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Mechanisms of intestinal dysbiosis: new insights into tuft cell functions

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ABSTRACT

Symbiosis between the host and intestinal microbial communities is essential for human health. Disruption in this symbiosis is linked to gastrointestinal diseases, including inflammatory bowel diseases, as well as extra-gastrointestinal diseases. Unbalanced gut microbiome or gut dysbiosis contributes in multiple ways to disease frequency, severity and progression. Microbiome taxonomic profiling and metabolomics approaches greatly improved our understanding of gut dysbiosis features; however, the precise mechanisms involved in gut dysbiosis establishment still need to be clarified. The aim of this review is to present new actors and mechanisms underlying gut dysbiosis formation following parasitic infection or in a context of altered Paneth cells, revealing the existence of a critical crosstalk between Paneth and tuft cells to control microbiome composition.

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Introduction



The gut microbiome is increasingly recognized as a key player in both host physiology and disease development. Indeed, alterations in gut microbiome composition, or dysbiosis, by environmental factors, impairment of host-microbiome interfaces, or perturbation in immune responses lead to gastrointestinal diseases as well as extra-gastrointestinal pathologies. Although the involvement of host intrinsic factors or environmental changes in altering gut microbiome composition and function has been well described, the underlying cellular and molecular mechanisms remain to be better defined. In this review, we aim to give a background of gut microbiome features and functions, as well as characteristics and mechanisms of gut dysbiosis, and discuss more specifically recent data on the control of bacterial homeostasis revealing a critical crosstalk between two intestinal epithelial cell types, the Paneth and the tuft cells.

Background of microbiome

Plasticity and functions of the gut microbiome

The human gut is the host of trillions of diverse microorganisms living in symbiosis with the

organism, including bacteria, viruses, fungi and parasites, collectively termed the microbiome. The collective genome of microbes is 150 times larger than the human genome¹. Recent advances in microbiome research revealed that the gut microbiome is not passive but is a powerful modifier of host biology, affecting nutritional responses,² metabolism and immunity²⁻⁴ as well as circadian rhythmicity.⁵⁻⁹ Moreover, the gut microbiome is highly dynamic and plastic; its composition varies cyclically with daily feeding/fasting periods.¹⁰ Throughout life, environmental and physiological factors such as hormonal changes, life style and aging also influence its composition.¹¹ The composition of the microbiome is complex; although most research to date has focused on bacteria, an increasing attention is given to non-bacterial microorganisms like viruses, fungi, and archaea within the gut microbiome. For example, alterations in the enteric virome of colorectal cancer (CRC) patients have been revealed by shotgun metagenomics analyses, suggesting potential interactions between viruses and bacteria that may contribute to CRC development.¹² Likewise, the mycobiome, though less studied, has shown distinct profiles in CRC patients, particularly elevations in *Basidiomycota* to *Ascomycota* ratios and

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enrichment of *Aspergillus* species.^{13,14} Moreover, commensal protists are also important elements of the gut microbiome however their contribution in host-microbiome interactions is still poorly studied. Nevertheless, they can profoundly affect the gut epithelium and immune responses, as demonstrated after mice colonization with *Tritrichomonas musculus* that is able to induce strong epithelial remodeling and type 2 immunity.^{15,16}

Microbial composition is relatively simple at birth, depending on the mode of delivery, and its complexity increases during the 2–3 y after birth in part due to nutrition changes.^{11,17,18} Early life is a crucial period for the maturation of the adult microbiome, and microbes participate to the education of the host's immune system that eventually leads to a functional symbiosis between the host and the microbiome. Indeed, a growing body of evidence indicates the existence of a "window of opportunity" in early life, during which both mice and humans need to be exposed to microbes to imprint their immune system and shape the host physiology.^{19–22} When the early crosstalk between the host and microbes is altered, the defects on development and education of specific immune subsets can be irreversible and lead to the development of disease later in life. Hence, repeated exposure of antibiotics in the first years of life is associated with an increased susceptibility to allergy and asthma,^{23,24} or an increased risk of developing inflammatory bowel disease (IBD).²⁵ Another example concerns malnourished children. Food is an important leads of microbiome composition, and malnourished Bangladeshi children display persistent gut microbiome immaturity, which can be partially improved by nutritional intervention but still prevents normal growth of those children.²⁶ Along the same line, the hygiene hypothesis proposes that modern life style has led to a loss of microbial diversity and a decreased exposure to microbes during early life. The reduced microbiome that colonize host's mucosal surfaces, in turn, leads to hyperstimulation of the immune system and might play a role in the increased incidence of chronic diseases in developed countries such as obesity, allergies including food allergy, IBD, among many others.²⁷ Given the complexity and the important roles played by the microbiome, a classification based on

compositional patterns would help to develop microbiota-based diagnosis, therapies and prevention, opening opportunities for personalized therapies via nutritional or microbial treatments. Yet, the identification and characterization of distinct patterns in human gut microbial community, termed "enterotypes", is challenging and remains in debate.²⁸ Three predominant ecosystems or enterotypes have been identified, with predominant abundance of *Bacteroides*, *Prevotella* and *Ruminococcus* genera, respectively, yet the concept of enterotypes is still under investigation. Enterotypes are not nation or continent specific, and host parameters such as ethnic origin, age, gender or health status do not govern the affiliation to a specific enterotype.²⁹

