blood® Review Series

HUMAN NEUTROPHILS

Cross talk between neutrophils and the microbiota

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The microbiota has emerged as an important regulator of the host immunity by the induction, functional modulation, or suppression of local and systemic immune responses. In return, the host immune system restricts translocation and fine tunes the composition and distribution of the microbiota to maintain a beneficial symbiosis. This paradigm applies to neutrophils, a critical component of the innate immunity, allowing their production and function to be influenced by microbial components and metabolites derived from the microbiota, and engaging them in the process of microbiota containment and regulation. The cross talk between neutrophils and the microbiota adjusts the magnitude of neutrophil-mediated inflammation on challenge while preventing neutrophil responses against commensals under steady state. Here, we review the major molecular and cellular mediators of the interactions between neutrophils and the microbiota and discuss their interplay and contribution in chronic inflammatory diseases and cancer. (*Blood*. 2019;133(20):2168-2177)

Introduction

The microbiota refers to the symbiotic microorganism communities that reside on the mucosal and skin surfaces of all vertebrates. With estimated 10^{14} microorganisms in the human intestine, the microbiota outnumbers host cells by almost 2 orders of magnitude, encoding 100 times more genes compared with the host genome, and comprises >1000 species, including bacteria, fungi, protozoa, and viruses.[1,2] In the past decade, rapidly growing understanding of the microbiota has significantly transformed the field of immunology, leading to increasing appreciation of the fundamental functions for the microbiota in the development and regulation of host immune system.

Remarkable diversity and spatial partitioning of the microbiota are observed on distinct barrier surfaces, including the gastrointestinal tract, skin, oral cavity, lung, and vaginal tract.[3] In the gastrointestinal tract, the composition and distribution of the microbiota is anatomically defined by barrier specificity and nutrient availability. In the small intestine, a discontinuous mucus layer separates the majority of the microbiota from the epithelium while allowing specific species to adhere and directly interact with epithelial cells.[4] The small intestine is also rich in monosaccharides, disaccharides, and amino acids, which support the growth of bacterial species that rely on simple sugars, including mainly Proteobacteria and Lactobacillales.[5] In the colon, 2 continuous layers of mucus structures mixed with anticommensal immunoglobulin-A (IgA) and antimicrobial peptides (AMPs) more strictly compartmentalize the microbiota. Different from the small intestine, the majority of nutrients in the colon comprise polysaccharides that the host cannot digest. Consequently, only bacterial species capable of breaking down fibers and mucin can survive in the colon, which leads to the enrichment of *Bacteroides* and *Clostridiales* as dominant populations.[5] The microbial communities in the gastrointestinal tract are shaped by the diet, host metabolic and inflammatory conditions and pathogen infection, and dysbiosis is often associated with inflammatory, autoimmune, and metabolic diseases.[5-7]

Given the pathogenic potential of large numbers of bacteria, the symbiosis between the host and microbiota is essential for health. In fact, the capacity of certain bacteria to act as a commensal or pathogen is highly dependent on host immune conditions, genetic predispositions, and coinfections. To maintain a safe union, the host must develop a complex regulatory system, involving epithelial cells, mucus, IgA, AMPs, and an array of innate and adaptive immune cells to control the composition and distribution of the microbiota.[8] These structural and immune components form a "mucosal firewall," which eliminates invading pathogens, selects commensal species, prevents microbial translocation, and places "ambivalent" microbial species with high pathogenic potential, such as *Escherichia coli* and segmented filamentous bacteria (SFB), under serveillance.[9] In turn, the microbiota also communicates with the host via microbial components and metabolites that diffuse into the host system, which have demonstrated broad influences on host immunity, metabolism, and tissue homeostasis.[1,8,10-12] For example, the microbiota is essential for the development of mucosal lymphoid structures, the establishment of commensal-specific adaptive immunity, and the induction of regulatory responses in mucosal tissues. Importantly, the influence of the microbiota goes beyond the area where these microorganisms reside, leading to systemic regulation of the production and function of innate immune cells. These findings also position the microbiota as a key modulator in various inflammatory diseases.[6-8]

