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The Critical Role of the Antimicrobial Peptide LL-37/CRAMP in Protection of Colon Microbiota Balance, Mucosal Homeostasis, Anti-Inflammatory Responses, and Resistance to Carcinogenesis

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Abstract

Mouse cathelin-related antimicrobial peptide (CRAMP) and its homologue human cathelicidin (LL-37) play active roles in innate immune responses, angiogenesis, and wound healing. In addition, LL-37/CRAMP fends off microbes and protects against infections in the colon, where the epithelium is exposed to myriad of enteric pathogens. It is increasingly recognized that LL-37/CRAMP maintains colon mucosal barrier integrity, shapes the composition of microbiota, and protects the host from tumorigenesis. In this review, we discuss the importance of LL-37/CRAMP in the homeostasis of the host, with novel findings derived from mice deficient in CRAMP that support the proposition for this natural antimicrobial peptide and an immune modulator as a drug lead for therapeutic development.

Keywords

CRAMP; homeostasis; colitis; cancer; microbiota

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I. INTRODUCTION

LL-37 and CRAMP are cathelicidin-related antimicrobial peptides, which belong to a family of host-derived antibacterial polypeptides. The first cathelicidin, cecropin, was isolated in 1980 from tissues of the *Hyalophora cecropia* moth.[1] The first mammalian cathelicidins (bactenecins) were isolated in the late 1980s from bovine neutrophils and were named Bac5 and 7.[2] However, some investigators considered that the first mammalian cathelicidin should be rabbit CAP18.[3] Until now, cathelicidins have been identified in a range of animals, including cattle, buffalo, horse, pig, sheep, goat, deer, chicken, fish, snake, rhesus monkey, guinea pig, mouse, rat, and human.[2,4–17]

One cathelicidin gene has been identified in humans which encodes LL-37 of 37 aa residues with a molecular weight of 18 kDa,[18,19] also known as hCAP-18, FALL-39, or CAMP—human cationic antimicrobial peptide.[18,19] In mice, the gene *Cramp* was mapped to chromosome 9 in a region of conserved synteny, homologous to the map locations of cathelicidins in human.[15]

LL-37 is expressed by various cells and tissues such as bone marrow (BM) myeloid cells, neutrophils, macrophages and epithelial cells. In human tissues, the expression of LL-37 is detected in the skin and gastrointestinal tract, including mouth, tongue, esophagus, and colon, as well as in the urinary tract and the lung (Table 1).[20–23] CRAMP was expressed abundantly by mouse granulocytes and bone marrow cells of the myeloid lineage, which agrees with the sites of expression of cathelicidins in humans and during embryogenesis as early as E12, the earliest stage of blood development.[15,18,24,25] CRAMP is detectable in adult mouse testis, spleen, stomach, and intestine but not in brain, liver, heart, or skeletal muscle.[15] Both LL-37 in human and mouse CRAMP possess intrinsic antimicrobial activity to act as “natural antibiotics” in the host. However, they are also able to activate host cells by interacting with cellular receptors.

II. RECEPTOR FOR LL-37/CRAMP

It has been demonstrated that LL-37 uses human formyl peptide receptor 2 (FPR2), a G-protein–coupled, seven-transmembrane domain receptor,[26] as the receptor to mediate its chemotactic and angiogenic effects on myeloid cells.[27,28] Fpr2 is the mouse homologue of FPR2. Mouse CRAMP utilizes Fpr2 to induce leukocyte chemotaxis and activation.[29] There is a significantly reduced recruitment of $Ly6C^+$ inflammatory dendritic cells (DC) into the bronchiolar area in the allergic inflammatory airway of Fpr2- or CRAMP-deficient mice.[30] Injection of mouse CRAMP into skin air pouches results in the accumulation of neutrophils and monocytes, confirming the capacity of CRAMP to act as a chemoattractant *in vivo* via Fpr2.[29]

Interestingly, LL-37 is also reported to interact with a $P2X_7$ receptor and epidermal growth factor receptor (EGFR).[31] $P2X_7$ receptors have been implicated in ATP-mediated cell death, in the regulation of receptor trafficking and inflammation.[32–34] LL-37 promotes high glucose–attenuated epithelial wound healing in cultured corneas[35] and activates innate immunity on airway epithelial surfaces by EGFR transactivation.[36] Furthermore, LL-37 is

able to activate insulin-like growth factor-1 receptor (IGF-1R) on cancer cells, which results in increased cell proliferation and the manifestation of a metastatic phenotype.[37] Therefore, LL-37 appears to activate multiple cellular receptors to exert biological effects.

III. LL-37/CRAMP IS REQUIRED FOR COLON EPITHELIAL BARRIER INTEGRITY

The colon mucosal barrier consists of epithelial and immune cells with participation of a balanced microbiota. LL-37/CRAMP as a natural antimicrobial peptide, produced by colon epithelial cells and macrophages, plays an important role in maintaining colon microbiota balance and supports mucosal homeostasis.