Definition and characteristics of gut dysbiosis

Intestinal homeostasis requires a permanent crosstalk between microbiome, intestinal epithelial cells and immune cells. When this balance is altered, by environmental perturbations, genetic defects or modifications in host-microbiome interfaces, gut dysbiosis develops. Dysbiosis was first commonly defined by any modification relative to the composition and function of resident microbial community found in healthy individuals.³⁰ Because microbiome composition between healthy individuals displays a high interindividual variability related to geography, age and diet, dysbiosis most often refers to changes in microbial communities that are associated with an altered physiological status such as inflammation or increased transepithelial permeability, or with diseases in which it participates to the pathogenesis, diagnosis or treatment.³¹ Importantly, disease-associated dysbiosis is usually able to reproduce diseases in models of fecal transplantation, as illustrated in axenic mice transplanted with microbiomes derived from obese, asthmatic or IBD patients which reproduce the respective pathologies.^{32–34} Gut dysbiosis can be linked to a reduction in beneficial microbial organisms which can in turn favor overgrowth of commensals potentially harmful (termed pathobionts), whose expansion can induce inflammation and pathology.^{35,36} Moreover, the diversity of microbial organisms is crucial to ensure maximum health benefits for the host since the various species

exert diverse and non-redundant functions.^{37,38} Although not fully established, some studies suggest that a loss in microbial diversity may participate to disease progression and severity.^{39–42} Of note, dysbiosis involves one or more of these non-mutually exclusive characteristics.

Mechanisms responsible for gut dysbiosis

The intestinal mucosa barrier is composed of several elements including the outer mucus layer, which comprises the commensal gut microbiome, antimicrobial peptides (AMPs), and secretory immunoglobulin A, the single layer of epithelial cells, and the inner *lamina propria* where immune cells reside. Deregulation of these elements compromises intestinal barrier integrity and promotes gut dysbiosis.^{43,44} Impaired expression of junctional complexes (adherent junctions, desmosomes, claudins) have been reported following exposition to several environmental factors. For example, a prolonged exposure to high fat diet (HFD) induces disruption in mucosal barrier integrity with a decrease in goblet cell differentiation and a loss in the tight junction protein claudin 1.⁴⁵ Deregulated expression of claudins, tight junction and adherens junction proteins have also been reported in Crohn's disease patients.^{46,47}

Importantly, the mucus layer provides nutrients and attachment sites to some commensals and prevents most microbes to reach the epithelial surface, and this interaction is necessary to maintain intestinal homeostasis.⁴⁴ This mucosal barrier is altered in pediatric IBD biopsies with a reduction in goblet cells and mucin secretion.⁴⁸ The essential role of mucus in epithelial protection has been revealed in Muc2-deficient mice which develop spontaneous colitis in young mice and display bacteria in the crypts in direct contact with the epithelial cells.^{49,50} Recently, mucus layer composition alterations have been found in intestinal tissues from type 1 diabetes patients, associated with AMPs reduction, bacterial translocation, and dysbiosis and inflammation.⁵¹ Similarly, a defect of the colonic mucus has been reported under a standard dietary regimen in pre-diabetic, genetically obese mice.⁵² Moreover, a recent study in IL10 deficient mice demonstrated that a fiber-deprived gut microbiota induces a deterioration of colonic

mucus, leading to lethal colitis.⁵³ This lethal colitis is caused by Th1 immune responses promoted by increased activities of mucin-degrading bacteria, with the inflammation starting first in regions with thinner mucus. Indeed, these changes in the mucosal barrier affect mucosal communities. Hence, an inverse correlation exists between the abundance of *Akkermansia muciniphila* and diseases such as IBD, obesity and diabetes. *A. muciniphila* is a mucolytic commensal that reduces diabetes, obesity and IBD symptoms in mice and its use as a probiotic gives promising results in obese and diabetic patients.⁵⁴ Interestingly, dysbiosis in the mucus layer precedes the onset of colitis in a murine model of spontaneous ulcerative colitis and is associated with a depletion in mucus layer.⁵⁵ These studies show the importance of an intact mucosal barrier to maintain homeostasis. Loss of compartmentalization of microbes and their presence nearby the epithelium leads in turn to immune responses initiation and inflammation.

Others mechanisms underlying gut dysbiosis have been reported such as oxidative stress, bacteriophages and secretion of bacterial toxins.⁵⁶ Intestinal inflammation is associated with mucosal leukocyte infiltration which produce reactive oxygen and nitrogen species. The latter leads, in turn, to killing of anaerobic bacteria and therefore to an important decrease in microbe populations. The role of bacteriophages in maintaining gut microbiome homeostasis is an emerging area, and is challenging to investigate due to the difficulty to provide accurate identification and classification because of their high diversity and the absence of conserved regions within their genomes. Gut inflammation leads to an environmental stress that can induce the lytic cycle of bacteriophages and therefore a depletion in bacteria abundances and reduced diversity by unknown mechanisms, as well as activation of innate immunity via the liberation of cellular toxins or nucleic acids which are recognized as pathogen-associated molecular patterns.⁵⁷ In the colon, bacteriophages can interact with the heavily O-glycosylated mucin Muc2 through Ig-like domains and protect the host from mucus-penetrating bacteria.⁵⁸ During inflammation, changes in mucus composition and glycosylation status can impact the interaction with

bacteriophages and profoundly affect this protective function and thus participate to dysbiosis establishment. Interestingly, oxidative stress and bacteriophages can act synergistically to increase dysbiosis.⁵⁹