The neutrophil is a critical component of innate immunity. They defend against pathogens by phagocytosis, releasing AMPs and reactive oxygen species (ROS), secreting inflammatory cytokines, and forming neutrophil extracellular traps. This arsenal eliminates effectively the invading pathogens, but may also promote tissue damage in inflammatory diseases.[13-15] In this review, we will discuss how the microbiota and neutrophils balance their bilateral cross talk to maintain a beneficial relationship that promotes health and prevents diseases.

Regulation of neutrophils by the microbiota

Similar to many other cell types in the body, neutrophils are regulated by microbial components and metabolites in health and disease conditions. How these signals are orchestrated to influence neutrophil production and functions, however, remains incompletely understood (Figure 1).

The microbiota regulates neutrophil production

Neutrophils are terminally differentiated cells produced in the bone marrow (BM), where hematopoietic stem cells give rise to progenitor cell types that hierarchically commit toward the neutrophilic lineage, including multipotent progenitors, granulocyte-macrophage progenitors, and unipotent neutrophil progenitors.[16] The gut microbiota plays a fundamental role in the tonic regulation of neutrophil production. It was noted >30 years ago that antibiotic usage was associated with reduced myelopoiesis in the BM.[17,18] Further evidence suggests that the reduction or absence of microbial content, as induced by antibiotics or germ-free state, profoundly reduces neutrophil numbers and their progenitors in neonates and adult mice, resulting in increased susceptibility to infections.[19-24] Neutrophil reductions in microbiota-depleted models can be rescued by heat-killed *E coli* strain, autoclaved cecal content, or lipopolysaccharide (LPS),[20,21] indicating that microbial components derived from the microbiota may mediate these effects. Indeed, ligands for the pattern recognition receptors, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-containing proteins (NODs), such as LPS and peptidoglycan, can be readily detected in human and murine circulation.[1,19,25] These molecules induce interleukin-17 (IL-17) production from innate lymphoid cells in the intestine via the TLR4/Myd88 pathway, resulting in the production of granulocyte colony-stimulating factor (G-CSF), a master regulator of neutrophil differentiation.[21] Interestingly, neutrophils can also be recruited to the mucosal system via CXCR2, leading to a feedback suppression of IL-17/G-CSF production.[26] Taken together, the production of neutrophils is tailored to a need controlled by the microbiota.

The microbiota also regulates neutrophil production by targeting the niche that supports hematopoiesis in the BM. For example, NOD1 ligands can be sensed by stromal cells, resulting in the expression of multiple hematopoietic cytokines including IL-7, FMS-like tyrosine kinase 3 ligand, stem cell factor, thrombopoietin, and IL-6.[27] Administration of NOD1 ligands in germ-free mice can thus restore the expression of these cytokines and promote hematopoiesis in both myeloid and lymphoid lineages.[27] In addition, a high-fat diet can induce a myeloid bias in hematopoiesis, which is mediated by deregulation of the niche characterized by increased mesenchymal stem cell (MSC) numbers with reduced expression of hematopoietic cytokines including C-X-C motif chemokine-12, IL-7, Notch2, vascular cell adhesion molecule-1, and osteopontin.[28] Interestingly, high-fat diet induces a shift in the microbiome, and fecal transplantation from obese to normal mice produces similar niche deregulation and myeloid bias,[28,29] suggesting a significant contribution of the microbiota in these phenomena. Microbiota-derived signals have also been found to affect macrophages, an important niche component in the BM, in the context of viral infection.[30] Further investigations into the regulation of macrophages and other niche constituents by the microbiota in the context of hematopoiesis or neutrophil production are needed to advance the understanding of these processes.

