# Antimicrobial peptides in intestinal inflammation, infection and cancer.

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Antimicrobial peptides (AMPs) form a significant part of innate immunity. AMP expression is often altered in response to colonic infection, inflammation and cancer. Over the last two decades, the roles of antimicrobial peptides have been discovered and explored. Certain AMPs such as alpha defensin HD 5-6 and beta defensin HBD1 are constitutively expressed while others including defensin HBD2-4 and bactericidal/permeability increasing protein (BPI) are associated with Inflammatory Bowel Disease (IBD). Cathelicidin expression is decreased in colon cancer tumors. Gene expression of several AMPs (beta defensin HBD2-4 and cathelicidin) is induced in response to invasion by gut microbes. Cathelicidin can modulate colitis while other AMPs such as lactoferrin and hepcidin can serve as biomarkers of IBD disease activity and/or colon cancer. Several endogenous host-based AMPs (cathelicidin, elafin and SLPI) may be delivered intravenously or intracolonically *in vivo*. Novel AMPs (synthetic or artificial non-human peptides) with potent antimicrobial, anti-inflammatory or anti-tumoral property have been developed but the application of AMPs for therapeutic purposes is still at an early stage of development. This report details the latest development of AMP-related research with emphasis on innate immunity and pathophysiology of colitis, intestinal infection and cancer.

**Keywords** Antimicrobial peptides, colitis, infection, microflora, protein, Crohn's disease, ulcerative colitis, cancer.

## 1. 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 intestinal epithelial cells, Paneth cells and immune cells in either a constitutive or inducible manner. Over the last decade, many endogenous AMPs have been studied for their expression and role during infections and cancer as well as during intestinal inflammation in Crohn's disease (CD) or Ulcerative colitis (UC). This book chapter includes recent findings of different antimicrobial peptides and proteins found in the gut and further discusses their role in intestinal infection, inflammation and cancer.

## 2. Natural antimicrobial peptides

### 2.1. Cathelicidin

Humans and mice have only one form of cathelicidin called LL-37 and mCRAMP, respectively [1, 2]. Although the exact antimicrobial mechanism of cathelicidin is not fully understood, it is known that LL-37 is able to form transmembrane pores on cell membrane of target organisms [3]. Subsequently, cathelicidin increases cell membrane permeability and inhibits bacterial cell wall biosynthesis [4]. Cathelicidin possesses significant antimicrobial activity against group A *Streptococcus*, *Staphylococcus aureus* and enteroinvasive *E. coli* O29 [5]. In addition, cathelicidins are found in amniotic fluid and breast milk. The mature form of LL-37 is present in human milk, suggestive of its role in conferring passive immunity in fetuses and newborns before autonomous immunity is fully established.

Colonic cathelicidin *Camp* mRNA expression is significantly increased in UC, but not CD patients as seen in biopsy samples from a pool of 89 normal and IBD patients [6]. The presence of NOD2 gene polymorphism or severity of inflammation have no significant influence on cathelicidin expression level in CD patients [6]. In the colonic mucosa, cathelicidin is typically found at the top of colonic crypts but not in deeper crypts and this expression pattern is similar among normal and IBD patients. The expression of cathelicidin and its distribution in the colon is not associated with pro-inflammatory cytokines in IBD patients, as TNFα, IFNγ, LPS, IL-4, IL-12 and IL-13 are not able to induce cathelicidin expression in human colonic epithelial HT-29 cells [6].

Short-chain fatty acid butyrate, a bacterial metabolite, is a well-established stimulant of cathelicidin expression. Sodium butyrate belongs to the histone deacetylase (HDAC) inhibitors family. Exposure to another HDAC inhibitor, Trichostatin A, leads to an increase in cathelicidin expression though this observed response was anticipated [7]. Moreover, the transcription factor PU.1 of the Ets family binds to the *Camp* promoter segment and mediates *Camp* gene expression in HT-29 cells [8]. Upon stimulation by specific agents, such as vitamin D, butyrate, or the secondary bile acid lithocholic acid, both PU.1 and Vitamin D receptor are recruited to the *Camp* promoter to facilitate cathelicidin gene transcription [9]. Curcumin, a curry spice ingredient, can also induce cathelicidin RNA and protein expression in HT-29 cells independent of vitamin D receptor pathway [10].

Ligands of TLR-2, TLR-4 and TLR-9 stimulate cathelicidin in several different cell types, including epithelial cells, macrophages and neutrophils [11]. Koon *et al* recently found that intracolonic administration of bacterial DNA induces colonic cathelicidin expression in normal mice as well as in mice with DSS-induced colitis. Cathelicidin deficient *Camp*$^{-/-}$ mice developed severe experimental acute DSS colitis compared to wild-type mice [12]. Furthermore, bone marrow transplantation experiments demonstrated that expression of cathelicidin from bone-marrow derived immune cells plays an important anti-inflammatory role in the development of DSS-induced colitis in mice [12].

As shown by Koon *et al*, endogenous cathelicidin exerts anti-inflammatory effects during the course of DSS-induced colitis in mice [12]. Tai *et al* administered cathelicidin mCRAMP intracolonically to treat mouse colitis in the same model [13]. Intrarectal administration of mCRAMP to mice with DSS-induced colitis led to a significant reduction in colonic histological damages and apoptosis while simultaneously restored colonic mucus thickness through increased expression of mucin genes (MUC1-4). Importantly, mCRAMP administration markedly reduced total fecal microflora loading further solidifying its role as a potent antimicrobial agent. Intrarectal delivery of mCRAMP-encoding plasmids and oral delivery of mCRAMP-encoding *Lactococcus* can also provide similar protective effects against DSS-induced colitis in mice [14, 15]. *In vitro* experiments showed that cathelicidin has no effect on cell proliferation, but can exert anti-apoptotic effect and promote wound healing in human intestinal epithelial HT-29 and Caco-2 cells [16]. One of the putative LL-37 receptors, P2X purinoceptor 7 (P2RX7), is expressed in primary intestinal epithelial cells and Caco-2 cells but not HT-29 cells [16]. LL-37 induces mucin gene expression via P2RX7-dependent pathway [16].

