Published in final edited form as:

Curr Opin Gastroenterol. 2015 March ; 31(2): 118–123. doi:10.1097/MOG.0000000000000143.

Host-microbe interactions in the small bowel

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Abstract

Purpose of Review—The intestine, home to a vast microbiome, balances its immune reactivity on a knife's edge. This review will summarize recent studies examining innate immune signals that shape the microbiota, and how pathogens can usurp protective responses to their advantage.

Recent findings—Innate signaling uses several pathways to maintain epithelial defense. Toll-like receptor signaling through myeloid differentiation factor 88 maintains segregation between bacteria and the epithelium through production of anti-microbial proteins and inflammasome signaling mediates efficient goblet cell release of mucus containing granules. Conversely, negative regulators of TLR signaling help maintain a healthy microbiota resistant to pathogen infection. Methods to evade immune elimination by pathogens associated with human infections and inflammatory bowel disease are described. Emerging evidence that pattern recognition receptors can differentiate between commensals and pathogens will be examined.

Summary—The balance of innate signaling in the intestine is crucial to homeostasis: too little and bacteria can directly contact the epithelium, too much depletes the protective microbiota creating a niche for pathogens. Understanding the dynamic interaction between the immune system and the microbiota in a variety of infection and inflammation models will hopefully translate to new therapies.

Keywords

toll-like receptor; microbiota; intestine; inflammasome; *Salmonella enterica* serovar Typhimurium

Introduction

Tolerance in the intestinal environment is a precarious balancing act between non-reactivity against commensal symbionts and active inflammation against pathogens. The single cell epithelial layer that forms the barrier to the lumen has a number of capabilities to defend its borders. Subsets of epithelial cells have secretory functions that produce mucus (goblet cells) or secrete anti-microbial peptides (Paneth cells) to constrain the growth of microbes in

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Conflict of Interest

MTA serves as a consultant and scientific board member of AbbVie Laboratories, GI Health Foundations and Asana Medical Inc, and consults for Hospira, Takeda, Merck, Pfizer, Sanofi Aventis, Janssen, Prometheus, Mucosal Health Board, Focus Medical Communications, Shire Pharmaceuticals, GSK Holding Americas Inc. and UCB. JMD declares no conflict of interest.

the epithelial vicinity. Epithelial cells are induced to secrete mucus and anti-microbial peptides in response to innate immune signaling initiated by lamina propria resident immune cells (1). Innate signaling in the intestine is tightly controlled, with anatomical segregation of receptors (2) and increased quantities of negative regulators (3, 4) to prevent inappropriate immune activation. However, how the host is able to maintain homeostasis in the face of the onslaught of pathogen derived molecular patterns that abound in the environment has yet to be fully elucidated.

In this review we will consider recent studies that have investigated the interaction between host defense mechanisms and their effect on the intestinal microbiota. We will consider new evidence that defines the roles for innate signaling in maintenance of the mucus layer and anti-microbial peptide synthesis and secretion. We will also review the evidence that supports the requirement for balanced inflammatory signaling in maintaining a homeostatic relationship with the microbiota. We will examine the evidence showing how some enteric pathogens are able to subvert immune responses to promote their own growth. Finally we will consider an exciting study of a new pattern recognition receptor, and a new purpose for a well-known one, and how they are re-defining our notion of innate recognition of commensals and pathogens.

Innate signaling is required for effective segregation of bacteria from the epithelium