Neutrophil "priming" by the microbiota

Microbial components from the microbiota can also regulate neutrophil functions and thus modulate the magnitude of inflammatory responses. These signals can be sensed by local and distal organs, which secrete factors that promote neutrophil recruitment or activation. For example, direct contact of SFB with the epithelium leads to the release of serum amyloid A, which induces Th17 differentiation in the intestine,[31] and results in low-grade activation of NF-κB signaling in circulating neutrophils, enhancing their migration and production of ROS and IL-1β upon stimulation.[32-34] In addition, very low doses of LPS can be sensed by MSCs in the BM, leading to rapid mobilization of the neutrophil and monocyte reservoir.[35] Further, germ-free mice exhibit increased IL-10 expression upon inflammatory challenges in distal organs, such as the lung, leading to reduced neutrophil recruitment and cytokine production.[36,37] Interestingly, pretreatment with LPS abrogates the increased IL-10 production and restores neutrophil responses in germ-free mice,[36,37] suggesting a "priming" of the host immune system to mount neutrophil reactions.

Neutrophils can also sense microbiota-derived signals with their own pattern recognition receptors, including most TLRs (except for TLR3), NODs, and inflammasome.[13,38] Neutrophils isolated from germ-free mice exhibit decreased myeloperoxidase activity,[39] and reduced capacity of chemotaxis toward diverse inflammatory stimuli in vivo, which requires microbiota signaling through Myd88.[40] Further, the absence of the microbiota leads to significant reductions in the phagocytic killing capacity in BM-derived neutrophils, which is mediated by NOD1, but not NOD2 or TLR4 signaling pathways.[25] Recently, it has been shown that neutrophil heterogeneity arises from their aging in the circulation, in which they acquire distinct phenotypic, transcriptional, and functional properties before they get cleared from blood.[19,41-43] Aged neutrophils, characterized by a $CD62L^{low}$ $CXCR4^{high}$ phenotype, represent a proinflammatory subset that exhibits enhanced migration, $\alpha_M\beta_2$ integrin activation, neutrophil extracellular trap formation, and phagocytosis under inflammatory conditions.[19,41] Neutrophil aging is regulated by the microbiota via TLR/Myd88 pathways. Depletion of the microbiota significantly reduces aged neutrophil numbers and dramatically improves the inflammation-associated organ damage of sickle cell disease and endotoxin-induced septic shock.[19] The microbiota thus provides a constitutive low-grade stimulation that primes neutrophils for a robust response to inflammatory stimuli. The "priming" of neutrophils by the microbiota serves as