Endogenous cathelicidin induction may produce similar therapeutic effects in animal models of infection. Oral administration of butyrate or phenylbutyrate to rabbits infected with *Shigella* resulted in increase expression of cathelicidin mRNA and protein level within colonic and rectal mucosa with improvement of symptoms [17]. However, both human and mouse cathelicidin failed to kill *Entamoeba histolytica* and did not ameliorate *Entamoeba histolytica*-associated colitis in mice because *Entamoeba histolytica* releases a cysteine protease that can cleave cathelicidin and subsequently lead to its degradation [18].

A recent report showed that LL-37 activated p53 dependent but caspase independent activation of apoptosis in human cancer cells including HCT116 and LoVo cells [19]. This anti-tumoral effect is G-protein coupled receptor dependent but does not involve formyl peptide receptor 2 as its antagonist WRW4 failed to block it. Cathelicidin deficient *Camp*$^{-/-}$ mice exhibited increased susceptibility to azoxymethane induced colon cancer development as their colonic mucosa have reduced expression of p53, Bax and Bak and increased expression of Bcl-2 with low basal apoptosis compared to wild-type mice. In addition, LL-37 expression in human colonic tumors is down-regulated while LL-37 is expressed strongly in normal colonic tissues [19]. Another group also showed cathelicidin analog peptide FF-CAP18 caused apoptosis and inhibited cell proliferation in HCT116 colon cancer but this effects is p53 independent [20].

In summary, cathelicidin may be a potential therapeutic treatment for colitis, at least in acute colitis, while its role in chronic colitis has yet been fully elucidated. Cathelicidin may also be a promising agent against colon cancer. However, the cytotoxicity and hemolytic activity of cathelicidin are major concerns that need to be addressed prior to clinical applications [21].

## 3. Defensin family

Human defensin family consists of a large group of ten antimicrobial peptides. Defensins are secreted from Paneth cells, epithelial cells and immune cells and they constitute an important part of the gut innate immune response. Defensins are divided into two groups, alpha defensin and beta-defensin, based on their amino acid cysteine distribution and orientation of disulfide bonds [22]. Defensins are further labeled as constitutive (unchanged expression during inflammation or infection) or inducible (increased expression during inflammation or infection).

### 3.1. *Human alpha defensin (HNP1-4)*

Human alpha defensins 1-4 (HNP1-4), also called human neutrophil peptides, are primarily secreted from neutrophils [23]. These neutrophil-derived defensins possess antibacterial activity against a broad spectrum of pathogens and modulates innate immunity to defend the host against infections [1, 2]. Intraperitoneal administration of HNP-1 to mice with DSS-induced colitis leads to more severe colitis with higher colonic cytokine expression compared to controls, suggesting a potential pro-inflammatory role for HNP-1 in colitis [24]. Interestingly, HNP1-3 protein expression is increased in active IBD intestinal mucosa, though this response may be related to increased neutrophil infiltration within the inflamed tissues [25]. Plasma concentrations of HNP1-3 are also significantly increased in IBD patients, but not in normal subjects, possibly as a result of increased HNP release from circulating neutrophils [2, 23-25]. HNP-1 has been shown to inhibit LPS-induced IL-1β release from monocytes, suggesting that it possesses an inherent anti-inflammatory effect against endotoxin [26]. HNP-1 and HNP-3 inhibit cytotoxicity and Rho glucosylation in Caco-2 cells caused by *C. difficile* toxin B, but not toxin A, while beta defensin has no such protective effect [27]. There is a lack of evidence supporting the role of HNP-4 in IBD or other form of colitis even though its antibacterial effects are noticeably prominent compared to HNP1-3 [28]. Based on a study of 100 colorectal cancer patients and 60 normal

 

volunteers, the plasma concentration of HNP-1, HNP-2 and HNP-3 are all significantly elevated [29]. Therefore, alpha defensin may serve a novel biomarker for colorectal cancer.

### 3.2. *Human alpha defensin (HD5 and HD6)*

Human alpha defensins (HD5 and HD6) are only expressed in Paneth cells of the human duodenum, jejunum and ileum [23]. The inactive form of HD-5 is stored in granules within Paneth cells and the enzyme trypsin cleaves it to active form upon secretion [30]. They are not expressed in normal adult colon due to the lack of Paneth cells [26]. Interestingly, HD-5 is found in metaplastic Paneth cells in the colon of IBD patients [30] and presumably it serves as a protective role in response to bacterial challenges during colitis. In Crohn's disease patients, ileal expression of HD-5 and HD-6 are reduced compared to those of control subjects [31]. One study suggested that NOD2 mutations may be associated with reduced expression of alpha defensin (HD-5 and HD-6) [31]; however, there is no direct association between NOD2 and Paneth cell alpha defensin expression [32]. Paneth cell alpha defensin HD-5 exerts multiple roles in inflammation and infection. Mature form of HD-5 possesses bactericidal activities against numerous bacterial strains that include *E. coli*, *S. aureus* and *S. typhimurium* [33-36] and can induce IL-8 expression in intestinal epithelial cells. Transgenic mice overexpressing HD-5 are highly resistant to enteric *Salmonella* infection [37]. HD-5 can also inhibit *C. difficile* toxin B cytotoxicity in intestinal epithelial Caco-2 cell monolayers by inhibiting toxin B-catalyzed Rho glucosylation [27]. Human alpha defensin HD-5 protein level is elevated in the serum of colon cancer patients. Its protein level is also increased in the colonic tumors versus normal intestinal samples [38]. Human alpha defensin HD-6 gene DEFA6 expression is only moderately increased in colonic carcinoma but many adenoma expressed DEFA6 more than 60 fold compared to normal colon tissues [39]. Thus DEFA6 may be suitable for diagnosis of adenoma but not carcinoma, i.e. early colon cancer development.

### 3.3. *Human beta-defensin 1 (HBD1)*

Human beta defensin 1 (protein: HBD-1; gene name: DEFB1) is constitutively expressed in both ileal and colonic epithelium of humans [1, 2]. Even with exposure to proinflammatory cytokine IL-1α or *E.coli*, the levels of DEFB1 mRNA expression in human colonic epithelial Caco-2 and HT-29 cells are unaffected. One study has demonstrated the role of peroxisome proliferator-activated receptor gamma (PPARγ) in directly regulating DEFB1 expression in human colonic Caco-2 cells [40]. PPARγ deficient mice expressed a marked decrease in beta-defensin mDefB10 within the colonic mucosa [40]. The cationic proteins extracted from colonic mucosal biopsies of PPARγ deficient mice showed a lack of bactericidal activity against *Candida albicans*, *Bacteroides fragilis*, *Enterococcus faecalis* and *E. coli* [40], suggesting that HBD-1 may play a role in colonic inflammation and infection. In contrast to HD-5, beta defensin 1 does not protect Caco-2 cells against the cytotoxicity effect of *C. difficile* toxin B [27].