Finally, bacteriocins share common features with bacteriophages. Bacteriocins possess antimicrobial properties. They are synthesized by ribosomes in Gram-positive and Gram-negative bacteria belonging mainly to the Firmicutes phylum, but others belong to Bacteroidetes and to Actinobacteria and Proteobacteria phyla.⁶⁰ They are able to kill pathogens by forming pores in membranes and cleaving nucleic acids, and possess also anti-viral, spermicidal, and anti-cancer properties.⁵⁶ Additionally, bacteriocins can impact the immune system, and regulate the niche competition among commensals and act as a probiotic by specifically reducing the abundance of pathogens.^{57,61–63} Notably, although bacteriocins display abilities to improve gut barrier function and host immune responses, oxidative stress can induce the expression of bacteriocins which participates to microbial composition shifts in the context of inflammation-induced oxidative stress.⁶⁴

In addition to the roles played by these distinct actors, that can interact to potentialize gut dysbiosis severity, on the host side, Paneth cell dysfunctions were shown to be major contributors in gut dysbiosis, and current knowledge will be presented in the next section.

Paneth cells/dysbiosis

Paneth cell functions

Paneth cells are located at the bottom of the small intestinal crypts in close vicinity with intestinal stem cells (ISCs), and have a longer life span (60 d) than other epithelial cells (3–5 ds). These highly specialized cells exhibit ultrastructural features of secretory cells with an extensive endoplasmic reticulum (ER) and Golgi network and numerous secretory granules, and as we will discuss later on, they are susceptible to ER stress and altered autophagy flux. Paneth cells are now recognized to play critical roles in the establishment of ISC niche^{65–70} and regulation of Wnt/ β -catenin and Notch signaling pathways.^{71,72} They secrete

growth factors such as EGF and Wnt ligands required for ISC stemness and proliferation.^{73–75} Moreover, the importance of Paneth cell metabolism on ISC fate regulation, especially via lactate secretion, was recently demonstrated.⁷⁶

Another key role played by Paneth cells is the regulation of innate immunity. Indeed, secretory granules contain AMPs such as α -defensins, lysozyme, secretory phospholipase A2, and RegIIIy, which can be secreted into the crypt lumen by exocytosis to protect the host against pathogens^{77–82} and to shape gut microbiome composition.⁸³ Paneth cells exert their sentinel role through Toll-like receptors such as TLR9,^{84,85} and NOD2 which is required for α -defensin expression.^{79,86} Likewise, TLR receptors and MyD88 signaling are required for RegIIIy expression.^{80,81} Recently, deletion of *Lyz1* in epithelial cells further illustrated the major role played by Paneth cells in the control of gut microbiome integrity.⁸⁷

Therefore, Paneth cells play major roles in host-microbiome interactions by controlling both homeostasis with commensals and innate immunity to ensure the host defense against enteric pathogens.

Altered Paneth cells

Given the importance of Paneth cells and their multiple roles, alterations in their functions are associated with failure of gut homeostasis. For example, HFD treatment induced Paneth cell alterations with abnormalities in secretory granules, decreased expression of α -defensins 5 and 6, and increased expression of ER stress and autophagy markers. These effects were induced by bile acids secreted in response to HFD treatment which promoted the upregulation of the G protein-coupled bile acid receptor TGR5 in Paneth cell membrane, and contributed to HFD-associated dysbiosis.⁸⁸ Similarly, overweight and obese subjects display Paneth cell defects,^{89,90} and consumption of a western diet in mice impacted Paneth cell function via the secondary bile acid deoxycholic acid produced by *Clostridium spp.* and subsequent activation of farnesoid X receptor and type I interferon signaling pathways.⁹⁰ Moreover, as mentioned above, deletion of *Lyz1* in epithelial

cells also impacted Paneth cell function and promoted remodeling in microbiome composition and intestinal inflammation.⁸⁷

Importantly, Paneth cells are highly susceptible to deregulation in three interrelated signaling pathways involved in IBD pathogenesis: autophagy, ER stress and intracellular sensing.⁹¹ Hence, starvation for 48 h in mice induced strong decreases in Paneth cell AMP levels, and abnormalities in cell ultrastructure elements with defects in secretory granules and late degenerative autophagolysosomes.⁹² Additionally, alterations in autophagy pathways can trigger major Paneth cell defaults with critical consequences on their function. Mutations or deficiency of *Atg16L1* led to Paneth cell impairments similar to that observed in IBD patients and Crohn's disease-like ileitis development.^{93–95} Intestinal epithelial specific deletion of *Atg7* also triggered secretory granules alterations and decreased AMP levels,^{95,96} and *Irgmm1* was shown to regulate intestinal inflammation in gut murine intestine, potentially via autophagy modulation.⁹⁷

In secretory cells, such as Paneth cells, secretory proteins undergo folding and maturation in the ER. In case the quantity of unfolded proteins exceeds the capacity of ER to process these proteins, ER stress occurs and triggers the unfolded protein response (UPR) that in turn reduces the rate of protein synthesis and ER load. If homeostasis cannot be restored, cell apoptosis is triggered to avoid secretion of altered proteins. Specific intestinal epithelial cells deletion of *Xbp1*, a transcription factor required for the UPR response, resulted in absence of Paneth cells and spontaneous enteritis in *Xbp1*^{-/-} mice. In *Xbp1*^{-/+} mice, Paneth cells were altered with ER stress and reduced number of secretory granules, and *XBPI* was identified as a major risk gene in IBD patients.⁹⁸ Moreover, Adolph et al. showed that severe spontaneous Crohn's disease-like transmural ileitis was established when both UPR and autophagy processes were disrupted.⁹⁵

Altogether, these studies showed that deregulated functions of Paneth cells, involving in particular alterations in autophagy process and UPR response, are associated with intestinal inflammation. Therefore, a link between Paneth cell-derived AMP, the microbiome and the inflammatory status

was established, yet the underlying cellular and molecular mechanisms responsible for gut dysbiosis and inflammation still remained to be clarified.