A. Contribution of CRAMP to Intact Colon Crypt Structure

In the colon, LL-37/CRAMP is detectable in epithelial cells located on the luminal surface and in upper crypts with little or no expression in deeper crypts.[38] The peptide is likely associated with the differentiation of colon epithelial cells because LL-37 mRNA and protein were upregulated in spontaneously differentiating Caco-2 human colon epithelial cells as well as in HCA-7 human colon epithelial cells treated with a differentiation-inducing agent, sodium butyrate.[38] In *CRAMP*$^{-/-}$ mice, the length of colonic crypts was significantly shortened, implying a consequence of reduced proliferation of epithelial cells due to lack of CRAMP as a possible differentiation stimulant.[39]

B. Contribution of CRAMP to Colon Mucus Integrity

Human normally live in symbiosis with ~ 10^{13} bacteria present in the colon.[40] Normal intestinal microbiota inhabits the colon mucus layer without penetrating an inner layer to trigger undesirable inflammatory responses.[41] The inner layer of the mucus is densely packed and firmly attached to the epithelium normally “free” of bacteria. The outer layer of the mucus is movable with an expanded volume and colonized by bacteria.[42] In a human colonic cell line, HT-29, LL-37/CRAMP directly stimulates mucus synthesis through MAP kinase activation and up-regulation of *MUC* gene transcription.[43] In the colon of mice deficient in *CRAMP*, the mucus layer is thinner and discontinuous with severe disruptions,[44,45] and therefore more easily colonized and penetrated by *E. coli* strain O157:H7.[44] *CRAMP*$^{-/-}$ mice exhibit defects in re-epithelialization of injured colon tissues due to lack of CRAMP stimulation.[46]

C. Contribution of CRAMP to Microbicidal Function of Macrophages

Macrophages represent the first line of defense against invading bacterial pathogens. Tissueresident macrophages patrol the colon epithelial layer of barrier, putative entry, and colonization sites for pathogens, to control invaders in addition to their functions in removal of dying cells by efferocytosis.[47,48] Our recent study revealed that myeloid cell–specific *CRAMP*$^{-/-}$ (LysMCre-*CRAMP*$^{F/F}$ KO) mice were more sensitive to DSS-induced colitis as compared with intestinal epithelial cell–specific *CRAMP*$^{-/-}$ (VillinMCre-*CRAMP*$^{F/F}$ KO) mice,[39] indicating that macrophage-derived CRAMP plays an important role in maintaining microbicidal function in colon mucosa. CRAMP expression in mouse macrophages was

increased after infection by an intracellular pathogen, *Salmonella typhimur*.[49] Mouse macrophage cell line J774A.1 and bone marrow–derived macrophages (BMMs) infected by *M. smegmatis* showed increased Camp (*CRAMP* gene) mRNA levels, coinciding with increases in their killing activity.[50] Macrophages infected with *S. typhimur* exhibited a punctate-patterned, yet increased, expression of CRAMP in the perinuclear region. CRAMP reduced *Salmonella* division in Wild type (WT) macrophages, but the bacteria showed enhanced survival within macrophages derived from CRAMP-deficient mice. Mechanistically, intracellular reactive oxygen intermediates and proteases in macrophages may be associated with CRAMP production and activity.[49] Some studies also showed that human LL-37 is not only directly bactericidal but serves also as a mediator of vitamin D3-induced autophagy in macrophages which activates the transcription of autophagy-related genes Beclin-1 and Atg5, in association with killing of intracellular bacteria.[51] Therefore, CRAMP/LL37 is critical for protecting the integrity of the colon mucosa, as illustrated in Fig. 1.

IV. CONTRIBUTION OF LL-37/CRAMP–COLON MICROBIOTA BALANCE

In the human gut, there are estimated to be > 1,000 species-level phylotypes of bacteria.[52] Most of these phylotypes belong to only a few phyla. In general, Bacteroidetes and Firmicutes are dominant, whereas Actinobacteria, Proteobacteria, and Verrucomicrobia are frequent but generally minor constituents.[53] A multitude of species of bacteria in the gut are in equilibrium because of control by many factors. Antimicrobial peptides (AMPs) are major players in maintaining microbiota balance in the gut.[54] LL-37/CRAMP, as one of the AMPs, plays an important role in intestinal microbe ecosystems.

In vitro, synthetic CRAMP exhibits antimicrobial activity against the murine enteric pathogen *Citrobacter rodentium*, which, like the clinically related human pathogens enteropathogenic *Escherichia coli* and enterohemorrhagic *E. coli*, adheres to the apical membrane of intestinal epithelial cells.[55] Synthetic CRAMP and LL-37 also kill *E. coli O157:H7 in vitro*.[44] *CRAMP*$^{-/-}$ mice infected with *C. rodentium* by oral inoculation suffer from increased bacterial colonization in the colon and developed significantly higher fecal counts of *C. rodentium*.[55] Those inoculated with *E. coli O157:H7* also exhibited higher fecal counts of this strain, and the bacteria penetrated the mucus layer, forming a higher number of attaching and effacing lesions.[44] Therefore, LL-37/CRAMP mediates innate intestinal defense against colonization by epithelium-adherent bacteria to maintain gut microbiota balance. Recent studies revealed that CRAMP acts as a limiting factor on dysbiosis to maintain ecologic balance in the colon.[39] Single-housed *CRAMP*$^{-/-}$ mice showed a significantly different microbiota composition in feces as compared to single-housed WT mice. However, after 4-wk cohousing, microbiota composition in the feces of WT mice shifted markedly toward that of *CRAMP*$^{-/-}$ mice. Meanwhile, WT mice cohoused with *CRAMP*$^{-/-}$ mice exhibited a phenotype similar to *CRAMP*$^{-/-}$ mice, indicating that the phenotype of *CRAMP*$^{-/-}$ mice is transferable to WT mice by cohousing, presumably through the transfer of pathogenic bacteria species that overgrow in the absence of CRAMP.[39] Sequencing of microbiota DNA in fecal pellets of mice revealed significantly different microbiota composition in non-cohoused WT and *CRAMP*$^{-/-}$ mice, in particular after DSS treatment, with increased *Mucispirillum schaedleri*, *Clostridium populeti*, and

Acetivibrio cellulosolvens in WT mice, but increased *Odoribacter laneus*, *Ruminococcus lactaris*, *Desulfovibrio piger*, *Desulfomicrobium orale*, *Mogibacterium neglectum*, and *Bacteroides acidifaciens* in *CRAMP*$^{-/-}$ mice. It is notable that some species, such as *M. neglectum, D. piger*, and *D. orale*, which are typically found in oral microbiota, were detected in *CRAMP*$^{-/-}$ mice after DSS treatment. The frequencies of *O. laneus*, *D. piger*, and *D. orale* were significantly increased in WT mouse feces after cohousing with *CRAMP*$^{-/-}$ mice.[39] Therefore, CRAMP deficiency was associated with severe dysbiosis in mice, in particular in chemically induced colitis. The role of CRAMP in colon microbiota balance is depicted in Fig. 2.