### 3.4. *Inducible human beta-defensin (HBD 2-4)*

Human beta-defensin 2 (HBD-2) is minimally expressed in normal colon but its expression is considerably elevated in inflamed colonic epithelium of IBD patients [41]. Despite this known response, plasma levels of HBD-2 in IBD patients remain unchanged [42]. Unlike constitutive HBD-1, exposure of human colonic epithelial Caco-2 and HT-29 cells to pro-inflammatory IL-1α or enteroinvasive *E.coli (O29:NM)* significantly induce HBD-2 expression, indicating that HBD2 plays a role in the pathogenesis of colonic inflammation and colitis-associated microflora. HBD-3 and HBD-4 expression are markedly increased in colonic crypts of UC, but not CD patients [43]. There is increase mouse beta defensin-3 expression, an analogue of HBD-2 in humans, in the colonic epithelium of mice with chronic experimental DSS-induced colitis [44].

## 4. Hepcidin

Hepcidin has antimicrobial properties and also acts as a major regulator in iron homeostatis [45]. Serum hepcidin level is notably elevated in both UC and CD patients, compared to normal, healthy subjects [46]. Serum hepcidin level is directly associated with disease activity among UC patients [45]. Moreover, serum hepcidin level is inversely correlated with hemoglobin level, further supporting the role of hepcidin in IBD-associated anemia [46]. There is also evidence suggesting that the expression of hepcidin is dependent on bone morphogenetic protein/interleukin-6 (BMP/IL-6). Anti-BMP reagents (BMP receptor blockers and anti-BMP antibody) are able to decrease hepcidin levels in mice with T-cell transfer colitis leading to an increase in serum iron level and modest reduction in severity of colitis [47]. Inhibition of hepcidin may lead to a reversal of IBD-associated anemia and colonic inflammation. The antimicrobial potential of hepcidin in IBD may be negligible. Among colorectal cancer patients, hepcidin level is not correlated to anemia but its mRNA expression is increased in colonic tumors with decreased ferropoetin expression [48]. Therefore, hepcidin may be involved in signaling pathway of colon cancer.

## 5. Natural antimicrobial proteins:

### 5.1. Protease inhibitors: Elafin and secretory leukocyte peptidase inhibitor (SLPI)

Elafin possesses antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Elafin is also a protease inhibitor with anti-bacterial effects [49] that modulates inflammation through its anti-protease activity [50]. A delicate relationship between proteases and anti-proteases is central in determining the development of inflammation during colitis [51]. Proteases damage tissues in inflammation while protease inhibitors minimize tissue damage and facilitate healing. In a microarray study of human colonic biopsies, UC patients expressed more elafin mRNA by 30-fold in comparison to healthy controls [46] but elafin expression is not evident in CD patients [52].

The expression of another anti-protease equivalent, secretory leukocyte protease inhibitor (SLPI), is toxic to *E. coli* and mycobacteria [53, 54]. SLPI is expressed in human jejunum and colonic biopsies as well as in human colonic epithelial Caco-2-BBE, T84, and HT29-Cl.19A cells [55]. SLPI level is elevated in inflamed UC colonic mucosa, but its level is unaffected in non-inflamed UC colonic mucosa or colon of CD patients [56]. It is probable that low expression of the anti-protease elafin and SLPI along with high expression of matrix metalloproteinases in CD patients may be associated with a risk of developing fistula as a result of increase protease activity. Elafin and SLPI levels are elevated in UC patients and may serve as a self-protective mechanism against colitis. SLPI is able to exert direct antimicrobial effects but it does not affect epithelial barrier integrity specifically against *Salmonella typhimurium* [56].

Adenoviral overexpression of elafin is associated with decreased colonic proteolytic activity, reduced NF-κB activation and diminished cytokine levels that subsequently lead to a marked reduction in DSS and TNBS models of colitis [57]. Elafin overexpression also inhibits TNFα induced permeability of Caco-2 cells *in vitro*, thus rendering a protective effect in maintaining the integrity of epithelial barrier. Elafin exerts potent anti-inflammatory effects by decreasing IL-8 secretion and NF-κB luciferase activity as seen in HT-29 cells exposed to TNFα or LPS [57]. SLPI can promote healing during colitis. Thymic stromal lymphopoetin (Tslp) deficient mice had reduced expression of SLPI with enhanced neutrophil elastase activity during inflammation [58]. These deficient mice developed similar degree of acute colitis like wild-type mice when exposed to DSS, but failed to recover from colitis resulting in higher mortality rates. Administration of recombinant SLPI to Tslp-deficient mice significantly reduced DSS-induced colitis associated mortality rates, thus indicating mucosal healing role of SLPI.

## 6. Bactericidal/permeability increasing protein (BPI)

BPI is stored inside secretory granules of neutrophils within the mucosa and stroma of colon [59]. BPI binds to LPS directly and inhibits LPS-induced cytoxicity. BPI mRNA expression can be found in human intestinal epithelial (Caco-2, T84 and SW480) cells and its over-expression in Caco-2 cells resulted in a reduction of *Salmonella* induced IL-8 secretion [60]. BPI expression appeared to be elevated in UC patients compared to normal, healthy patients. BPI level is correlated with disease activity in UC [59]. One single nucleotide polymorphism (SNP) genotype (GLU216Lys) is associated with impaired defense against gram-negative bacteria in CD patients [61]. Some IBD patients have elevated anti-neutrophil cytoplasmic (ANCA) antibodies levels which neutralize the antimicrobial effects of BPI and are associated with severe symptoms of IBD patients [62].