Role in gut dysbiosis

Paneth cells play a crucial role in shaping gut microbiome composition via the secretion of AMPs; however as discussed above this epithelial cell type is highly vulnerable to genetic mutations or environmental perturbations. Impairment in Paneth cell function greatly impacts the equilibrium of the crosstalk between microbiome, epithelial cells and the immune system, and compromises gut homeostasis. Furthermore, in several of the murine models reported above, Paneth cell disruption was associated with gut dysbiosis development.^{88,90,99} Along the same line, the antimicrobial lectin RegIIIγ expressed in Paneth cells is required to maintain the mucus barrier and restrain microbe access to the epithelium, and thus prevent the activation of adaptive immunity.¹⁰⁰

Hence, altered Paneth cells were considered for many years as the guards of microbiome integrity, and their defects as critical players in gut dysbiosis and inflammation.^{95,101} However, two recent studies further explored the underlying mechanisms and revealed that communication between Paneth cells and another important epithelial cell type, tuft cells, is required to promote dysbiosis and inflammation.^{102,103}

Tuft cells

Functions

Tuft cells represent a rare intestinal epithelial cell type (0.4% of epithelial cells), which nevertheless plays a crucial role of sentinel by recognizing various luminal factors and participates to the regulation of mucosal immunity.^{104–107} These cells can be activated by the presence of helminths or protozoa in the gut lumen and, via the secretion of Interleukin 25 (IL25), they trigger the activation and recruitment of type-2 T helper cells (Th2) and innate lymphoid cells 2 (ILC2). ILC2 then produce Interleukin 13 (IL13) and Interleukin 4 (IL4) that, in turn, act on epithelial cells through

the IL4 and IL13 receptors to induce profound remodeling of the epithelium with goblet cell and tuft cell hyperplasia, thus facilitating parasites expulsion.^{15,108,109} The initiation of the type 2 immune response requires the cation channel Trpm5, and the metabolite succinate derived from the protist *Tritrichomonas muris* (*Tm*) can activate tuft cells through the succinate receptor Sucnr1.¹⁶ An important study recently revealed an unappreciated diversity in commensal parabasalids from the genus *Tritrichomonas* and showed that two closely related species, *Tm* and *T. casperi*, differ in the excretion of the metabolite succinate and in their nutritional niches within the gut microbiota.¹¹⁰ These metabolic differences result in divergent immune responses in the small intestine, and differential preference for mucus or fiber, leading in turn to distinct competition with commensal bacteria. Interestingly, type 2 immunity induced by *Tm* can be modulated by dietary fiber composition. Of note, tuft cell activation by the helminth *Nippostrongylus brasiliensis* (*Nb*) does not involve succinate receptor signaling, suggesting the existence of alternative mechanisms. Taste receptors are also implicated in pathogen recognition, with Tas1r3 receptor involved in tuft cells activation following *Tm* colonization¹¹¹ and members of the Tas2r family are required to initiate IL25 production in presence of the parasite *Trichinella spiralis*.¹¹² Thus, pathogen recognition presumably relies on different receptor families expressed by tuft cells.

Tuft cells are also sensitive to other components of the microbiome such as viruses. Tuft cells specifically express the Cd300lf receptor for murine norovirus;¹¹³ nevertheless, the human CD300LF is not the receptor for human norovirus,¹¹⁴ suggesting that the tropism of murine norovirus for tuft cells may exclusively concern rodents. Recently, another study showed that, unexpectedly, the enteric virus rotavirus is able to infect tuft cells, which respond by inducing interferon-related pathways.¹¹⁵ This suggests that tuft cells may be able to modulate immune responses differentially, depending on luminal triggers.

Finally, tuft cells were recently shown to play also an important role in bacterial eradication.¹¹⁶ The authors showed that *Shigella*, a common bacterium involved in human diarrhea, induced

a hyperplasia of the subtype tuft-2 cells, independent from IL13 signaling. Tuft-2 cells sense the bacterial metabolite N-undecanoylglycine (N-C11-G) through vomeronasal receptor Vmn2r26. The activation of the receptor Vmn2r26 led to the production of prostaglandin D2 that in turn increased mucus secretion by goblet cells and initiated antibacterial immunity. Yet, opposite results regarding tuft cell-derived prostaglandin D2 have been reported in studies assessing the role of NAIP – NLRC4 inflammasome activation in tuft cells, and demonstrating that this pathway induces a prostaglandin D2 – ILC3 signaling pathway that contributes to bacterial pathogen clearance in the small intestine.^{117,118} In addition, administration of N-C11-G to intestinal epithelial monolayers failed to induce a release of leukotrienes or ion influx showing that tuft cells were not activated by this ligand in this *ex vivo* context.^{117,118} Thus, further studies are required to explore the recognition of pathogenic bacteria specific extracellular ligands by tuft cells. Noteworthy, microbes and parasites coexist in the same environment, interact and influence each other, and several studies reported an impact of helminth infection on microbiome composition.^{119–122}

Currently, tuft cells are recognized as key actors in the initiation of type 2 immune responses in a context of parasitic infection, yet other functions will be probably discovered in the future.

