkers [79]. In CD patients, lysozyme mRNA is also found in colonic, but not ileal epithelial cells and chronic colonic inflammation results in increased lysozyme expression [18]. In a model of colitis in pigs, hen egg lysozyme infusion reduces DSS-induced colitis, a response associated with reduced colonic TNFα and IL-6 expression [80]. Like cathelicidin, hen egg lysozyme also increases mucin gene expression which promotes colonic barrier integrity [80].

LACTOFERRIN

Lactoferrin is also called lactotransferrin as it belongs to transferring family which consists of protein and iron. Lactoferrin is abundant in milk, earning its lacto- prefix [78]. With its iron binding property, lactoferrin exert its multiple antibacterial effects via deprivation of iron from pathogens [81]. Lactoferrin binds to the LPS layer of bacterial cell wall and causes increased membrane permeability and bacterial cell lysis [82]. Iron in the bacterial cell may kill the cell via peroxide formation [83]. Alternatively, lactoferrin may also stimulate phagocytosis in immune cells [84].

Multiple reports suggest that fecal lactoferrin is a non-invasive biomarker of IBD as its levels are significantly increased in IBD, but not IBS patients [85]. Like another IBD marker, calprotectin, both are neutrophil derived-indicators of disease activity [86]. Lactoferrin is simple and inexpensive to detect and has excellent stability in feces for an extended period of time. One report showed that a decrease of fecal lactoferrin may be correlated to mucosal healing and response to therapy [87]. Apart from IBD, fecal lactoferrin levels are also increased along with levels of IL-1β and IL-8 in patients with severe *C. difficile* colitis [88], as well as patients with Enterohemorrhagic *E. coli* infection [89].

The therapeutic value of oral lactoferrin administration is underscored by its ability to dose-dependently ameliorate DSS-induced colitis in rats [90]. After oral bovine lactoferrin treatment, colonic expression of anti-inflammatory cytokines, IL-4 and IL-10 are increased, while expression of proinflammatory cytokines TNFα, IL-1β and IL-6, histology damage and MPO levels are improved [90]. Such oral bovine lactoferrin treatment has similar beneficial effects to TNBS colitis in rats [91]. Lactoferrin-derived lactoferricin and lactoferrampin had been shown to kill *Entamoeba histolytica* in and this may help reduce use of the antibiotic metronidazole that is associated with several side effects [92]. Bovine Lactoferrin also exert anti-inflammatory effects as it reduces IL-8 secretion from Caco-2 cells infected with *E. coli* HB101 [93].

HEPCIDIN

Hepcidin plays an important regulatory role in iron homeostasis while it also possesses antimicrobial properties [94]. It limits the availability of iron to bacteria, thus withholding the growth of invading pathogens [95]. Hepcidin can also control intracellular *Salmonella and Mycobacteria* by modulating iron availability within macrophages. Prohepcidin is a precursor form of hepcidin. Acting as a hormone, hepcidin is generally secreted from the liver and inhibits iron absorption from the gut. Although hepcidin has antibacterial effects [81], no anti-bacterial role in colitis has been reported.

One study showed that serum hepcidin levels are significantly higher in both UC and CD patients, compared to healthy subjects [96]. Serum hepcidin was also correlated with disease activity and C-reactive protein levels in UC patients [96]. Serum hepcidin is negatively correlated, while serum prohepcidin is positively correlated with hemoglobin levels [96], suggesting that hepcidin and prohepcidin may be related to anemia associated with IBD. However, another group found that hepcidin expression to be dependent on bone morphogenetic protein (BMP) and IL-6. Using the T-cell transfer mouse model of colitis, an anti-BMP reagent could inhibit hepcidin expression, thus increasing serum iron levels in mice with colitis [97]. Therefore, inhibition of hepcidin may correct IBD associated anemia and help reduce colonic inflammation.

NOVEL OR ARTIFICIAL AMPS IN INFLAMMATION

Apart from natural endogenous antimicrobial peptides, there are many non-host and artificial ant-microbial peptides possessing anti-inflammatory effects in various kinds of colitis. The nine amino acid peptide coprisin (LLCIALRKK), that derives from Korean dung beetle, exerts antimicrobial activity and prevents mice from *C. difficile* -associated inflammation and mucosal damage in mice [98]. A modified coprisin analogue does not affect commensal bacteria such as *lactobacillus* and *bifidobacterium*, but inhibits colonization of *C. difficile* in mice via a mechanism that involves disruption of the bacterial membrane [98]. Two semi-synthetic glycopeptides, telavancin and dalbavancin with antibacterial activity against Gram-positive bacteria have been evaluated in clinical trials for their oral effectiveness in *C. difficile* colitis and digestive tract decontamination [99]. A novel antimicrobial peptide (wrwycr) was recently found to inhibit bacterial DNA repair –associated mechanisms [100]. This peptide significantly reduces survival of Shiga toxin producing O157-H7 *E. coli* in acid stress [100]. Although data from animal experiments or clinical trials are not available, this peptide may be another potential candidate for prevention of intestinal bacterial infection.