## 7. Hepatocarcinoma-intestine-pancreas (HIP) / pancreatitis-associated protein (PAP)

HIP/PAP, as a C-type lectin of Rag family, can directly damage bacteria by binding to peptidoglycan carbohydrate [63]. HIP/PAP is expressed in Paneth cells within the base of intestinal pits and endocrine cells of jejunum, ileum and ascending colon [64]. HIP/PAP mRNA expression exists in colonic epithelial cells of IBD patients while its level is increased upon exposure to bacteria in germ free mice or DSS-induced colitis [65]. Although HIP/PAP possesses antibacterial activity, its role in the development of colitis is still not fully understood. HIP/PAP is also found in colorectal cancer tissues [66].

## 8. Lactoferrin

Lactoferrin is a protein found in milk [67]. Lactoferrin binds to the LPS layer of bacterial cell wall and causes increased membrane permeability that ultimately promotes bacterial cell lysis [68]. Moreover, lactoferrin can stimulate the phagocytosis of immune cells and assist in regulating the inflammatory response [69]. Lactoferrin exerts its antibacterial effect by depriving iron supply to the offending pathogens [70]. Multiple reports suggest that fecal lactoferrin is a non-invasive biomarker of IBD as its level is significantly increased in IBD, but not irritable bowel syndrome patients [71]. One report indicates that a decrease in fecal lactoferrin may be correlated to mucosal healing and positive response to therapy [72]. In certain gut infections such as C. *difficile* colitis and enterohemorrhagic *E. coli*, fecal lactoferrin levels are elevated along with other inflammatory markers such as IL-8 and IL-1β [73 74]. Oral lactoferrin administration minimizes

 

DSS-induced colitis in rats and its effect appears to be dose-dependent [75]. 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), mucosal damage and MPO levels are reduced [75]. The same oral bovine lactoferrin treatment showed similar beneficial effects in TNBS-induced colitis in rats [76]. Bovine lactoferrin has anti-inflammatory effects as it inhibits IL-8 secretion from human colonic epithelial Caco-2 cells infected with *E. coli* HB101 [77]. Lactoferrin-derived lactoferricin and lactoferrampin have the ability to directly damage *Entamoeba histolytica* making them a good candidate as an alternative treatment if antibiotics cannot be tolerated [78].

Lactoferrin inhibits cell proliferation by lowering cyclin E1 expression in human colon cancer Caco-2 cells [79]. This later showed lactoferrin derived peptide lactoferricin(4-14) can reduce the ultraviolet light induced DNA damages in Caco-2 cells. A clinical trial demonstrated that 104 patients treated with oral administration of bovine lactoferrin showed retarded colonic adenomatous polyp growth [80]. Lactoferrin also enhances Fas expression and apoptosis in the colonic musosa of azoxymethane exposed rats [81]. Therefore, lactoferrin may be a preventive measure against colon cancer. On the other hand, 50% of colorectal cancer and 16% of colorectal polyps patients have positive fecal lactoferrin finding [82]. High fecal lactoferrin levels may be useful for colorectal cancer diagnosis.

## 9. Lysozyme

Lysozyme functions by hydrolyzing bacterial peptidoglycans cell wall [83]. Lysozyme is secreted from polymorphonuclear (PMN) cells and exists in multiple forms of secreted product, such as mucus [84], tears [85] and milk [67]. Lysozyme mRNA is elevated in colonic, but not ileal, epithelial cells [86]. Increased lysozyme expressions have also been detected in chronic colonic inflammation [86]. Colonic epithelial cells of UC patients have significantly higher lysozyme mRNA expression than controls [86] and its level is correlated with disease activity [87]. The exogenous administration of lysozymes derived from hen eggs in pigs with colitis resulted in a down-regulation of inflammatory response and reduction in TNFα and IL-6 expression [88]. Like cathelicidin, hen egg-derived lysozyme also increases mucin gene expression and promotes colonic barrier integrity in the DSS-induced colitis [88]. Lyzozyme production is elevated in colonic adenoma and adenocarcinoma [89, 90] but its biological significance in colon cancer development is not fully understood.

## 10. Synthetic antimicrobial peptides:

One major challenge in developing new antimicrobial peptides stems from the lack of consensus regarding the essential structure of these peptides and exact mechanism of their antimicrobial and anti-inflammatory effects. At the present time, there is no standard algorithm for developing synthetic antimicrobial peptides. New synthetic antimicrobial peptides are being developed. Several modified cathelicidin analogs are discussed in this section.

Tritrpticin (VRRFPWWWPFLRR) is a cathelicidin-derived peptide found in porcine cDNA [91]. The Trp, Pro and Arg amino acids within the peptide function by disrupting cell membrane of target organisms rendering them a potential agent for antimicrobial therapy. Tritrpticin can also prevent LPS-mediated lethality in rats [92]. Unfortunately, its hemolytic effect rendered it less suitable for clinical therapy [93].

Indolicidin (ILPWKWPWWPWRR-NH2) is derived from bovine neutrophils with antimicrobial effects against many Gram-positive bacteria, Gram-negative bacteria and fungi. Indolicidin functions by increasing membrane permeability and inhibiting DNA synthesis but does not cause bacterial cell lysis [94]. Indolicidin reduces the mortality rates of *E. coli* infection and LPS-mediated septic shock in rats [95]. Similar to Tritrpticin, the Trp amino acid within indolicidin is responsible for its hemolytic activity [96].

SMAP-29, a sheep-derived cathelicidin analog, can inhibit *E. coli* and LPS-mediated septic shock resulting in a lower mortality rate in rats [97]. The pro-19 amino acid of SMAP-29 plays a central role in determining its antibacterial property. The N-terminal amphipathic region and C-terminal hydrophobic region are also responsible for both its antibacterial activity and hemolytic activity [98].

A new class of non-peptide based cathelicidin analogs called Ceragenins has been shown to possess antimicrobial properties [99]. Ceragenins are cationic steroid cholic acid derived compounds developed by N8 medical Inc. Like cathelicidin, they are cationic amphiphiles that can form complexes with phospholipids and cause rapid membrane depolarization [99, 100]. However, their roles in intestinal infection, inflammation and cancer are still under investigation.