Signaling molecules associated with tuft cell activation

Besides the importance of IL25, other molecules can be secreted by tuft cells to initiate a type 2 immune response following parasitic infection.

Eicosanoids are bioactive lipids derived from arachidonic acid that exhibit both pro- and anti-inflammatory properties. Although leukotriene synthesis occurs in hematopoietic cells, tuft cells also express genes required for leukotriene and prostaglandin synthesis, such as arachidonate 5-lipoxygenase (Alox5), Ptgs1 and Ptgs2, leukotriene C4 synthase (Ltc4s), Pla2g4a and Hpgds.¹²³ A recent study showed that tuft cell cysteinyl leukotrienes (cysLTs) act in synergy with IL25 to activate type 2 immunity in response to helminth

infection, while cysLTs are dispensable for immune responses induced by protist colonization.¹²⁴ Interestingly, on the opposite, prostaglandin D2 and its receptor CRTH2 act on intestinal epithelial cells to restrain the effects of cytokines produced by ILC2 since mice deficient for CRTH2 show increased parasite expulsion following infection with *Nb*.¹¹⁷ This evidence suggests that tuft cells play dual effector roles, on one hand by their capacity to induce a type 2 immunity following parasitic infection, and on the other hand by exerting a negative feedback loop to avoid the consequences of an uncontrolled intestinal inflammation.

Another tuft cell factor, acetylcholine (ACh), has been shown to be implicated in response to helminth infection. Indeed, tuft cells are the only intestinal epithelial cells expressing the major enzyme required for ACh biosynthesis, choline acetyltransferase (ChAT).¹²⁵ The role of the ILC2 ChAT-ACh pathway in promoting type 2 immunity in response to helminth infection had already been identified, since specific ChAT deletion in ILC2 altered ILC2 responses and immunity against parasite infection.¹²⁶ However, the role of non-neuronal ACh production by tuft cells has long remained unknown until two recent studies illuminated critical functions of tuft cell-derived ACh in clearing helminth infections. Both studies reported delayed worm expulsion in mice with ChAT-deficient tuft cells, without a global impairment of the type 2 immune response, and identified distinct and complementary mechanisms. Following worm infections, one of the studies reported increased ACh concentrations not only in tuft cells, but also in the gut lumen, suggesting a direct action of ACh on parasites that purposely secrete in their neighborhood acetylcholinesterase (AChE), the enzyme responsible of ACh breakdown.¹²⁷ Using *ex vivo* worm culture, this study also demonstrated a direct effect of ACh on worm fecundity, through worm muscarinic ACh receptors.¹²⁸ The other study found that, in infected mice, tuft cell-derived ACh stimulates neighboring enterocytes to rapidly increase fluid secretion in the lumen to contribute to the weep and sweep response that expulses worms out of the gut.¹²⁹ Together, these two reports met the identical conclusion that the role of tuft cells cannot be limited to their sentinel function but also includes an effector function

revealed by the multifaceted role of ACh secretion by tuft cells, following their dramatic amplification during type 2 responses.

Besides its role during worm infections, tuft cell-derived ACh might also be involved in neuroimmune crosstalk in type 2 immunity. The neuroimmune crosstalk is an emerging field that involves a complex cellular environment including specific gut immune populations and specific neurons subsets, and how these two systems interact together in a coordinated manner is still not clear. Interestingly, recent studies highlight bidirectional interactions between immune cells, especially innate lymphoid cells, and the nervous system and provide a better understanding of the mechanisms underlying the communication between those two systems to regulate tissue homeostasis and host defense.¹³⁰⁻¹³² Hence, a crosstalk between cholinergic neurons that express the neuropeptide U (NMU) and ILC2 that selectively express the NMU receptor 1, participates to the type 2 immune response induced by the infection with the helminth *Nb* to increase host defense and worm clearance. This crosstalk therefore constitutes one of the mechanisms through which the enteric nervous system and the immune system integrate signals to maintain gut homeostasis.^{133,134} Additionally, the mammalian nervous system displays dual functions to rapidly activate or repress type 2 immunity to prevent deleterious effects of prolonged ILC2-dependent inflammation. Thereby, β 2-adrenergic signaling exerts a negative feedback on activated ILC2 to restrain ILC2 proliferation and effector function against *Nb* infection,¹³⁵ and the neuropeptide α -CGRP, mainly produced by ChAT⁺ enteric neurons, inhibits ILC2 proliferation in an IL-25-induced *in vivo* activation model.¹³⁶ Therefore, the neuroimmune field needs to be further explored, for example the role of tuft cell-derived ACh deserves attention. Hence, while many evidences support the communication between neurons and immune cells, how gut immune cells or epithelial cells signal to neurons to trigger defensive responses is a key remaining question.

Thus, tuft cells are key sentinels able to sense different luminal cues, including the metabolite succinate, and exert immunomodulatory effects

via the secretion of various mediators. The intestinal microbial ecosystem is highly complex, and whether tuft cells might be involved in the control of its composition remained unexplored.