Recognition by innate immune sensors in the intestinal epithelium contribute to maintaining separation of the niche environments of both the host and microbiota. Myeloid differentiation factor 88 (MyD88) functions as an adaptor molecule for signaling initiated by Toll-like receptors (TLRs) and the Interleukin-1R (IL-1R). TLRs recognize conserved pathogen associated molecular patterns (PAMPs) and activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)-dependent transcription of pro-inflammatory mediators (2). In the epithelium, TLR activation is important in driving production of antimicrobial peptides and mice which lack MyD88 specifically in the epithelium (MyD88^{IEC}) have decreased production of Muc2 (Mucin), Defa-rs1 (alpha Defensin) and Reg3g (C-type lectin) compared to control MyD88^{fl/fl} mice (5). In normal conditions, the mucus layer prevents luminal bacteria from coming into contact with the epithelium (6). Specific loss of MyD88 in epithelial cells (MyD88^{IEC}) reduces the distance between the bacterial biofilm and the apical border of the epithelium. This allows bacteria to come directly into contact with the epithelium, which doesn't occur in wild-type mice. The importance of epithelial MyD88 signaling was found to lie in its ability to induce secretion of the anti-microbial peptide RegIII γ . Loss of RegIII γ was most important in the small intestine, as RegIII γ expression is low in the colon (6). Epithelial MyD88 signaling was also important in containing pathogenic microbiota to the lumen, as MyD88^{IEC} mice had increased translocation of *Klebsiella pneumoniae* to the mesenteric lymph nodes (5). In the colon, loss of IEC MyD88 also increased susceptibility to dextran sodium sulfate (DSS)-induced colitis through increased destruction of the epithelial layer and increased inflammatory cell infiltrate despite lower levels of inflammatory cytokines TNF, IL-1 β and the chemokine CXCL2 (5). Thus, MyD88 signaling in intestinal epithelial cells impacts both

the protective mucus layer and the production of antimicrobial factors that directly control the proximity and survival of the microbiota at the interface of the luminal environment and the host tissues.

Other pattern recognition receptors play a similar role to MyD88 in maintaining segregation of bacteria from the epithelial layer. NOD-like receptor family pyrin domain containing 6 (NLRP6) is a member of the NOD-like receptor family that forms an inflammasome complex in association with apoptosis-associated speck-like protein containing a CARD and Caspase 1. Loss of NLRP6 results in spontaneous hyperplasia and increased inflammatory infiltrate in these mice. These mice also have an altered microbiota that transfers worse colitis risk to co-housed WT mice. Like MyD88^{IEC} mice, NLRP6^{-/-} mice have bacteria in contact with the crypt and epithelium in the colon, and antibiotic treatment decreased the Prevotellaceae and TM7 bacterial classes and resulted in better colitis scores. Indicating that the dysbiotic bacteria was driving the inflammation in this model (7). In a follow-up study by the same group, the NLRP6^{-/-} mice were found to have a profound defect in secretion of goblet cell granules to the lumen which impacted the mucus layer, and the luminal secretion of anti-microbial peptides. They found that inflammasome signaling was required for efficient induction of autophagy, which was a requirement for the fusion of goblet cell granules with the plasma membrane for their release into the lumen. Without this fusion, the granules were found intact in the lumen, which was unproductive in creating a mucus layer and preventing bacterial contact with the epithelium and release of the anti-microbial peptides. Mice with defective autophagy (Atg5^{+/-}) also have this goblet cell functional defect, demonstrating the connection between the two pathways (8). These studies have been crucial to understanding the specific defect in goblet cell production of an effective mucus layer in the absence of inflammasome signaling, because these mice produce ample gene transcripts of Muc2 and anti-microbial peptides, but were still unable to generate an effective mucus layer, and control access of the microbiota to the epithelium. Additionally, these studies drive home the inter-connectedness of autophagy and inflammasome activation – defects in both of these pathways are highly significantly associated with the development of inflammatory bowel disease (9). These studies highlight how these two pathways connect to regulate the environment at the interface of the epithelium.

The above-mentioned studies demonstrate that epithelial innate signaling promotes an effectively anti-microbial mucus layer. Epithelial MyD88 signaling induces RegIII γ and establishes a protective segregation between bacteria and the epithelium, and NLRP6 inflammasome signaling in combination with autophagy is required for proper goblet cell granule maturation and release.