V. ANTI-INFLAMMATORY EFFECT OF LL-37/CRAMP IN COLITIS

Disturbance in colon homeostasis results in altered composition of the colon microbiota, or dysbiosis. Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of IBD, are characterized by chronic relapsing inflammation of the digestive tract. IBD is caused by complex interaction of genetic, microbial, and immunological factors. Several risk genes identified for IBD are linked to innate immune recognition of bacteria such as NOD2 and NLRP3 or processing and elimination of bacteria.

Colon bacteria have a potentially pathogenic role in intestinal inflammation,[56] as shown by evidence that germ-free animals do not develop intestinal inflammation. It is well-established that interaction between the intestinal microbiome and colon mucosa initiates inflammatory bowel disease and impaired healing. Our recent investigation revealed that *CRAMP*$^{-/-}$ mice are highly sensitive to DSS-induced colitis associated with more extensive mucosal injury, higher-level production of proinflammatory cytokines, and increased infiltration of inflammatory cells in the gut, culminating in decreased mouse survival. As stated earlier, CRAMP deficiency also alters the composition of microbiota in the colon, as shown by the observation that antibiotics alleviated the severity of DSS-induced colitis in *CRAMP*$^{-/-}$ mice. In addition, the colon phenotype found in *CRAMP*$^{-/-}$ mice was transferable to WT mice after cohousing (Fig. 2). Furthermore, administration of synthetic CRAMP significantly reduced the development of DSS-induced ulcerative colitis in mice with a reduction in the number of fecal bacteria.[57] Administration of plasmid[58] or *Lactococcus lactis* encoding *CRAMP* gene[59] alleviated DSS-induced colitis in mice, emphasizing the protective role of CRAMP in chemically induced colitis via its antimicrobial activity.

Interestingly, LL-37/CRAMP also has antifibrogenic effects on murine colitis-associated fibrosis by directly inhibiting collagen synthesis in colonic fibroblasts. Chronic colitis induced by trinitrobenzene sulphonic acid (TNBS) was associated with increased colonic collagen (col1a2) mRNA expression. Intracolonic CRAMP administration or intravenous delivery of the lentivirus-overexpressing *CRAMP* gene significantly reduced colonic collagen (col1a2) mRNA expression in TNBS-exposed mice. Cecal inflammation associated with increased collagen (col1a2) mRNA expression is also caused by *Salmonella* infection, which was prevented by intravenous delivery of the *Camp* (*CRAMP*-) –expressing lentivirus. A mechanism study revealed that LL-37/CRAMP inhibited TGF-β1– and/or

IGF-1–induced collagen synthesis in colon fibroblasts.[60] Thus, LL-37/CRAMP attenuates colitis associated with acute and chronic inflammation.

VI. PROTECTION AGAINST COLON TUMORIGENESIS BY LL-37/CRAMP

LL-37/CRAMP is a double-edged sword in promoting and inhibiting tumor growth. On the one hand, LL-37/CRAMP acts as a ligand for different cell membrane receptors whose expression on cancer cells varies. Overexpression of LL-37/CRAMP was found to promote the development and progression of ovarian,[28,61–64] lung,[31,65,66] and breast cancers[67,68] but to suppress gastric[69] and colon cancer.[70]

On the other hand, LL-37 is highly expressed in normal colon mucosa but is down-regulated in colon cancer tissues.[70,71] Therefore, some investigators suggest that low levels of LL-37 may serve as a biomarker for colon cancer. In mice, CRAMP protects the animals from carcinogenesis with AOM-DSS–induced colitis, which may be caused by increased epithelial cell turnover and leukocyte infiltration in colon mucosa following more severe damage and slower recovery.[39]

The mechanisms by which LL-37/CRAMP suppresses the development of colon cancer is not completely clear. But lines of evidence suggest that (1) LL-37 induces the death of colon cancer cells by activation of caspase-independent apoptosis and autophagy[72]; (2) LL-37 induces apoptotic death of colon cancer cells by regulating metabolic profile, especially purine metabolism, glycolysis, and the tricarboxylic acid cycle[73]; (3) LL-37/CRAMP inhibits colon cancer development by interfering with epithelial mesenchymal transition (EMT) and fibroblast-supported cancer cell proliferation.[74] These observations support the notion that CRAMP deficiency confers on mice increased susceptibility to chemically induced colitis and cancer. Therefore, LL-37/CRAMP may constitute a plausible candidate of therapeutics agent(s).

VII. PERSPECTIVES

LL-37/CRAMP as important host defense peptides are multifunctional, exhibiting broad-range antimicrobial and immune regulatory activity. Currently, the possibility of applying LL-37/CRAMP as therapeutic agents has been explored. For example, CRAMP encoded by plasmid and *Lactococcus lactis* have been used for treating colitis.[58,59] PG-1 (pig peptide protegrin) offers 100% protection of rats against infections caused by intraperitoneal injection of *P. aeruginosa*, *S. aureus*, and methicillin-resistant *S. aureusin*.[75] Ovine cathelicidins SMAP29 and SMAP34 are potential candidates for human therapy against bacterial infection and immune suppression.[76] However, the significance of LL-37/CAMP and related peptides in human immune responses and cancer development remains to be fully recognized. Further understanding of the biological activity of LL-37 and CRAMP as well as other related host-derived microbicidal peptides will be beneficial for development of novel therapeutic agents combating infectious, inflammatory, and cancerous diseases.