## 11. Conclusion

Expression of several endogenous antimicrobial peptides is increased during colitis or colonic infection (Table 1). Certain AMPs serve as disease markers of colitis to predict disease activity or response to therapy. The administration of exogenous AMPs has shown potential therapeutic effects in animal models. More research is needed to address the stability of antimicrobial peptides, modes of delivery, toxicity and efficacy *in vivo*. Further investigation, including

human clinical trials, is needed to elucidate the effects of synthetic and endogenous AMPs as a novel approach in the treatment of inflammatory bowel diseases, intestinal infection, and colorectal cancer.

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**Table 1** Overview of natural antimicrobial peptides and proteins in colitis, infection and cancer.

| AMP(s) | Expression | Roles in IBD/colitis | Roles in bacterial infection | Roles in intestinal cancer |
|---|---|---|---|---|
| **Natural antimicrobial peptides** | | | | |
| Cathelicidin | Colon epithelium, increased expression in UC but not CD. | Camp$^{-/-}$ mice have more serious DSS colitis in mice. Exogenous cathelicidin is anti-inflammatory and inhibit DSS colitis in mice. | Bactericidal against many strains of bacteria including Shigella but not *Entamoeba histolytica;* cathelicidin is not protective against *C. difficile* toxin B. | Down-regulated in colon tumors. Inhibit colon cancer proliferation and induce apoptosis. |
| Alpha defensin 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 *C. difficile* toxin B. | HNP-1-3 increase in tumors and serum of colon cancer patients. May serve as biomarker. |
| Alpha defensin 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 *C. difficile* toxin B | HD-5 increase in serum and tumors. HD-6 increase in adenoma. |
| Beta defensin HBD-1 | Constitutive in colonic epithelium | Not changed in colitis | Against many strains of bacteria | Not known |
| Beta defensin HBD-2-4 | Colonic epithelium, increase in colitis | Stimulated by IL-1$\alpha$. | Stimulated by LPS, not protective against *C. difficile* toxin B. | Not known |
| Hepcidin | From liver, act like hormone, serum hepcidin level increases in IBD | Associated with IBD anemia. | May be not important in antimicrobial role. | Increased in colonic tumors. |
| **Natural antimicrobial proteins** | | | | |
| BPI | Colonic epithelium, increase in UC | Compromised by ANCA antibody, possibly anti-inflammatory. | Kill gram negative bacteria. | Not known |
| Elafin/SLPI | Colonic epithelium, increase in UC but not CD | Protective against DSS colitis, possibly anti-inflammatory. | At least against *Salmonella* | 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 | Increased expression in colonic tumors. |
| Lactoferrin | Neutrophils, Increased in IBD, *E. coli* and *C. difficile* infection | Protective against DSS and TNBS colitis | Protective against *Entamoeba histolytica* and *E. coli* | Inhibit adenomatous polyp growth. May serve as biomarker. |

 

## References


[1] Ho S, Pothoulakis C, Koon HW. Antimicrobial peptides and colitis. Curr Pharm Des 2013;19:40-7.
[2] Wehkamp J, Schauber J, Stange EF. Defensins and cathelicidins in gastrointestinal infections. Curr Opin Gastroenterol 2007;23:32-8.
[3] Lee CC, Sun Y, Qian S, Huang HW. Transmembrane pores formed by human antimicrobial peptide LL-37. Biophys J 2011;100:1688-96.
[4] Sochacki KA, Barns KJ, Bucki R, Weisshaar JC. Real-time attack on single Escherichia coli cells by the human antimicrobial peptide LL-37. Proc Natl Acad Sci U S A 2011;108:E77-81.
[5] Murakami M, Dorschner RA, Stern LJ, Lin KH, Gallo RL. Expression and secretion of cathelicidin antimicrobial peptides in murine mammary glands and human milk. Pediatr Res 2005;57:10-5.
[6] 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;18:615-21.
[7] Schauber J, Iffland K, Frisch S, Kudlich T, Schmausser B, Eck M, Menzel T, Gostner A, Luhrs H, Scheppach W. Histone-deacetylase inhibitors induce the cathelicidin LL-37 in gastrointestinal cells. Mol Immunol 2004;41:847-54.
[8] Termen S, Tollin M, Rodriguez E, Sveinsdottir SH, Johannesson B, Cederlund A, Sjovall J, Agerberth B, Gudmundsson GH. PU.1 and bacterial metabolites regulate the human gene CAMP encoding antimicrobial peptide LL-37 in colon epithelial cells. Mol Immunol 2008;45:3947-55.
[9] Schauber J, Oda Y, Buchau AS, Yun QC, Steinmeyer A, Zugel U, Bikle DD, Gallo RL. Histone acetylation in keratinocytes enables control of the expression of cathelicidin and CD14 by 1,25-dihydroxyvitamin D3. J Invest Dermatol 2008;128:816-24.
[10] Guo C, Rosoha E, Lowry MB, Borregaard N, Gombart AF. Curcumin induces human cathelicidin antimicrobial peptide gene expression through a vitamin D receptor-independent pathway. J Nutr Biochem 2012.
[11] Pinheiro da Silva F, Gallo RL, Nizet V. Differing effects of exogenous or endogenous cathelicidin on macrophage toll-like receptor signaling. Immunol Cell Biol 2009;87:496-500.
[12] .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.
[13] 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;104:251-8.
[14] Tai EK, Wu WK, Wang XJ, Wong HP, Yu L, Li ZJ, Lee CW, Wong CC, Yu J, Sung JJ, Gallo RL, Cho CH. Intrarectal administration of mCRAMP-encoding plasmid reverses exacerbated colitis in Cnlp(-/-) mice. Gene Ther 2012.
[15] Tai EK, Wu WK, Wong HP, Lam EK, Yu L, Cho CH. A new role for cathelicidin in ulcerative colitis in mice. Exp Biol Med (Maywood) 2007;232:799-808.
[16] Otte JM, Zdebik 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;156:104-17.
[17] Raqib R, Sarker P, Bergman P, Ara G, Lindh M, Sack DA, Nasirul Islam KM, Gudmundsson GH, Andersson J, Agerberth B. Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic. Proc Natl Acad Sci U S A 2006;103:9178-83.
[18] Cobo ER, He C, Hirata K, Hwang G, Tran U, Eckmann L, Gallo RL, Reed SL. Entamoeba histolytica Induces Intestinal Cathelicidins but Is Resistant to Cathelicidin-Mediated Killing. Infect Immun 2011;80:143-9.
[19] .Ren SX, Cheng AS, To KF, Tong JH, Li MS, Shen J, Wong CC, Zhang L, Chan RL, Wang XJ, Ng SS, Chiu LC, Marquez VE, Gallo RL, Chan FK, Yu J, Sung JJ, Wu WK, Cho CH. Host immune defense peptide LL-37 activates caspase-independent apoptosis and suppresses colon cancer. Cancer Res 2012;72:6512-23.
[20] Kuroda K, Fukuda T, Yoneyama H, Katayama M, Isogai H, Okumura K, Isogai E. Anti-proliferative effect of an analogue of the LL-37 peptide in the colon cancer derived cell line HCT116 p53+/+ and p53. Oncol Rep 2012;28:829-34.
[21] Sorensen OE, Borregaard N. Cathelicidins--nature's attempt at combinatorial chemistry. Comb Chem High Throughput Screen 2005;8:273-80.
[22] Cederlund A, Gudmundsson GH, Agerberth B. Antimicrobial peptides important in innate immunity. FEBS J 2011;278:3942-51.
[23] Cunliffe RN. Alpha-defensins in the gastrointestinal tract. Mol Immunol 2003;40:463-7.
[24] 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 2011;18:667-75.
[25] 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.
[26] Shi J, Aono S, Lu W, Ouellette AJ, Hu X, Ji Y, Wang L, Lenz S, van Ginkel FW, Liles M, Dykstra C, Morrison EE, Elson CO. A novel role for defensins in intestinal homeostasis: regulation of IL-1beta secretion. J Immunol 2007;179:1245-53.
[27] Giesemann T, Guttenberg G, Aktories K. Human alpha-defensins inhibit Clostridium difficile toxin B. Gastroenterology 2008;134:2049-58.
[28] Ericksen B, Wu Z, Lu W, Lehrer RI. Antibacterial activity and specificity of the six human {alpha}-defensins. Antimicrob Agents Chemother 2005;49:269-75.
[29] Kemik O, Kemik AS, Sumer A, Begenik H, Purisa S, Tuzun S. Human neutrophil peptides 1, 2 and 3 (HNP 1-3): elevated serum levels in colorectal cancer and novel marker of lymphatic and hepatic metastasis. Hum Exp Toxicol 2011;32:167-71.