A “Paneth cell-succinate-tuft cell-immune system” circuit shapes the microbiome landscape

Paneth cells were proposed to be the guardians of gut microbiome; however, two important studies recently converge to demonstrate, in two different contexts, that tuft cells are also key regulators of gut microbiome, at least in part in a crosstalk with Paneth cells.^{102,103}

The metabolite succinate is a key activator of tuft cells,^{16,137,138} and is produced by host cells, commensal bacteria, parasitic worms and tritrichomonads, suggesting roles of tuft cell succinate sensing other than antiparasitic effects.^{16,138–140} Fung et al. tackled this question and showed that microbial-derived succinate can activate tuft cells and induce a type 2 immune response that leads to a profound remodeling of intestinal epithelium with a hyperplasia of tuft cells, goblet cells as well as Paneth cells. Paneth cells displayed structural alterations with decreased secretory granules reminiscent to that observed in Crohn’s disease patients and *Atg16L1*-deficient murine models,⁹³ leading to strong alterations in AMP production. Succinate alone was sufficient to induce AMP remodeling, but not in mice deficient for tuft cell signaling (*Trpm5*^{-/-} mice) or succinate sensing (*Sucnr1*^{-/-} mice). In addition, type 2 immunity without tuft cell activation can trigger AMP remodeling, via a direct effect of the type cytokine IL13 on intestinal epithelium to remodel AMP expression. In return, the microbiome composition is strongly impacted and the abundance of mucosa-associated microbes is reduced in the ileum.

Finally, the authors questioned tuft cell capacity to sense luminal succinate levels and to control in return bacterial homeostasis. Succinate is produced by commensals during carbohydrate fermentation and its level remains normally low due to rapid consumption by others bacteria. Evidence provided by colitis models suggested that succinate could have beneficial effects on the host. Tuft cell

activation by the metabolite succinate reduced chronic intestinal inflammation in mice.¹⁴¹ Along the same line, lysozyme deficiency specifically in Paneth cells led to microbiota remodeling and increased succinate, and reduced inflammation in a colitis model.⁸⁷ Increased succinate luminal levels are associated with antibiotics- or Polyethylene Glycol (PEG)-induced dysbiosis.¹³⁷ In PEG-treated mice, the authors showed that altered AMP expression was present and this remodeling required the presence of tuft cells. These data suggest that the metabolite succinate, in a context of parasitic infection as well as gut dysbiosis, exerts deleterious effects on the host. Importantly, these results demonstrate that tuft cells can sense dysbiosis through an elevation of luminal succinate and in turn modulate AMP expression to alter microbial composition.

In summary (Figure 1), this study highlighted a novel mechanism by which tuft cells can be activated by increased levels of the luminal metabolite succinate, linked to *Tm* colonization or dysbiosis, to trigger a type 2 immune response. The type 2 cytokine IL13 then induces epithelial remodeling and profoundly alters AMP expression in Paneth and goblet cells, thus reshaping microbiome composition. This study shows that tuft cells not only play a crucial role in type 2 immunity initiation and antiparasitic action but may have also evolved to sense succinate-associated dysbiosis and regulate gut microbial landscape via important shifts in AMP production.

Importantly, another study revealed the existence of a crosstalk between Paneth and tuft cells to modulate gut microbial homeostasis.¹⁰² As discussed previously, Paneth cells regulate gut microbiome and prevent host alteration by pathogen colonization. Altered Paneth cells are associated with obesity and IBD,^{89,142} and deficiency in defensins¹⁴³ or lysozyme⁸⁷ showed Paneth cell critical role in gut microbiome integrity since impaired Paneth cells are drivers of gut dysbiosis and inflammation. However, although gut dysbiosis and inflammation are associated with various pathologies, the mechanisms linking these processes to altered Paneth cells are missing. Moreover, a potential cooperation between impaired Paneth and tuft cells has been suggested in IBD models in which an artificial increase in tuft