Loss of regulation of innate signaling breaks the balance with commensal microbiota

Balanced innate signaling is vital to a peaceful co-existence between microbes and the host in the intestine. Too little, and there is ineffective mucus production and antimicrobial peptide production. However, too much is equally perilous to homeostasis. TLR signaling is important to keep bacteria segregated from the epithelium, but negative regulators of TLR signaling are also important in maintaining balance in the intestine. Single Ig IL-1 Related

Receptor (SIGIRR) is a membrane inhibitor of TLR and IL-1R signaling (10). Loss of SIGIRR results in increased inflammatory gene expression in response to *Citrobacter rodentium* infection compared to WT mice, but the inflammation did not reduce bacterial burdens, and actually lead to increased bacterial loads in the cecum at early time points. Loss of SIGIRR increased anti-microbial peptide expression including: RegIII γ , β -defensin-III, and cathelin-related antimicrobial peptide (CRAMP) (11). How is it that increased anti-microbial peptide production didn't protect against *C. rodentium* infection? The authors postulated that increased anti-microbial peptide levels actually decreased the commensal bacteria which were sensitive to their actions, and this opened up a niche that *C. rodentium* was able to fill. Thus it was the loss of competition from other commensals that allowed the outgrowth of *C. rodentium* in this model (11). Studies from the same laboratory have investigated the role of SIGIRR in the course of infection with the human pathogen *Campylobacter jejuni*. In this infection model increased inflammation is driven by TLR4 signaling since double knock outs of SIGIRR and TLR4 have decreased colonic pathology and inflammatory gene expression. Conversely, TLR2 was protective in the same model and SIGIRR $-/-$ TLR2 $-/-$ double knock outs were more susceptible to infection, having increased pathology and cytokine secretion than the SIGIRR $-/-$ mice (12).

Loss of another negative regulator of TLR4 signaling Toll-interacting protein (TOLLIP) leads to increased intestinal permeability during DSS colitis compared to WT mice indicating a role for microbial sensing in maintaining tight junctions barriers in the intestine. Bone marrow chimeras suggest that the IEC TOLLIP contribution is most important to increased colitis severity, but there was no baseline inflammation in this model, nor was there a role for the microbiota in driving increased colitis susceptibility (13). Loss of IRAK-M in IL-10 $-/-$ mice also increases colitis susceptibility while TLR4 $-/-$ IL10 $-/-$ double knock outs and MyD88 $-/-$ IL-10 $-/-$ double knock outs had decreased severity, suggesting that de-regulation of TLR signaling in the absence of the regulatory cytokine IL-10 was driving inflammation in this model (14). IRAK-M was found to be induced in response to the microbiota, as germ-free mice had low levels of IRAK-M expression which was increased following colonization (14).

So, the intestine employs a delicately balanced system in which innate signaling responses in epithelial cells are crucial to host-microbe homeostasis. Innate signaling that alters the mucus layer or production of commensal-modifying anti-microbial peptides has a huge impact on keeping the microbiota constrained to its anatomical niche and out of direct contact with the epithelial layer. But what happens when pathogens learn the rules?

Pathogens out-fox epithelial defenses

There is a point at which the effectiveness of host-derived anti-microbial peptides becomes hazardous. Certain pathogens have evolved to take advantage of the lack of competition that occurs after anti-microbial peptides have cleaned-out the commensal microbiota. They use this opportunity to gain a foothold and take over the niche as evidenced in the above-mentioned studies in SIGIRR $-/-$ mice infected with *C. rodentium*. *Salmonella enterica* serovar Typhimurium (*S.typh*) is one such human pathogen that uses immune responses to its advantage. *S.typh* is a colonizing human pathogen that induces inflammatory diarrhea