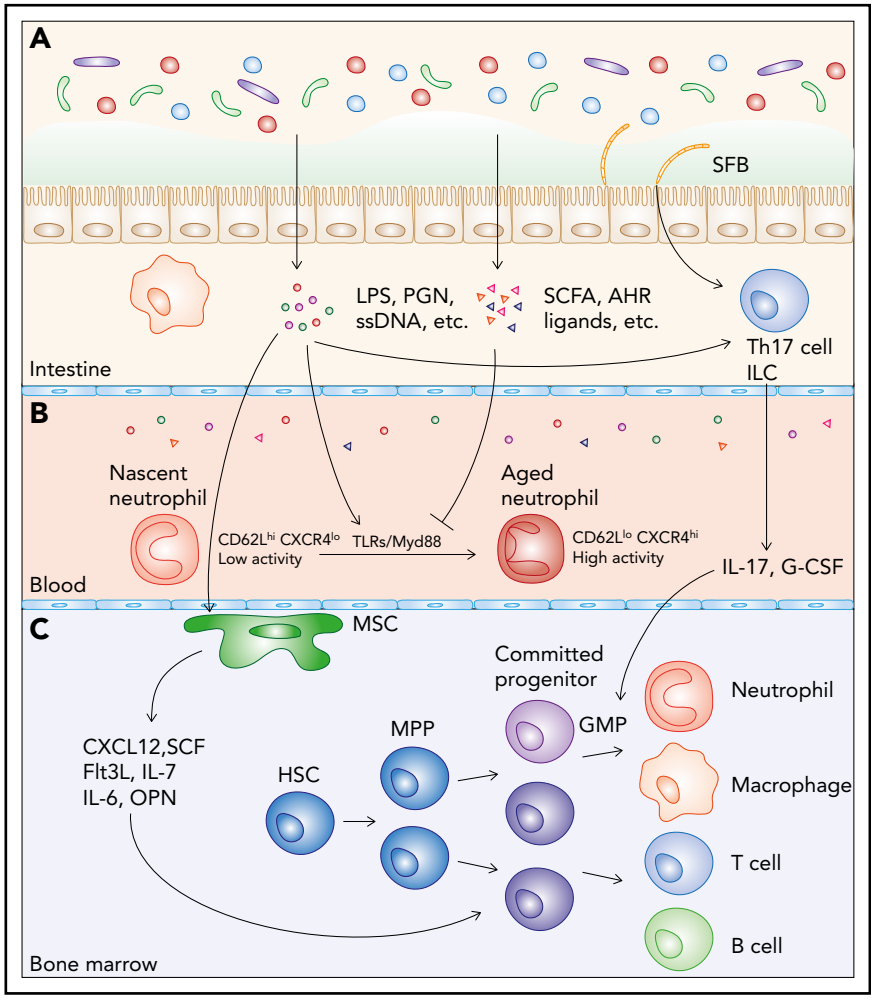

Figure 1. Regulation of neutrophils by the microbiota. (A) All vertebrates host a large and diverse bacterial community in their intestine. The microbiota produces a variety of small molecules that can communicate with the host, including microbial components and metabolites. Microbial components, as well as direct contact between specific species such as SFB and the epithelium, induces IL-17 secretion from Th17 cells and ILCs, which induces the synthesis of G-CSF, a master regulator of neutrophil production. (B) The microbial products and metabolites can diffuse into the circulation, directly regulating the function of neutrophils in blood. Nascent neutrophils released from the BM exhibit limited proinflammatory activity. They acquire enhanced capacities of migration, integrin activation, ROS production, and NET formation as they age and sense microbiota-derived signals in the circulation. This process requires the presence of microbiota, and is dependent on TLR/Myd88 pathways. Conversely, microbiota-derived metabolites including SCFAs exhibit anti-inflammatory properties. (C) Microbial products from the microbiota can also diffuse into the BM, and be sensed by MSCs, which produce cytokines that support lineage differentiation from HSCs. In addition, the phagocytic capacities of BM neutrophils are also regulated by microbiota-derived NOD1 ligands. GMP, granulocyte-monocyte progenitors; HSC, hematopoietic stem cell; ILC, innate lymphoid cell; LPS, lipopolysaccharide; PGN, peptidoglycan; MPP, multipotent progenitor; NET, neutrophil extracellular trap; ssDNA, single-stranded DNA.

a double-edged sword that improves defense against invading pathogens, but also amplifies neutrophil-mediated organ damage in inflammatory diseases.

Microbial products do not always produce proinflammatory effects. For example, formyl peptides are well known to activate neutrophils,[15] but can also signal through distinct formyl peptide receptors based on their length, structure, and concentration, leading to diverse responses in different cell types.[44,45] In a *Staphylococcus aureus* infection model, bacterial *N*-formylated peptides activate sensory neurons via formyl peptide receptor 1 (FPR1), resulting in inflammatory pain and the release of nociceptor neuropeptide, calcitonin gene-related peptide, which significantly inhibits neutrophil recruitment and activation.[46,47] These microbial products thus create an anti-inflammatory environment that favors bacterial propagation in this specific model. The microbiota also produces formyl peptides that contribute to the maintenance of epithelial homeostasis.[48,49] Whether these peptides promote or suppress neutrophil activity remains unclear.