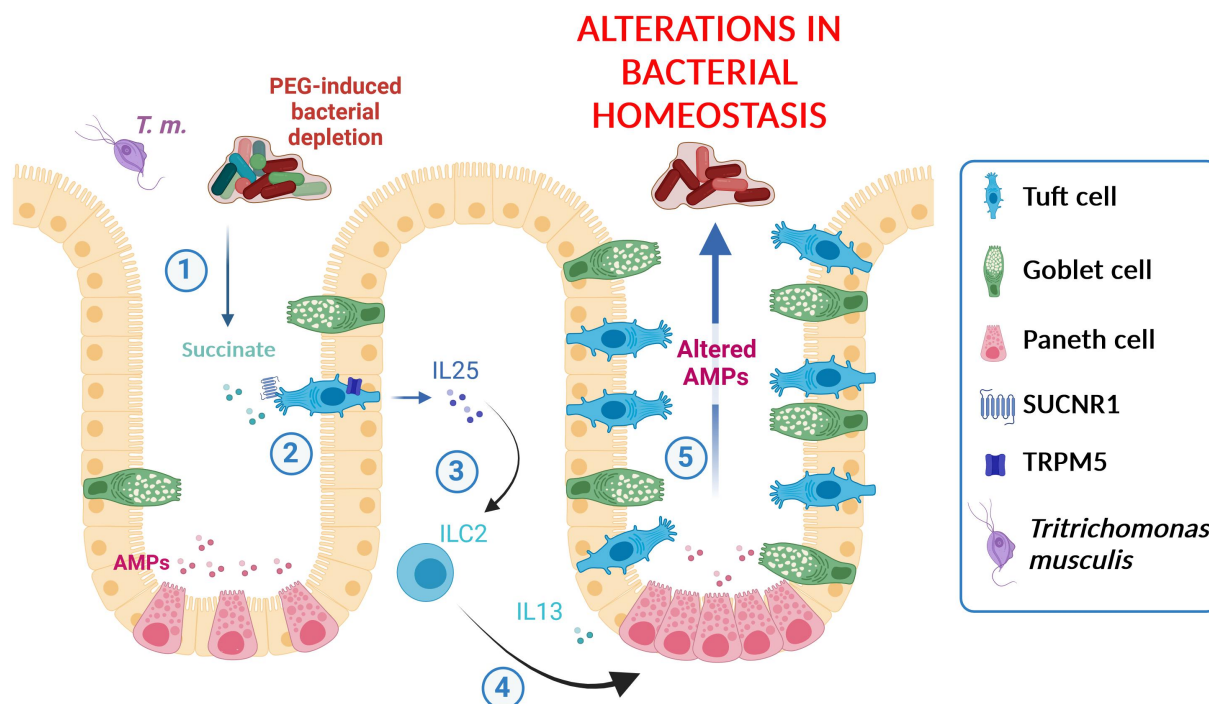


Figure 1. Tuft cell-induced intestinal epithelium remodeling and AMP shifts leading to microbiota reshaping following tuft cell activation by microbial-derived succinate. The presence of *Tm* in the distal intestine or bacterial depletion induced by PEG administration (1) lead to enhanced levels of succinate in the lumen that activate tuft cells through *Sucnr1* and opening of the ion channel *Trpm5* (2). IL25, secreted by tuft cells, then activate ILC2 located in the *lamina propria* (3). Activated ILC2 liberate IL13 which can act on stem cells to force cellular differentiation toward tuft, Paneth and goblet cells, thus leading to hyperplasia of these three cell types (4). In addition, IL13 induces strong alterations in AMP expression in both Paneth and goblet cells, causing killing of commensals and thus promoting alterations in microbiota composition (5). Created with [BioRender.com](https://www.biorender.com).

cell numbers improves their inflammatory status.¹⁴¹ The authors therefore investigated the hypothesis that a crosstalk between both epithelial cell types may play a role in gut dysbiosis formation and inflammation with the help of a murine model with dysfunctional Paneth cells. The transcription factor *Sox9* is required for Paneth cell differentiation,^{144,145} and it was found that *Sox9* deletion in adult Paneth cells (*Sox9^{LoxP/LoxP}; Villin-Cre^{ERT2}* mice leading to *Sox9* deletion in intestinal epithelial cells) leads to profound alterations in their differentiation status with mixed features of Paneth and goblet cells, impaired secretory granules and altered AMP production. Therefore, the *Sox9^{LoxP/LoxP}; Villin-Cre^{ERT2}* model constitutes a relevant model to address the consequences of dysfunctional Paneth cells on intestinal physiology.

Further kinetic investigation of the consequences of altered Paneth cells on intestinal homeostasis, especially on gut microbiome composition, intestinal permeability, immune status, and intestinal epithelium composition, by using various

murine models and intestinal organoid models over time led to the discovery of a multistep mechanism responsible for gut dysbiosis and inflammation (Figure 2). In a first step, initial Paneth defects characterized by a decreased expression of lysozyme, *Mmp7* and defensins, and ectopic expression of *Muc2* and *Klf4* were rapidly induced by *Sox9* loss. These impairments in Paneth cell function led to mild remodeling of gut microbes with an increased in succinate production potential, in the absence of physiological parameters alteration. In a second step, tuft cells were activated via a succinate-*Sucnr1* signaling pathway and triggered the activation of ILC2. Strikingly, the results showed that tuft cell-dependent type 2 immune response following Paneth cell alterations and reshaping of microbiome was independent from *Trpm5*, suggesting that distinct triggers potentially activate specific tuft cell signaling pathways, with putative different impacts on mucosal immunity. In a third step, epithelial cell remodeling was induced with tuft and goblet hyperplasia and