 

[30] Cunliffe RN, Rose FR, Keyte J, Abberley L, Chan WC, Mahida YR. Human defensin 5 is stored in precursor form in normal Paneth cells and is expressed by some villous epithelial cells and by metaplastic Paneth cells in the colon in inflammatory bowel disease. Gut 2001;48:176-85.
[31] Wehkamp J, Harder J, Weichenthal M, Schwab M, Schaffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schroder JM, Bevins CL, Fellermann K, Stange EF. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut 2004;53:1658-64.
[32] Simms LA, Doecke JD, Walsh MD, Huang N, Fowler EV, Radford-Smith GL. Reduced alpha-defensin expression is associated with inflammation and not NOD2 mutation status in ileal Crohn's disease. Gut 2008;57:903-10.
[33] Ouellette AJ. Paneth cell alpha-defensins: peptide mediators of innate immunity in the small intestine. Springer Semin Immunopathol 2005;27:133-46.
[34] Ouellette AJ. Paneth cell alpha-defensin synthesis and function. Curr Top Microbiol Immunol 2006;306:1-25.
[35] Ouellette AJ. Paneth cell alpha-defensins in enteric innate immunity. Cell Mol Life Sci 2011;68:2215-29.
[36] Ouellette AJ, Satchell DP, Hsieh MM, Hagen SJ, Selsted ME. Characterization of luminal paneth cell alpha-defensins in mouse small intestine. Attenuated antimicrobial activities of peptides with truncated amino termini. J Biol Chem 2000;275:33969-73.
[37] Salzman NH, Ghosh D, Huttner KM, Paterson Y, Bevins CL. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature 2003;422:522-6.
[38] Bukurova Iu A, Nikitina SL, Khankin SL, Krasnov GS, Lisitsin NA, Karpov VL, Beresten SF. [Identification of protein markers for serum diagnosis of cancer based on microRNA expression profiling]. Mol Biol (Mosk) 2011;45:376-81.
[39] Radeva MY, Jahns F, Wilhelm A, Glei M, Settmacher U, Greulich KO, Mothes H. Defensin alpha 6 (DEFA 6) overexpression threshold of over 60 fold can distinguish between adenoma and fully blown colon carcinoma in individual patients. BMC Cancer 2010;10:588.
[40] Peyrin-Biroulet L, Beisner J, Wang G, Nuding S, Oommen ST, Kelly D, Parmentier-Decrucq E, Dessein R, Merour E, Chavatte P, Grandjean T, Bressenot A, Desreumaux P, Colombel JF, Desvergne B, Stange EF, Wehkamp J, Chamaillard M. Peroxisome proliferator-activated receptor gamma activation is required for maintenance of innate antimicrobial immunity in the colon. Proc Natl Acad Sci U S A 2010;107:8772-7.
[41] Wehkamp J, Fellermann K, Herrlinger KR, Baxmann S, Schmidt K, Schwind B, Duchrow M, Wohlschlager C, Feller AC, Stange EF. Human beta-defensin 2 but not beta-defensin 1 is expressed preferentially in colonic mucosa of inflammatory bowel disease. Eur J Gastroenterol Hepatol 2002;14:745-52.
[42] 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;58:192-7.
[43] Fahlgren A, Hammarstrom S, Danielsson A, Hammarstrom ML. beta-Defensin-3 and -4 in intestinal epithelial cells display increased mRNA expression in ulcerative colitis. Clin Exp Immunol 2004;137:379-85.
[44] Rahman A, Fahlgren A, Sundstedt C, Hammarstrom S, Danielsson A, Hammarstrom ML. Chronic colitis induces expression of beta-defensins in murine intestinal epithelial cells. Clin Exp Immunol 2010;163:123-30.
[45] Ganz T. Hepcidin and iron regulation, 10 years later. Blood 2011;117:4425-33.
[46] Oustamanolakis P, Koutroubakis IE, Messaritakis I, Malliaraki N, Sfiridaki A, Kouroumalis EA. Serum hepcidin and prohepcidin concentrations in inflammatory bowel disease. Eur J Gastroenterol Hepatol 2011;23:262-8.
[47] Wang L, Trebicka E, Fu Y, Ellenbogen S, Hong CC, Babitt JL, Lin HY, Cherayil BJ. The bone morphogenetic protein-hepcidin axis as a therapeutic target in inflammatory bowel disease. Inflamm Bowel Dis 2011;18:112-9.
[48] Ward DG, Roberts K, Brookes MJ, Joy H, Martin A, Ismail T, Spychal R, Iqbal T, Tselepis C. Increased hepcidin expression in colorectal carcinogenesis. World J Gastroenterol 2008;14:1339-45.
[49] Wiesner J, Vilcinskas A. Antimicrobial peptides: the ancient arm of the human immune system. Virulence 2010;1:440-64.
[50] Williams SE, Brown TI, Roghanian A, Sallenave JM. SLPI and elafin: one glove, many fingers. Clin Sci (Lond) 2006;110:21-35.
[51] Medina C, Radomski MW. Role of matrix metalloproteinases in intestinal inflammation. J Pharmacol Exp Ther 2006;318:933-8.
[52] Flach CF, Eriksson A, Jennische E, Lange S, Gunnerek C, Lonnroth I. Detection of elafin as a candidate biomarker for ulcerative colitis by whole-genome microarray screening. Inflamm Bowel Dis 2006;12:837-42.
[53] Nishimura J, Saiga H, Sato S, Okuyama M, Kayama H, Kuwata H, Matsumoto S, Nishida T, Sawa Y, Akira S, Yoshikai Y, Yamamoto M, Takeda K. Potent antimycobacterial activity of mouse secretory leukocyte protease inhibitor. J Immunol 2008;180:4032-9.
[54] Miller KW, Evans RJ, Eisenberg SP, Thompson RC. Secretory leukocyte protease inhibitor binding to mRNA and DNA as a possible cause of toxicity to Escherichia coli. J Bacteriol 1989;171:2166-72.
[55] Si-Tahar M, Merlin D, Sitaraman S, Madara JL. Constitutive and regulated secretion of secretory leukocyte proteinase inhibitor by human intestinal epithelial cells. Gastroenterology 2000;118:1061-71.
[56] 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;81:907-15.
[57] Motta JP, Magne L, Descamps D, Rolland C, Squarzoni-Dale C, Rousset P, Martin L, Cenac N, Balloy V, Huerre M, Frohlich LF, Jenne D, Wartelle J, Belaaouaj 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;140:1272-82.
[58] Reardon C, Lechmann M, Brustle A, Gareau MG, Shuman N, Philpott D, Ziegler SF, Mak TW. Thymic stromal lymphopoetin-induced expression of the endogenous inhibitory enzyme SLPI mediates recovery from colonic inflammation. Immunity 2011;35:223-35.
[59] Monajemi H, Meenan J, Lamping R, Obradov DO, Radema SA, Trown PW, Tytgat GN, Van Deventer SJ. Inflammatory bowel disease is associated with increased mucosal levels of bactericidal/permeability-increasing protein. Gastroenterology 1996;110:733-9.