Metabolites from the microbiota modulate neutrophil functions

The microbiota produces a diverse repertoire of metabolites from the fermentation of dietary compounds, or conversion of endogenous compounds secreted by the host. These metabolites mainly include short-chain fatty acids (SCFAs), secondary bile acids, tryptophan metabolites, and amines, which have broad influence on host physiology and diseases.[8,10,12,50,51] In this section, we will focus on the roles of these metabolites in the regulation of neutrophil functions.

SCFAs are the most studied metabolites that can affect neutrophil recruitment and activation. These molecules are produced in the colon by bacterial fermentation of polysaccharides, and are rapidly absorbed with only 5% being secreted in the feces.[52] High concentrations of SCFAs, mainly butyrate, propionate, and acetate, are detected in the colon (80-131 mM) and in peripheral blood (79-375 μM),[53] and these levels are dramatically diminished in germ-free animals.[54] SCFAs can serve as an important energy source for intestinal epithelial cells,[55] and promote antibody responses by fueling B-cell metabolism.[56] However, there is currently no evidence supporting a similar role for SCFAs in neutrophils, which have long been known to exclusively rely on glycolysis.[57,58] SCFAs can also regulate the immune system by acting as inhibitors of histone deacetylases (HDACs),[59,60] or ligands for G protein–coupled receptors, including mainly GPR43, GPR41, and GPR109A.[51] For neutrophils, SCFA-mediated HDAC inhibition inactivates NF-κB signaling, resulting in reduced expression of proinflammatory cytokines and recruitment to inflamed tissues on challenge.[61-64] In

addition, SCFAs can induce apoptosis of neutrophils via HDAC inhibition, promoting the resolution of inflammation.[65]

The effects of SCFAs on neutrophils through G protein–coupled receptors are more complex and contextual. Neutrophils express GRP43 and GPR109A, but not GPR41.[51] Signaling through GPR43 has been shown to trigger calcium flux in neutrophils, induce chemotaxis toward SCFAs at high concentrations, and promote ROS production and phagocytosis.[66-70] However, neutrophils from *Gpr43*$^{-/-}$ mice exhibit enhanced ROS production and chemotaxis on microbial stimulation in vitro and show increased recruitment and activation in inflamed tissues in the models of colitis, inflammatory arthritis, allergic airway disease, and infection,[66,71] suggesting a suppressive role of GPR43 signaling on neutrophils. In addition, SCFA treatments have also been reported to reduce ROS production and proinflammatory cytokine secretion from neutrophils.[67,72] These contradictory observations lead to an SCFA paradox; it remains unclear how SCFA produces opposite effects on neutrophils via the same receptor. One possible explanation is that SCFAs at low concentrations (plasma levels) suppress neutrophil recruitment and activation via GPR43 signaling and HDAC inhibition to prevent immune response against commensals, but that at high concentrations (colon levels), they promote neutrophil elimination of the pathogens. Further studies are needed to reach a definitive explanation.

Emerging evidence suggests that several other metabolite species are involved in neutrophil regulation. First, the microbiota plays an essential role in regulating bile acid homeostasis by converting primary bile acids into secondary bile acids, and by modulating the conjugation of bile acids.[10] Although evidence supporting a direct effect on neutrophils is lacking, bile acids have been shown to mediate anti-inflammatory effects on monocytes, macrophages, natural killer T (NKT) cells, and endothelial and epithelial cells, which creates an environment that suppresses neutrophil recruitment and activation.[73-77] For example, bile acids, particularly secondary bile acid lithocholic acid, can suppress NLRP3 inflammasome-mediated IL-1β secretion, leading to reduced neutrophil recruitment during inflammation.[75] In addition, mice deficient in Farnesoid X receptor, the receptor that senses bile acids, exhibit enhanced osteopontin production from NKT cells in the liver, leading to increased neutrophil-mediated liver injury in a model of autoimmune hepatitis.[73,74] The microbiota can also metabolize dietary tryptophan into indole derivatives, such as indole-3-acetate, indole-3-aldehyde, and indole-propionic acid, which act as ligands for aryl-hydrocarbon receptor (AHR) in host tissues.[50] Microbiota-derived AHR ligands promote the immune-suppressive functions of Tregs during inflammation, which indirectly affects neutrophil recruitment and activation.[78,79] Although host-derived AHR ligands have been suggested to act on neutrophils to suppress chemotaxis during inflammation,[80,81] whether microbiota-derived AHR ligands directly communicate with neutrophils remains unknown.