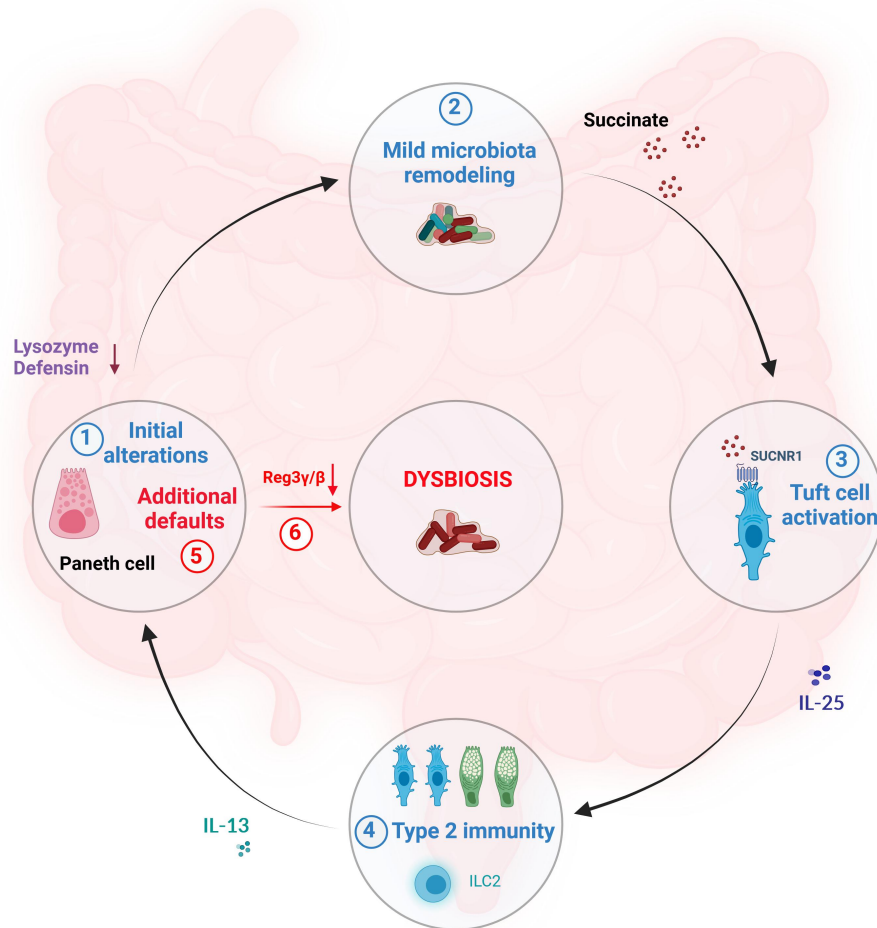


Figure 2. Multi-step mechanism responsible for gut dysbiosis and inflammation establishment following a crosstalk between altered Paneth cells and tuft cells, involving a succinate-Sucnr1 axis and type 2 cytokines. In absence of Sox9, Paneth cell function is altered with reduction of Lysozyme and defensins (1), which leads to mild remodeling of microbiota and increased succinate production potential (2). Tuft cells sense succinate through Sucnr1 and release IL25 (3). Type 2 cytokines liberated by IL-25-activated ILC2 then induce IL4ra-dependent remodeling of epithelial cells including goblet and Paneth cells (4). IL13 secretion also causes reduction of *RegIII* levels, which aggravates Paneth cell defaults (5), eventually leading to gut dysbiosis (6). Hence, tuft cells cooperate with altered Paneth cells to drive remodeling in microbiome landscape and intestinal inflammation. Created with [BioRender.com](https://www.biorender.com).

ectopic expression in the small intestine of the antimicrobial molecule Resistin-like β (dependent from the presence of IL4ra in intestinal epithelial cells). This remodeling was associated with a concomitant reduction in RegIII γ / β levels, induced by the type 2 cytokine IL13, which thus aggravated initial Paneth cell defects and eventually triggered dysbiosis. This study revealed a novel function of tuft cells as essential actors in gut microbiome regulation, via a crosstalk with altered Paneth cells. Furthermore, it also highlights an unrecognized role of Paneth cells in the maintenance of a balanced microbiome, preventing the

outgrowth of a succinate-producing altered microbiota that may inadequately activate the tuft cell anti-parasite response and inflammation, potentially leading to chronic dysbiosis.

Together, these two studies brought major conceptual advances in the understanding of the cellular and molecular mechanisms involved in the host-microbiome communication. They revealed a major new mechanism by which tuft cells sense succinate and initiate a response that leads to altered production of Paneth cell-derived AMPs, which in turn drives intestinal dysbiosis and inflammation. Therefore, the crosstalk between

Paneth and tuft cells, and not Paneth cells alone, orchestrate the gut microbiome landscape and inflammation.

Conclusion

Recent advances in microbial metagenomics have provided compelling evidence that disruption in host-microbiota communication is central to the frequency, severity, and progression of multiple diseases. Yet, the nature of gut dysbiosis is highly variable and imbalance in microbial communities exhibit a myriad of aspects. This suggests that a variety of mechanisms may underlie dysbiosis establishment. Moreover, several processes may cause the associations between dysbiosis and disease development: dysbiosis can originate directly from the disease itself and in turn contributes to disease severity/progression, or dysbiosis resulting from impaired communication between environment-host-microbiome could be a direct trigger of diseases. Future studies performed in humans or in preclinical animal models are thus required to unravel complex and multifactorial mechanisms responsible for gut dysbiosis establishment.

Importantly though, two recent studies performed in distinct contexts converge to provide new insights into the mechanisms underlying the altered communication between the gut microbiome, the epithelium and the immune system. Tuft cells are able to sense succinate produced by an altered microbiome, and in turn impact microbiome composition via major reshaping in AMP production by Paneth cells, thus promoting a more severe reorganization in gut microbial landscape. Hence, a crosstalk between Paneth and tuft epithelial cells happens to be at the core of this communication, thus opening new opportunities to identify novel tools to target tuft cell-Paneth cell interactions with the ultimate goal to prevent and correct gut dysbiosis.

Of note, gut microbiome functions in physiology and pathology is a large research domain that we could not cover exhaustively. We therefore apologize to our colleagues, whose valuable work could not be included or cited in this review.

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Data availability statement

The data that support the findings of this study are openly available in Proc Natl Acad Sci U S A. 2023 Jun 20;120(25):e2219431120; doi: 10.1073/pnas.2219431120; Epub 2023 Jun 12; PMID: 37307458, and Proc Natl Acad Sci U S A. 2023 Jun 6;120(23):e2216908120; doi: 10.1073/pnas.2216908120; Epub 2023 May 30; PMID: 37253002.

Shotgun metagenomic data are fully available under BioProject ID: PRJEB40719 for shotgun metagenomic data and PRJNA944985 for 16S rRNA sequencing data (<http://www.ncbi.nlm.nih.gov/bioproject/944985>). The 16S rRNA gene sequencing data are deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject ID PRJNA873680). Original code for 16S rRNA gene sequencing analysis is deposited at https://github.com/MCW-CMR/Salzman-Howitt_collaboration.

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