and thrives in the inflamed gut. In fact, *S.typh* *requires* inflammation in order to colonize (15). *S.typh* has developed several competitive advantages over the commensal microbiota to survive host defenses (16–18). Infection with *S.typh* induces a huge increase in IL-22, which acts on epithelial cells to increase anti-microbial gene expression including *Lcn2* (lipocalin2), *s100A8*, *s100A9* and *Reg3g* and oxidative stress proteins *Nos2* (inducible nitric oxide synthase 2) and *Duox2* (dual oxidase 2) (19) – which are all ineffective against *S.typh*. *S100A8* and *S100A9* form a heterodimer called calprotectin which functions to mop up zinc in the lumen; metal chelation is a common anti-microbial strategy to limit bacterial growth. *S.typh* uses a zinc sequestration protein *ZnuABC* to grow in the presence of calprotectin (20). Similarly, *S.typh* is able to use neutrophil-derived oxidation by-products as an energy source. Hydrogen sulfate, normally produced by the microbiota is oxidized to tetrathionate after neutrophil activation of *gp91^{phox}* in the NADPH pathway and release of oxygen radicals. *S.typh* can use tetrathionate as an energy source using genes encoded by the *ttrSR* and *ttrBCA* gene cluster (15). *S.typh* can also outcompete iron-dependent bacteria in the presence of lipocalin2 using its *iroN* siderophore genes (21). IL-22^{-/-} mice are protected from *S.typh* colonization without any increase in inflammatory cell recruitment. Protection is mediated through changes in the commensal microbiota communities that occur after infection with *S.typh*. Without IL-22 induced increases in anti-microbial peptides which would normally wipe out members of the Enterobacteriaceae group, these bacteria are able to compete with *S.typh* for growth (19). These data highlight the potential role of fecal transplants in treating conditions in which normal commensals can compete with pathogens for niche space.

Other pathogenic bacteria have also developed similar immune evasion strategies. Adherent-invasive *Escherichia coli* (AIEC) is more frequently found in Crohn's disease patients with ileal disease than in controls, or patients without ileal disease. AIEC strains have been found in the lamina propria of CD patients and can persist and replicate in macrophages and as such are candidate as instigating agents in CD (22). Following phagocytosis, AIECs have been shown to induce the secretion of TNF which is dose dependent on their own proliferation inside of the phagolysosomes (23). The mechanism by which TNF secretion by macrophages allows intracellular proliferation of the bacteria is still unclear. However, treatment of infected macrophages with anti-TNF antibodies decreased their proliferation, and may represent an otherwise unconsidered aspect of the efficacy of anti-TNF treatment in CD and UC patients. That TNF promotes the intracellular proliferation of AIEC is not common to all intracellular pathogens. *S. typh* was not able to increase its intracellular growth following TNF treatment inside macrophages (23). AIEC strains isolated from CD patients with ileal disease have also been found to have genomic islands that encode genes able to evade anti-microbial peptides including LL-37, two other alpha helical cationic proteins chemotactic protein 10A (CP10A) and CP28 and is also somewhat resistant to alpha and beta defensins. The gene islands allow the AIEC to outcompete other strains lacking parts of the gene island in vivo and also to colonize better over time (24).

Taken together these new studies have provided insight into the way in which the immune system interacts with the commensal microbiota; through modulation of the mucus layer and secretion of proteins that constrain the growth of bacteria. This has a direct impact on the

composition of the microbiota – and influences the interactions amongst commensal and pathogenic populations. Broad spectrum immune responses such as those induced by *S. typh* may limit systemic dissemination of the pathogen (25), but allow it to gain a foothold in the intestinal niche through eradication of its competitors. *S. typh* has gained the ability to evade host responses, and even use them to their own advantage. It may be of interest for future studies to investigate the types of commensal bacteria that directly compete with *S. typh* including members of the Enterobacteriaceae family to determine if therapies aimed at providing competition for the pathogenic bacteria may be a beneficial treatment strategy.

Emerging pattern recognition receptors discriminate between pathogens and commensals

A central tenet of innate PRRs is that they recognize broad classes of structures present in commensals and pathogens alike and are not sufficiently sensitive to distinguish between the two. This has proved a paradox in the intestine where the large majority of the bacteria are commensal and well tolerated by the immune system, and do not actively induce inflammatory reactions. It has been postulated that this anergy toward commensal microbiota lies in the inability of commensals to breach the epithelial barrier and induce tissue damage, and is maintained through the physical segregation of PRRs to the apical surface of epithelial cells. Lately, several new classes of PRRs have been identified that may be able to do just that - distinguish commensals from pathogens.