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ABBREVIATIONS:

AMPs	antimicrobial peptides
BM	bone marrow
CRAMP	cathelin-related antimicrobial peptide
DC	dendritic cells
EGFR	epidermal growth factor receptor
EMT	epithelial mesenchymal transition
FPR2	formyl peptide receptor 2
Fpr2	mouse FPR2
hCAP	human cathelicidin protein
IGF-1R	insulin-like growth factor-1 receptor
Ly6C	lymphocyte antigen 6 complex
TNF-α	tumor necrosis factor-α
UC	ulcerative colitis
WT	wild type

REFERENCES

1. Hultmark D, Steiner H, Rasmuson T, Boman HG. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Hyalophora cecropia. Eur J Biochem. 1980;106:7–16. [PubMed: 7341234]
2. Gennaro R, Skerlavaj B, Romeo D. Purification, composition, and activity of two bactenecins, antibacterial peptides of bovine neutrophils. Infect Immun. 1989;57:3142–6. [PubMed: 2777377]
3. Hirata M, Yoshida M, Inada K, Kirikae T. Investigation of endotoxin binding cationic proteins from granulocytes: agglutination of erythrocytes sensitized with Re-LPS. Adv Exp Med Biol. 1990;256:287–99. [PubMed: 2183554]
4. Das H, Sharma B, Kumar A. Cloning and characterization of novel cathelicidin cDNA sequence of Bubalus bub-alis homologous to Bos taurus cathelicidin-4. DNA Seq. 2006;17:407–14. [PubMed: 17381041]
5. Scocchi M, Bontempo D, Boscolo S, Tomasinsig L, Giulotto E, Zanetti M. Novel cathelicidins in horse leukocytes (1). FEBS Lett. 1999;457:459–64. [PubMed: 10471829]
6. Kokryakov VN, Harwig SS, Panyutich EA, Shevchenko AA, Aleshina GM, Shamova OV, Korneva HA, Lehrer RI. Protegrins: leukocyte antimicrobial peptides that combine features of corticostatic defensins and tachyplesins. FEBS Lett. 1993;327:231–6. [PubMed: 8335113]

7. Brogden KA, Kalfa VC, Ackermann MR, Palmquist DE, McCray PB, Tack BF. The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection. Antimicrob Agents Chemother. 2001;45:331–4. [PubMed: 11120991]
8. Shamova O, Brogden KA, Zhao C, Nguyen T, Kokryakov VN, Lehrer RI. Purification and properties of proline-rich antimicrobial peptides from sheep and goat leukocytes. Infect Immun. 1999;67:4106–11. [PubMed: 10417180]
9. Fernandez de Mera IG, Perez de la Lastra JM, Ayoubi P, Naranjo V, Kocan KM, Gortazar C, de la Fuente J. Differential expression of inflammatory and immune response genes in mesenteric lymph nodes of Iberian red deer (Cervus elaphus hispanicus) naturally infected with Mycobacterium bovis. Dev Comp Immunol. 2008;32:85–91. [PubMed: 17604102]
10. Xiao Y, Cai Y, Bommineni YR, Fernando SC, Prakash O, Gilliland SE, Zhang G. Identification and functional characterization of three chicken cathelicidins with potent antimicrobial activity. J Biol Chem. 2006;281:2858–67. [PubMed: 16326712]
11. Uzzell T, Stolzenberg ED, Shinnar AE, Zasloff M. Hagfish intestinal antimicrobial peptides are ancient cathelicidins. Peptides. 2003;24:1655–67. [PubMed: 15019197]
12. Wang Y, Hong J, Liu X, Yang H, Liu R, Wu J, Wang A, Lin D, Lai R. Snake cathelicidin from Bungarus fasciatus is a potent peptide antibiotic. PLoS One. 2008;3:e3217. [PubMed: 18795096]
13. Bals R, Lang C, Weiner DJ, Vogelmeier C, Welsch U, Wilson JM. Rhesus monkey (Macaca mulatta) mucosal antimicrobial peptides are close homologues of human molecules. Clin Diagn Lab Immunol. 2001;8:370–5. [PubMed: 11238224]
14. Nagaoka I, Tsutsumi-Ishii Y, Yomogida S, Yamashita T. Isolation of cDNA encoding guinea pig neutrophil cationic antibacterial polypeptide of 11 kDa (CAP11) and evaluation of CAP11 mRNA expression during neutrophil maturation. J Biol Chem. 1997;272:22742–50. [PubMed: 9278433]
15. Gallo RL, Kim KJ, Bernfield M, Kozak CA, Zanetti M, Merluzzi L, Gennaro R. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Bio Chem. 1997;272:13088–93. [PubMed: 9148921]
16. Termen S, Tollin M, Olsson B, Svenberg T, Agerberth B, Gudmundsson GH. Phylogeny, processing and expression of the rat cathelicidin rCRAMP: a model for innate antimicrobial peptides. Cell Mol Life Sci. 2003;60:536–49. [PubMed: 12737313]
17. Durr UH, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochim Biophys Acta. 2006;1758:1408–25. [PubMed: 16716248]
18. Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, Gallo RL. Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. J Invest Dermatol. 2001;117:91–7. [PubMed: 11442754]
19. Tomasinsig L, Zanetti M. The cathelicidins—structure, function and evolution. Curr Protein Peptide Sci. 2005;6:23–34. [PubMed: 15638766]
20. Bals R, Wang X, Zasloff M, Wilson JM. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc Natl Acad Sci U S A. 1998;95:9541–6. [PubMed: 9689116]
21. Frohm M, Agerberth B, Ahangari G, Stahle-Backdahl M, Liden S, Wigzell H, Gudmundsson GH. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. J Bio Chem. 1997;272:15258–63. [PubMed: 9182550]
22. Menard S, Forster V, Lotz M, Gutle D, Duerr CU, Gallo RL, Henriques-Normark B, Putsep K, Andersson M, Glocker EO, Hornef MW. Developmental switch of intestinal antimicrobial peptide expression. J Exp Med. 2008;205:183–93. [PubMed: 18180308]
23. Chromek M, Slamova Z, Bergman P, Kovacs L, Podracka L, Ehren I, Hokfelt T, Gudmundsson GH, Gallo RL, Agerberth B, Brauner A. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nature Med. 2006;12:63641.
24. Gallo RL, Ono M, Povsic T, Page C, Eriksson E, Klagsbrun M, Bernfield M. Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds. Proc Natl Acad Sci U S A. 1994;91:11035–9. [PubMed: 7972004]