 

[60] Levy O, Canny G, Serhan CN, Colgan SP. Expression of BPI (bactericidal/permeability-increasing protein) in human mucosal epithelia. Biochem Soc Trans 2003;31:795-800.
[61] Klein W, Tromm A, Folwaczny C, Hagedorn M, Duerig N, Epplen J, Schmiegel W, Griga T. A polymorphism of the bactericidal/permeability increasing protein (BPI) gene is associated with Crohn's disease. J Clin Gastroenterol 2005;39:282-3.
[62] Schinke S, Fellermann K, Herlyn K, Reichel PH, Fundke R, Stange EF, Gross WL, Schultz H. Autoantibodies against the bactericidal/permeability-increasing protein from inflammatory bowel disease patients can impair the antibiotic activity of bactericidal/permeability-increasing protein. Inflamm Bowel Dis 2004;10:763-70.
[63] Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science 2006;313:1126-30.
[64] 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.
[65] 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;9:162-70.
[66] Zheng HC, Sugawara A, Okamoto H, Takasawa S, Takahashi H, Masuda S, Takano Y. Expression profile of the REG gene family in colorectal carcinoma. J Histochem Cytochem 2011;59:106-15.
[67] Clare DA, Catignani GL, Swaisgood HE. Biodefense properties of milk: the role of antimicrobial proteins and peptides. Curr Pharm Des 2003;9:1239-55.
[68] Drago-Serrano ME, de la Garza-Amaya M, Luna JS, Campos-Rodriguez R. Lactoferrin-lipopolysaccharide (LPS) binding as key to antibacterial and antiendotoxic effects. Int Immunopharmacol 2011;12:1-9.
[69] Kanyshkova TG, Buneva VN, Nevinsky GA. Lactoferrin and its biological functions. Biochemistry (Mosc) 2001;66:1-7.
[70] Johnson EE, Wessling-Resnick M. Iron metabolism and the innate immune response to infection. Microbes Infect 2011;14:207-16.
[71] Langhorst J, Elsenbruch S, Mueller T, Rueffer A, Spahn G, Michalsen A, Dobos GJ. Comparison of 4 neutrophil-derived proteins in feces as indicators of disease activity in ulcerative colitis. Inflamm Bowel Dis 2005;11:1085-91.
[72] Gisbert JP, McNicholl AG, Gomollon F. Questions and answers on the role of fecal lactoferrin as a biological marker in inflammatory bowel disease. Inflamm Bowel Dis 2009;15:1746-54.
[73] Steiner TS, Flores CA, Pizarro TT, Guerrant RL. Fecal lactoferrin, interleukin-1beta, and interleukin-8 are elevated in patients with severe Clostridium difficile colitis. Clin Diagn Lab Immunol 1997;4:719-22.
[74] Steiner TS, Lima AA, Nataro JP, Guerrant RL. Enteroaggregative Escherichia coli produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. J Infect Dis 1998;177:88-96.
[75] 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;17:1291-8.
[76] Togawa J, Nagase H, Tanaka K, Inamori M, Umezawa T, Nakajima A, Naito M, Sato S, Saito T, Sekihara H. Lactoferrin reduces colitis in rats via modulation of the immune system and correction of cytokine imbalance. Am J Physiol Gastrointest Liver Physiol 2002;283:G187-95.
[77] Berlutti F, Schippa S, Morea C, Sarli S, Perfetto B, Donnarumma G, Valenti P. Lactoferrin downregulates pro-inflammatory cytokines upexpressed in intestinal epithelial cells infected with invasive or noninvasive Escherichia coli strains. Biochem Cell Biol 2006;84:351-7.
[78] Lopez-Soto F, Leon-Sicairos N, Nazmi K, Bolscher JG, de la Garza M. Microbicidal effect of the lactoferrin peptides lactoferricin17-30, lactoferrampin265-284, and lactoferrin chimera on the parasite Entamoeba histolytica. Biometals 2010;23:563-8.
[79] Freiburghaus C, Janicke B, Lindmark-Mansson H, Oredsson SM, Paulsson MA. Lactoferricin treatment decreases the rate of cell proliferation of a human colon cancer cell line. J Dairy Sci 2009;92:2477-84.
[80] Kozu T, Iinuma G, Ohashi Y, Saito Y, Akasu T, Saito D, Alexander DB, Iigo M, Kakizoe T, Tsuda H. Effect of orally administered bovine lactoferrin on the growth of adenomatous colorectal polyps in a randomized, placebo-controlled clinical trial. Cancer Prev Res (Phila) 2009;2:975-83.