Uracil recognition

Initial studies in *Drosophila* have elucidated a role for reactive oxygen species (ROS) generation in mounting protective responses against gut pathogens which are not activated by commensals. DUOX2 is a member of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase family and produces ROS to kill invading microbes. Screening studies in *Drosophila* identified, by NMR structural analysis, the ROS-inducing secreted factor from the gut pathogen *Erwinia carotovora* subsp. *carotovora-15* as uracil. Symbiont bacteria that normally colonize the *Drosophila* gut were generally found to be low producers of uracil and subsequently unable to induce ROS whereas opportunistic pathogens including *Klebsiella pneumoniae*, *Shigella sonnei* and *Pseudomonas aeruginosa* all produced significant quantities of uracil. Studies in the human Caco2 epithelial cell line also determined that uracil activates ROS generation (26). The induction of ROS by pathogens and not commensals while seemingly at cross-purpose to their survival may prove to be a strategy to deplete competing commensal as we have seen above with *S. typh*.

Bacterial double-stranded RNA

TLR3 is usually considered a viral double-stranded RNA (dsRNA) sensor however, a recent study has found that heat killed commensal lactic acid bacteria (LAB) have abundant dsRNA available to activate TLR3 whereas pathogens have low levels of dsRNA. Selective RNase treatment of the heat killed bacteria to reduce dsRNA levels significantly reduced the amount of IFN β induced by bone marrow-derived dendritic cells, but not other inflammatory cytokines like IL-6 and TNF α which were assumed to be induced by other TLR engagement. LAB strains induced IFN β but not IFN α . IFN β has previously been

shown to mediate anti-inflammatory reactions in several auto-immune experimental models including collagen-induced arthritis and delayed-type hypersensitivity (27). Mice orally fed with LAB were protected from DSS colitis having less inflammatory infiltrate, lower myeloperoxidase concentrations (measure of neutrophil accumulation), and this protection was lost in TLR3^{-/-} mice. CD11c⁺ dendritic cells were the probable source of IFN β . Luminal contents from the ileum were shown to contain the highest quantity of dsRNA even compared to the colon, demonstrating the importance of TLR3 in regulating immune responses in the small intestine (28).

The innate immune system in the intestine may not be as blunt an instrument as previously thought. Subtle differences in the secretion of uracil or the presence of dsRNA may be just enough for the innate immune system to be able to differentiate between commensals and pathogens. Careful studies may yet uncover new recognition systems that are able to discriminate between friend and foe.

Concluding Remarks

The above studies have shown us the tight regulation of immune activation in the intestine. Insufficient innate immune signaling allows the microbiota to contact the epithelium but; over-activation of innate pathways is just as hazardous. Any process that alters production of anti-microbial peptides has the potential to significantly impact the vulnerable microbiota to the advantage of resistant and potentially pathogenic species. Future research in this area should focus on understanding the microbial population changes occurring during infection to determine how input from the immune system alters interactions amongst the microbiota. Finally, ongoing studies of newly identified PRRs that can differentiate between commensal and pathogen may prove an attractive testing model in the drive to discover novel probiotics. There is tremendous potential in the studies that are ongoing in this field for application to human therapies for infection and dysbiosis.

Acknowledgments

The authors would like to acknowledge the support of the members of the Abreu laboratory in the preparation of this review.

Financial Support and Sponsorship

Funding for this review was provided by grants to support the laboratory of MTA (National Institute of Health, National Cancer Institute 5R01CA137869-05, National Institute of Diabetes and Digestive and Kidney Diseases 2R01DK099076-06A1; Crohn's and Colitis Foundation of America Senior Investigator Award).