25. Shi J, Ross CR, Chengappa MM, Blecha F. Identification of a proline-arginine-rich antibacterial peptide from neutrophils that is analogous to PR-39, an antibacterial peptide from the small intestine. J Leukocyte Biol. 1994;56:807–11. [PubMed: 7996056]
26. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. Trends Immunol. 2002;23:541–8. [PubMed: 12401407]
27. Koczulla R, von Degenfeld G, Kupatt C, Krotz F, Zahler S, Gloe T, Issbrucker K, Unterberger P, Zaiou M, Lebherz C, Karl A, Raake P, Pfosser A, Boekstegers P, Welsch U, Hiemstra PS, Vogelmeier C, Gallo RL, Clauss M, Bals R. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. J Clin Invest. 2003;111:1665–72. [PubMed: 12782669]
28. De Y, Chen Q, Schmidt AP, Anderson GM, Wang JM, Wooters J, Oppenheim JJ, Chertov O. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exper Med. 2000;192:1069–74. [PubMed: 11015447]
29. Kurosaka K, Chen Q, Yarovinsky F, Oppenheim JJ, Yang D. Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant. J Immunol. 2005;174:6257–65. [PubMed: 15879124]
30. Chen K, Liu M, Liu Y, Wang C, Yoshimura T, Gong W, Le Y, Tessarollo L, Wang JM. Signal relay by CC chemo-kine receptor 2 (CCR2) and formylpeptide receptor 2 (Fpr2) in the recruitment of monocyte-derived dendritic cells in allergic airway inflammation. J Bio Chem. 2013;288:16262–73. [PubMed: 23603910]
31. von Haussen J, Koczulla R, Shaykhiev R, Herr C, Pinkenburg O, Reimer D, Wiewrodt R, Biesterfeld S, Aigner A, Czubayko F, Bals R. The host defence peptide LL-37/hCAP-18 is a growth factor for lung cancer cells. Lung Cancer. 2008;59:12–23. [PubMed: 17764778]
32. Kawano A, Tsukimoto M, Noguchi T, Hotta N, Harada H, Takenouchi T, Kitani H, Kojima S. Involvement of P2X4 receptor in P2X7 receptor-dependent cell death of mouse macrophages. Biochem Biophys Res Commun. 2012;419:374–80. [PubMed: 22349510]
33. Qu Y, Dubyak GR. P2X7 receptors regulate multiple types of membrane trafficking responses and non-classical secretion pathways. Purinergic Signal. 2009;5:163–73. [PubMed: 19189228]
34. Lee BH, Hwang DM, Palaniyar N, Grinstein S, Philpott DJ, Hu J. Activation of P2X(7) receptor by ATP plays an important role in regulating inflammatory responses during acute viral infection. PLoS One. 2012;7:e35812. [PubMed: 22558229]
35. Yin J, Yu FS. LL-37 via EGFR transactivation to promote high glucose-attenuated epithelial wound healing in organ-cultured corneas. Invest Ophthalmol Visual Sci. 2010;51:1891–7. [PubMed: 19797203]
36. Tjabringa GS, Aarbiou J, Ninaber DK, Drijfhout JW, Sorensen OE, Borregaard N, Rabe KF, Hiemstra PS. The antimicrobial peptide LL-37 activates innate immunity at the airway epithelial surface by transactivation of the epidermal growth factor receptor. J Immunol. 2003;171:6690–6. [PubMed: 14662872]
37. Girnita A, Zheng H, Gronberg A, Girnita L, Stahle M. Identification of the cathelicidin peptide LL-37 as agonist for the type I insulin-like growth factor receptor. Oncogene. 2012;31:352–65. [PubMed: 21685939]
38. 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. Infection Immunity. 2002;70:953–63. [PubMed: 11796631]
39. Yoshimura T, McLean MH, Dzutsev AK, Yao X, Chen K, Huang J, Gong W, Zhou J, Xiang Y, O'Huigin C, Thovarai V, Tessarollo L, Durum SK, Trinchieri G, Bian XW, Wang JM. The antimicrobial peptide CRAMP is essential for colon homeostasis by maintaining microbiota balance. J Immunol. 2018;200:2174–85. [PubMed: 29440355]
40. Johansson P, Wiltschi B, Kumari P, Kessler B, Vonrhein C, Vonck J, Oesterhelt D, Grininger M. Inhibition of the fungal fatty acid synthase type I multienzyme complex. Proc Natl Acad Sci U S A. 2008;105:12803–8. [PubMed: 18725634]

41. Johansson ME, Larsson JM, Hansson GC. The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):4659–65. [PubMed: 20615996]

42. Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc Natl Acad Sci U S A. 2008;105:15064–9. [PubMed: 18806221]

43. 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. [PubMed: 18059019]