[81] Fujita K, Matsuda E, Sekine K, Iigo M, Tsuda H. Lactoferrin enhances Fas expression and apoptosis in the colon mucosa of azoxymethane-treated rats. Carcinogenesis 2004;25:1961-6.
[82] Hirata I, Hoshimoto M, Saito O, Kayazawa M, Nishikawa T, Murano M, Toshina K, Wang FY, Matsuse R. Usefulness of fecal lactoferrin and hemoglobin in diagnosis of colorectal diseases. World J Gastroenterol 2007;13:1569-74.
[83] Callewaert L, Michiels CW. Lysozymes in the animal kingdom. J Biosci 2010;35:127-60.
[84] Widdicombe J. Relationships among the composition of mucus, epithelial lining liquid, and adhesion of microorganisms. Am J Respir Crit Care Med 1995;151:2088-92; discussion 2092-3.
[85] .McClellan KA. Mucosal defense of the outer eye. Surv Ophthalmol 1997;42:233-46.
[86] .Fahlgren A, Hammarstrom S, Danielsson A, Hammarstrom ML. Increased expression of antimicrobial peptides and lysozyme in colonic epithelial cells of patients with ulcerative colitis. Clin Exp Immunol 2003;131:90-101.
[87] Hidaka M, Sudoh I, Miyaoka M, Saito T. [Measurement of fecal proteins in inflammatory bowel disease--usefulness as an activity index]. Nihon Shokakibyo Gakkai Zasshi 2000;97:161-9.
[88] Lee M, Kovacs-Nolan J, Yang C, Archbold T, Fan MZ, Mine Y. Hen egg lysozyme attenuates inflammation and modulates local gene expression in a porcine model of dextran sodium sulfate (DSS)-induced colitis. J Agric Food Chem 2009;57:2233-40.
[89] Rubio CA. Colorectal adenomas produce lysozyme. Anticancer Res 2003;23:5165-71.
[90] Yuen ST, Wong MP, Chung LP, Chan SY, Cheung N, Ho J, Leung SY. Up-regulation of lysozyme production in colonic adenomas and adenocarcinomas. Histopathology 1998;32:126-32.

[91] Chan DI, Prenner EJ, Vogel HJ. Tryptophan- and arginine-rich antimicrobial peptides: structures and mechanisms of action. Biochim Biophys Acta 2006;1758:1184-202.
[92] Ghiselli R, Cirioni O, Giacometti A, Mocchegiani F, Orlando F, Silvestri C, Licci A, Della Vittoria A, Scalise G, Saba V. The cathelicidin-derived tritrpticin enhances the efficacy of ertapenem in experimental rat models of septic shock. Shock 2006;26:195-200.
[93] Yang ST, Yub Shin SY, Kim YC, Kim Y, Hahm KS, Kim JI. Conformation-dependent antibiotic activity of tritrpticin, a cathelicidin-derived antimicrobial peptide. Biochem Biophys Res Commun 2002;296:1044-50.
[94] Subbalakshmi C, Sitaram N. Mechanism of antimicrobial action of indolicidin. FEMS Microbiol Lett 1998;160:91-6.
[95] Giacometti A, Cirioni O, Ghiselli R, Mocchegiani F, Del Prete MS, Viticchi C, Kamysz W, E LE, Saba V, Scalise G. Potential therapeutic role of cationic peptides in three experimental models of septic shock. Antimicrob Agents Chemother 2002;46:2132-6.
[96] Ando S, Mitsuyasu K, Soeda Y, Hidaka M, Ito Y, Matsubara K, Shindo M, Uchida Y, Aoyagi H. Structure-activity relationship of indolicidin, a Trp-rich antibacterial peptide. J Pept Sci 2010;16:171-7.
[97] Giacometti A, Cirioni O, Ghiselli R, Mocchegiani F, D'Amato G, Circo R, Orlando F, Skerlavaj B, Silvestri C, Saba V, Zanetti M, Scalise G. Cathelicidin peptide sheep myeloid antimicrobial peptide-29 prevents endotoxin-induced mortality in rat models of septic shock. Am J Respir Crit Care Med 2004;169:187-94.
[98] Shin SY, Park EJ, Yang ST, Jung HJ, Eom SH, Song WK, Kim Y, Hahm KS, Kim JI. Structure-activity analysis of SMAP-29, a sheep leukocytes-derived antimicrobial peptide. Biochem Biophys Res Commun 2001;285:1046-51.
[99] Epand RM, Epand RF, Savage PB. Ceragenins (cationic steroid compounds), a novel class of antimicrobial agents. Drug News Perspect 2008;21:307-11.
[100] Lai XZ, Feng Y, Pollard J, Chin JN, Rybak MJ, Bucki R, Epand RF, Epand RM, Savage PB. Ceragenins: cholic acid-based mimics of antimicrobial peptides. Acc Chem Res 2008;41:1233-40.

 