One difficulty in developing new antimicrobial peptides relates to the lack of consensus about the essential structure contributing to their antimicrobial and/or anti-inflammatory activity. Among all of the antimicrobial peptides discussed above, their amino acid sequence, molecular size and structure are largely different [101]. It is possible to alter individual amino acid(s) in a short AMP sequence to understand how the peptide sequence affects its antimicrobial activity, but this approach is very difficult for larger protein molecules [102, 103]. As cathelicidin is short (37 amino acids) and easy to manipulate, there are many reports studying antimicrobial effects of altered forms of cathelicidin [104–106]. Up to now, however, there is no clear way to predict how to design a novel antimicrobial peptide based on our current knowledge of AMPs.

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CONCLUDING REMARKS

Expression of most of endogenous antimicrobial peptides and proteins are increased during colitis or colonic infection (Table 1). Some AMPs act as disease markers of colitis that predicts disease activity or response to therapy. The intimate association of AMP, microflora and immune regulation in the gut is still being extensively investigated. A few of AMPs had shown potential therapeutic effects in animal models by administration of exogenous antimicrobial peptides or protein. Development of new antimicrobial peptides for treating colitis is at an early stage and more information on peptide/protein stability, delivery method, efficacy and safety is much needed. Since many AMPs are unstable in blood, AMP gene therapy via viral vectors may be another possible way for prolonged expression of AMP in the host for long-term treatment. But safety of AMPs and its delivery methods still require more research as no human clinical trial data of AMP in colitis is available. Alternatively, induction of endogenous AMPs may be another interesting future research direction of innate immunity and protection of the host. The associated anti-inflammatory effects of antimicrobial peptides and proteins represent an important correlative advantage in developing new AMPs for pharmaceutical purposes.

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Acknowledgments

ACKNOWLEDGEMENT OF FUNDING

NIH funding DK084256, CCFA career development grant to Hon Wai Koon.

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Table 1

Overview of Antimicrobial Peptides in Colitis

AMP(s)	Expression	Roles in IBD/colitis	Roles in bacterial infection	Roles in *C. difficile* toxin inflammation
HNP1-4	Neutrophils: Increase possibly due to neutrophil infiltration in colons.	HNP-1 is protective against DSS colitis in mice.	Protective against many strains of bacteria and LPS	HNP-1 and HNP-3 are protective against toxin B
HD-5, HD-6	Constitutive expression in ileal Paneth cells. Decreased in CD, further reduced with NOD2 mutation.	Protective against DSS colitis in mice.	Against all strains	HD-5 is protective against toxin B.
Cryptdin	Ileal Paneth cells in mice only	Not known	Against *Salmonella*	Not known
HBD-1	Constitutive in colonic epithelium	Not changed in colitis	Against many strains of bacteria	Not known
HBD-2-4	Colonic epithelium, increase in colitis.	Stimulated by IL-1α	Stimulated by LPS	Not protective against toxin B
Theta defensin	Not expressed in humans	Not known	*Against Listeria monocytogenes* by retrocyclin	Not known
Cathelicidin	Colon epithelium, increase in UC but not CD	Inhibit DSS colitis, Camp$^{-/-}$ mice have more serious DSS colitis. Anti-inflammatory.	Bactericidal against many strains of bacteria including Shigella but not *Entamoeba histolytica*	Not protective against toxin B
Elafin/SLPI	Colonic epithelium, increase in UC but not CD	Protective against DSS colitis, possibly anti- inflammatory.	At least bactericidal against *Salmonella*	Not known
BPI	Colonic epithelium, increase in UC	Compromised by ANCA antibody, possibly anti-inflammatory.	Kill gram negative bacteria.	Not known
HIP/PAP	Paneth cells, endocrine cells	Increased in IBD/colitis	Against many strains of bacteria	Not known
Lyzozyme	PMN cells, increased in IBD	Protective against DSS colitis	Against many strains of bacteria	Not known
Lactoferrin	Neutrophils, Increased in IBD, *E. coli* and *C. difficile* infection	Protective against DSS and TNBS colitis	Protective against *Entamoeba histolytica* and *E. coli*	Not known
Hepcidin	From liver, act like hormone, serum hepcidin level increases in IBD.	Associated with IBD anemia	May be not important	Not known