44. Chromek M, Arvidsson I, Karpman D. The antimicrobial peptide cathelicidin protects mice from Escherichia coli O157:H7-mediated disease. PLoS One. 2012;7:e46476. [PubMed: 23077510]

45. Pullan RD, Thomas GA, Rhodes M, Newcombe RG, Williams GT, Allen A, Rhodes J. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. Gut. 1994;35:353–9. [PubMed: 8150346]

46. Wu WK, Wong CC, Li ZJ, Zhang L, Ren SX, Cho CH. Cathelicidins in inflammation and tissue repair: potential therapeutic applications for gastrointestinal disorders. Acta Pharmacol Sin. 2010;31:1118–22. [PubMed: 20676121]

47. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proc Natl Acad Sci U S A. 2014;111:2247–52. [PubMed: 24390544]

48. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunol Rev. 2015;264:182–203. [PubMed: 25703560]

49. Rosenberger CM, Gallo RL, Finlay BB. Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular Salmonella replication. Proc Natl Acad Sci U S A. 2004;101:2422–7. [PubMed: 14983025]

50. Sonawane A, Santos JC, Mishra BB, Jena P, Progida C, Sorensen OE, Gallo R, Appelberg R, Griffiths G. Cathelicidin is involved in the intracellular killing of mycobacteria in macrophages. Cell Microbiol. 2011; 13(10):1601–17. [PubMed: 21790937]

51. Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe. 2009;6:231–43. [PubMed: 19748465]

52. Claesson MJ, O'Sullivan O, Wang Q, Nikkila J, Marchesi JR, Smidt H, de Vos WM, Ross RP, O'Toole PW. Comparative analysis of pyrosequencing and a phylogenetic microarray for exploring microbial community structures in the human distal intestine. PLoS One. 2009;4:e6669. [PubMed: 19693277]

53. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science. 2005;308:1635–8. [PubMed: 15831718]

54. Ostaff MJ, Stange EF, Wehkamp J. Antimicrobial peptides and gut microbiota in homeostasis and pathology. EMBO Mol Med. 2013;5:1465–83. [PubMed: 24039130]

55. Iimura M, Gallo RL, Hase K, Miyamoto Y, Eckmann L, Kagnoff MF. Cathelicidin mediates innate intestinal defense against colonization with epithelial adherent bacterial pathogens. J Immunol. 2005, 174:4901–7. [PubMed: 15814717]

56. Kucharzik T, Maaser C, Lugering A, Kagnoff M, Mayer L, Targan S, Domschke W. Recent understanding of IBD pathogenesis: implications for future therapies. Inflam Bowel Dis. 2006;12:1068–83.

57. Ahluwalia A, Tarnawski AS. Cathelicidin gene therapy: a new therapeutic option in ulcerative colitis and beyond? Gene Ther. 2013;20:119–20. [PubMed: 22456327]

58. 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. 2013;20:187–3. [PubMed: 22378344]

59. Wong CC, Zhang L, Li ZJ, Wu WK, Ren SX, Chen YC, Ng TB, Cho CH. Protective effects of cathelicidin-encoding Lactococcus lactis in murine ulcerative colitis. J Gastroenterol Hepatol. 2012;27:1205–12. [PubMed: 22507188]

60. Yoo JH, Ho S, Tran DH, Cheng M, Bakirtzi K, Kukota Y, Ichikawa R, Su B, Tran DH, 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. [PubMed: 25729764]
61. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res. 2002;62:3603–8. [PubMed: 12097260]
62. Coffelt SB, Marini FC, Watson K, Zwezdaryk KJ, Dembinski JL, LaMarca HL, Tomchuck SL, Honer zu Bentrup K, Danka ES, Henkle SL, Scandurro AB. The pro-inflammatory peptide LL-37 promotes ovarian tumor progression through recruitment of multipotent mesenchymal stromal cells. Proc Natl Acad Sci U S A. 2009;106:3806–11. [PubMed: 19234121]
63. Viswanathan A, Painter RG, Lanson NA, Wang G. Functional expression of N-formyl peptide receptors in human bone marrow-derived mesenchymal stem cells. Stem Cells. 2007;25:1263–9. [PubMed: 17234990]
64. Lim R, Lappas M, Riley C, Borregaard N, Moller HJ, Ahmed N, Rice GE. Investigation of human cationic antimicrobial protein-18 (hCAP-18), lactoferrin and CD163 as potential biomarkers for ovarian cancer. J Ovarian Res. 2013;6:5. [PubMed: 23339669]
65. Piktel E, Niemirowicz K, Wnorowska U, Watek M, Wollny T, Gluszek K, Gozdz S, Levental I, Bucki R. The role of cathelicidin ll-37 in cancer development. Arch Immunol Ther Exper. 2016;64:33–46.
66. Li D, Beisswenger C, Herr C, Schmid RM, Gallo RL, Han G, Zakharkina T, Bals R. Expression of the antimicrobial peptide cathelicidin in myeloid cells is required for lung tumor growth. Oncogene. 2014;33:2709–16. [PubMed: 23812430]
67. Heilborn JD, Nilsson MF, Jimenez CI, Sandstedt B, Borregaard N, Tham E, Sorensen OE, Weber G, Stahle M. Antimicrobial protein hCAP18/LL-37 is highly expressed in breast cancer and is a putative growth factor for epithelial cells. Int J Cancer. 2005;114:713–9. [PubMed: 15609314]
68. Weber G, Chamorro CI, Granath F, Liljegren A, Zreika S, Saidak Z, Sandstedt B, Rotstein S, Mentaverri R, Sanchez F, Pivarcsi A, Stahle M. Human antimicrobial protein hCAP18/LL-37 promotes a metastatic phenotype in breast cancer. Breast Cancer Res. 2009;11:R6. [PubMed: 19183447]
69. Wu WK, Sung JJ, To KF, Yu L, Li HT, Li ZJ, Chu KM, Yu J, Cho CH. The host defense peptide LL-37 activates the tumor-suppressing bone morphogenetic protein signaling via inhibition of proteasome in gastric cancer cells. J Cell Physiol. 2010;223:178–86. [PubMed: 20054823]
70. 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. [PubMed: 23100468]
71. Chen X, Zou X, Qi G, Tang Y, Guo Y, Si J, Liang L. Roles and mechanisms of human cathelicidin LL-37 in cancer. Cell Physiol Biochem. 2018;47:1060–73. [PubMed: 29843147]
72. Ren SX, Shen J, Cheng AS, Lu L, Chan RL, Li ZJ, Wang XJ, Wong CC, Zhang L, Ng SS, Chan FL, Chan FK, Yu J, Sung JJ, Wu WK, Cho CH. FK-16 derived from the anti-cancer peptide LL-37 induces caspase-independent apoptosis and autophagic cell death in colon cancer cells. PLoS One. 2013;8:e63641. [PubMed: 23700428]
73. Kuroda K, Fukuda T, Isogai H, Okumura K, Krstic-Demonacos M, Isogai E. Antimicrobial peptide FF/CAP18 induces apoptotic cell death in HCT116 colon cancer cells via changes in the metabolic profile. Int J Oncol. 2015;46:1516–26. [PubMed: 25672949]
74. Cheng M, Ho S, Yoo JH, Tran DH, Bakirtzi K, Su B, Tran DH, Kubota Y, Ichikawa R, Koon HW. Cathelicidin suppresses colon cancer development by inhibition of cancer associated fibroblasts. Clin Exp Gastroenterol. 2015;8:13–29. [PubMed: 25565877]
75. Reddy KV, Yedery RD, Aranha, C. Antimicrobial peptides: premises and promises. Int J Antimicrob Agents. 2004;24:536–47. [PubMed: 15555874]
76. Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, McCray PB, Lehrer RI, Welsh MJ, Tack BF. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect Immun. 2000;68:2748–55. [PubMed: 10768969]