References

1. Cunliffe RN, Mahida YR. Expression and regulation of antimicrobial peptides in the gastrointestinal tract. *Journal of leukocyte biology*. 2004; 75(1):49–58. [PubMed: 14525966]
2. Abreu MT. Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function. *Nat Rev Immunol*. 2010; 10(2):131–144. [PubMed: 20098461]
3. Shibolet O, Podolsky DK. TLRs in the Gut. IV. Negative regulation of Toll-like receptors and intestinal homeostasis: addition by subtraction. *Am J Physiol Gastrointest Liver Physiol*. 2007; 292(6):G1469–G1473. [PubMed: 17554134]

4. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol*. 2005; 5(6):446–458. [PubMed: 15928677]
5. Frantz AL, Rogier EW, Weber CR, Shen L, Cohen DA, Fenton LA, et al. Targeted deletion of MyD88 in intestinal epithelial cells results in compromised antibacterial immunity associated with downregulation of polymeric immunoglobulin receptor, mucin-2, and antibacterial peptides. *Mucosal immunology*. 2012; 5(5):501–512. [PubMed: 22491177]
6. Vaishnav S, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, et al. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science*. 2011; 334(6053):255–258. [PubMed: 21998396]
7. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*. 2011; 145(5):745–757. [PubMed: 21565393]
8. Wlodarska M, Thaiss CA, Nowarski R, Henao-Mejia J, Zhang JP, Brown EM, et al. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014; 156(5):1045–1059. [PubMed: 24581500] This study demonstrates the defect in goblet cell granule fusion with the cell membrane in the absence of NLRP6 signaling. Loss of effective goblet cell release of mucus and antimicrobial peptides allowed the luminal microbiota to contact the epithelium.
9. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012; 491(7422):119–124. [PubMed: 23128233]
10. Garlanda C, Anders HJ, Mantovani A. TIR8/SIGIRR: an IL-1R/TLR family member with regulatory functions in inflammation and T cell polarization. *Trends in immunology*. 2009; 30(9): 439–446. [PubMed: 19699681]
11. Sham HP, Yu EY, Gulen MF, Bhinder G, Stahl M, Chan JM, et al. SIGIRR, a negative regulator of TLR/IL-1R signalling promotes Microbiota dependent resistance to colonization by enteric bacterial pathogens. *PLoS pathogens*. 2013; 9(8):e1003539. [PubMed: 23950714] In this paper the authors describe the susceptibility of SIGIRR^{-/-} mice to *C. rodentium* and *S. typh* infection. They found that increased antimicrobial peptide production in the SIGIRR^{-/-} mice depleted the commensal microbiota which compete with *S. typh* for niche space.
12. Stahl M, Ries J, Vermeulen J, Yang H, Sham HP, Crowley SM, et al. A novel mouse model of *Campylobacter jejuni* gastroenteritis reveals key pro-inflammatory and tissue protective roles for Toll-like receptor signaling during infection. *PLoS pathogens*. 2014; 10(7):e1004264. [PubMed: 25033044] SIGIRR^{-/-} mice are more susceptible to *C. jejuni* infection in a TLR4 dependent manner.
13. Maillard MH, Bega H, Uhlig HH, Barnich N, Grandjean T, Chamaillard M, et al. Toll-interacting protein modulates colitis susceptibility in mice. *Inflammatory bowel diseases*. 2014; 20(4):660–670. [PubMed: 24572204] Loss of TOLLIP in IL-10^{-/-} mice worsens colitis.
14. Biswas A, Wilmanski J, Forsman H, Hrcir T, Hao L, Tlaskalova-Hogenova H, et al. Negative regulation of Toll-like receptor signaling plays an essential role in homeostasis of the intestine. *European journal of immunology*. 2011; 41(1):182–194. [PubMed: 21182089]
15. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, et al. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature*. 2010; 467(7314): 426–429. [PubMed: 20864996]
16. Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, et al. *Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS biology*. 2007; 5(10):2177–2189. [PubMed: 17760501]
17. Lawley TD, Bouley DM, Hoy YE, Gerke C, Relman DA, Monack DM. Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. *Infection and immunity*. 2008; 76(1):403–416. [PubMed: 17967858]
18. Barman M, Unold D, Shifley K, Amir E, Hung K, Bos N, et al. Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infection and immunity*. 2008; 76(3):907–915. [PubMed: 18160481]
19. Behnsen J, Jellbauer S, Wong CP, Edwards RA, George MD, Ouyang W, et al. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. *Immunity*. 2014;