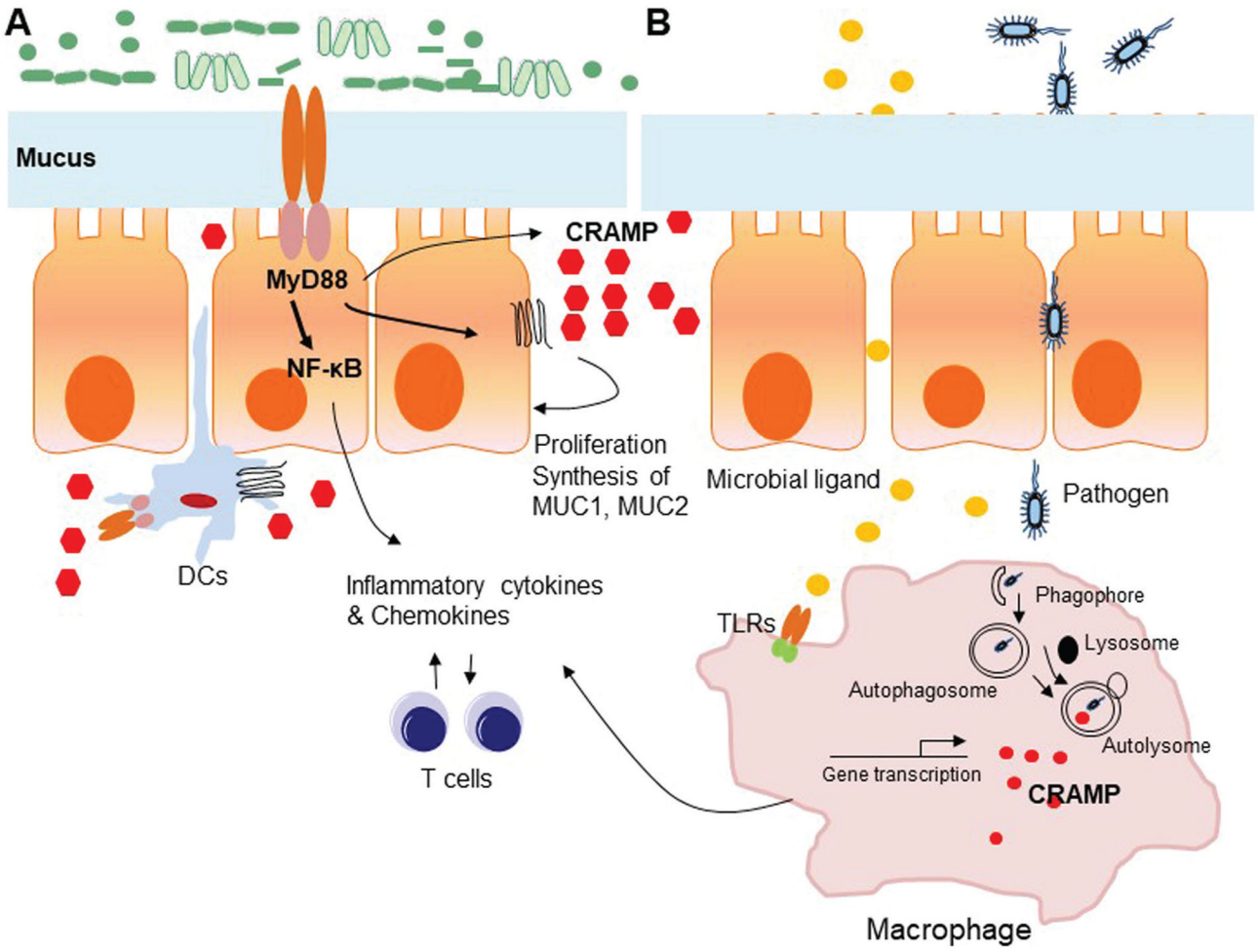

FIG. 1:

Contribution of CRAMP to colon epithelial barrier integrity. A. The contribution of CRAMP to colon crypt structure and mucus layer integrity–Colon mucosal barrier consists of epithelial and immune cells, which partner with resident microbiota to form a barrier fending off harmful substances. Epithelial cell–derived CRAMP is detectable in cells located on the mucosal surface and in upper crypts, with little or no expression seen within the deeper colon crypts. CRAMP stimulates the proliferation and differentiation of colon epithelial cells through Fpr2 espressed by epithelial cells. CRAMP also directly stimulates mucus synthesis through up-regulation of MUC1 and MUC2 and MAP kinase activation in colon epithelial cells. B. The contribution of CRAMP to the antimicrobicidal function of macrophages–resident macrophages in colon mucosa patrol and moniter pathogen invaders in the mucosal barrier. Upon stimulation by bacteria and their products, macrophages increase CRAMP production, which directly or in cooperation with autophagy kill and clear intracellular bacteria.

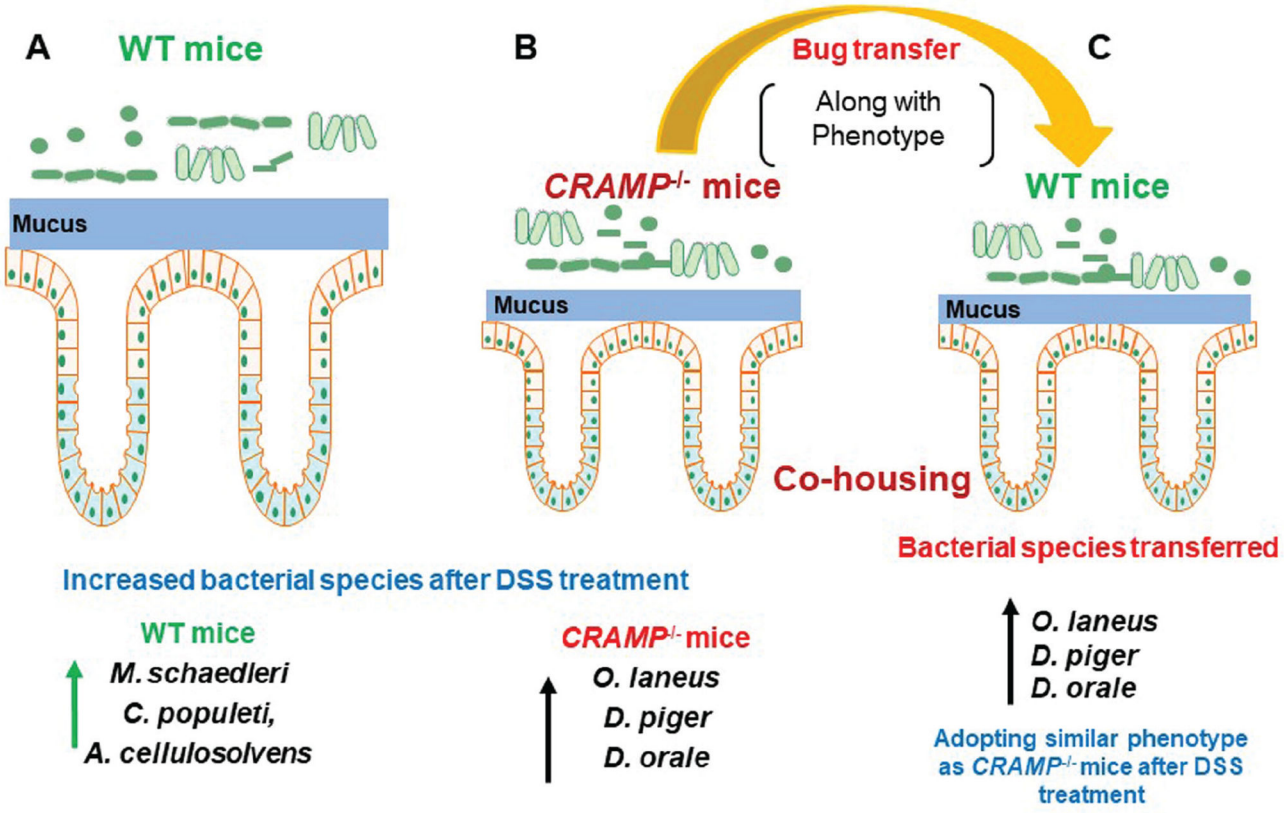

FIG. 2:

Contribution of CRAMP to colon microbiota balance. CRAMP-deficient mice show severe dysbiosis in the colon. This is verified by cohousing and single housing of WT mice (A) and *CRAMP*$^{-/-}$ mice (B). Single-housed *CRAMP*$^{-/-}$ mice show a significantly altered phenotype, including shortened colon crypts, thinner mucus layer, and dysbiosis with a different microbiota composition in the feces from that of single housed WT mice. WT mice cohoused with *CRAMP*$^{-/-}$ mice adopted a similar phenotype and microbiota composition from *CRAMP*$^{-/-}$ mice (C).

TABLE 1:

Distribution of LL-37/CRAMP in cells and tissues

Leukocytes	Neutrophils
	Macrophages
	B cells
	γδ T cells
Epithelial cells	Mast cells
	Lung
	Stomach
	Colon
	Urinary tract
	Cervix
Body fluids	Inflamed skin
	Bronchoalveolar lavage fluid
	Seminal plasma
	Cervicovaginal secretion
	Saliva
	Plasma