- 40(2):262–273. [PubMed: 24508234] *S. typh* infection of IL-22^{-/-} mice was attenuated due to reduction in anti-microbial peptides. IL-22 induces secretion of commensal-depleting anti-microbial peptides that allows. *S. typh* to colonize the intestine.
20. Liu JZ, Jellbauer S, Poe AJ, Ton V, Pesciaroli M, Kehl-Fie TE, et al. Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut. *Cell host & microbe*. 2012; 11(3):227–239. [PubMed: 22423963]
 21. Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio SP, Paixao TA, et al. Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. *Cell host & microbe*. 2009; 5(5):476–486. [PubMed: 19454351]
 22. Strober W. Adherent-invasive *E. coli* in Crohn disease: bacterial "agent provocateur". *J Clin Invest*. 2011; 121(3):841–844. [PubMed: 21339637]
 23. Bringer MA, Billard E, Glasser AL, Colombel JF, Darfeuille-Michaud A. Replication of Crohn's disease-associated AIEC within macrophages is dependent on TNF-alpha secretion. *Laboratory investigation; a journal of technical methods and pathology*. 2012; 92(3):411–419.
 24. McPhee JB, Small CL, Reid-Yu SA, Brannon JR, Le Moual H, Coombes BK. Host defense peptide resistance contributes to colonization and maximal intestinal pathology by Crohn's disease-associated adherent-invasive *Escherichia coli*. *Infection and immunity*. 2014; 82(8):3383–3393. [PubMed: 24866805] AIEC strains from ileal CD patients have genomic islands encoding resistance to anti-microbial peptides which confers a growth advantage to those strains.
 25. Godinez I, Raffatellu M, Chu H, Paixao TA, Haneda T, Santos RL, et al. Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype Typhimurium in the intestine. *Infection and immunity*. 2009; 77(1):387–398. [PubMed: 18955477]
 26. Lee KA, Kim SH, Kim EK, Ha EM, You H, Kim B, et al. Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. *Cell*. 2013; 153(4):797–811. [PubMed: 23663779] This is the first report that uracil is secreted by bacteria and recognized in *Drosophila*. Recognition of uracil initiates *DUOX2* activation and production of ROS. Pathogenic strains produce increased amounts of commensals in the strains tested. Thus the authors conclude that through detection of uracil the innate immune system in *Drosophila* can differentiate between commensal and pathogens.
 27. Billiau A. Anti-inflammatory properties of Type I interferons. *Antiviral research*. 2006; 71(2-3): 108–116. [PubMed: 16626815]
 28. Kawashima T, Kosaka A, Yan H, Guo Z, Uchiyama R, Fukui R, et al. Double-stranded RNA of intestinal commensal but not pathogenic bacteria triggers production of protective interferon-beta. *Immunity*. 2013; 38(6):1187–1197. [PubMed: 23791646] This study determined that commensal LAB strains have increased quantities of dsRNA which is recognized by TLR3 and TLR9 in the intestine to induce protective IFN β secretion, probably by dendritic cells. Pathogenic strains have less dsRNA than LAB strains, and through this difference the innate immune system differentiates between commensals and pathogens.

Key Points

- Balanced innate signaling in the intestine is required to maintain homeostasis with the microbiota
- Loss of MyD88 in epithelial cells allow bacteria to contact the epithelium through decreased production of anti-microbial peptides, and loss of inflammasome signaling impairs goblet cell secretory function
- Pathogens can use the innate immune responses against them to gain a foothold in the intestinal niche
- Novel pattern recognition receptors have been reported that can differentiate between commensal and pathogen.
- Understanding how microbial populations change during inflammation and infection may provide new therapeutic options for treating infections and inflammatory bowel disease.