Further, the microbiota can convert dietary amino acids into bioactive amines, such as histamine, which is well known to modulate inflammation.[82] Host cells can also produce amines, making it difficult to specify the contribution from microbiota-derived sources. Recently, histamine-secreting microbes have been identified in the intestine,[83,84] and histamine from the microbiota has been shown to suppress the production of tumor necrosis factor-α (TNF-α) in human monocytic cells,[84] and regulate AMP production via the NLRP6 inflammasome in the mucosal system.[85] In addition, trimethylamine, an amino acid metabolite exclusively derived from the microbiota, and its derivative trimethylamine-*N*-oxide, have been shown to promote thrombosis and atherosclerosis,[86,87] although its effects on neutrophils have not yet been explored. Last, there is increasing appreciation of the complex interactions among the microbiota, nervous system, and immunity.[88,89] Although direct evidence linking the "gut-brain axis" with neutrophil biology is still lacking, it will not be surprising if such connections exist, given that neutrophils are under the control of neural signals in both steady state and inflammatory conditions.[90]

Neutrophil response toward the microbiota

The immune system maintains a delicate balance between tolerance toward the microbiota and immune reactions against pathogens. Under steady state, commensal-derived signals induce a regulatory network that suppresses immune activation and leukocyte recruitment,[8] thus preventing immune responses against the commensals. When the host does not recognize an existing microorganism, both the innate and adaptive branches of the immune system are rapidly activated to eliminate the potential liabilities. Neutrophils contribute to the regulation of microbiota as a major effector cell type that eliminates unwanted species (Figure 2).

Neutrophils contribute to microbiota containment

Given that activation of neutrophils may cause damage to the commensals and healthy host tissues, neutrophil recruitment to the mucosal epithelium is tightly controlled by the local immune system and the microbiota. In the intestine, mononuclear phagocytes, including macrophages and dendritic cells, produce high amounts of Pro-IL1β, an inactive precursor of the proinflammatory cytokine IL-1β, but are not responsive to microbial products from commensals. The presence of pathogenic species, such as *Salmonella* or *Pseudomonas*, can trigger the conversion of these precursor proteins into active cytokines through the NLRC4 inflammasome, leading to upregulation of endothelial adhesion molecules followed by robust neutrophil recruitment.[91] Recruited neutrophils can migrate into the intestinal lumen to generate an organized structure that prevents direct contact between commensals and the epithelium via FPR1. Neutrophils in these "luminal casts" produce high amounts of ROS, constraining microbial translocation and overgrowth during infection.[92]

The recruitment of neutrophils to the mucosal epithelium depends on neutrophil chemotaxis receptor CXCR2, as suggested by reduced recruitment of neutrophils to peripheral tissues, and impaired immune defense against intestinal infections in *Cxcr2*$^{-/-}$ mice.[26,93-95] Interestingly, deficiency in CXCR2 alone leads to significant shifts of the microbiota composition.[26,96] In addition, IL-17, a cytokine enriched in the mucosal system, has been shown to promote neutrophil recruitment to the epithelium.[94,97] Neutrophils recruited via IL-17/CXCR2 can produce IL-22,[94,98] a cytokine known to contribute to the containment of the microbiota by inducing AMP and

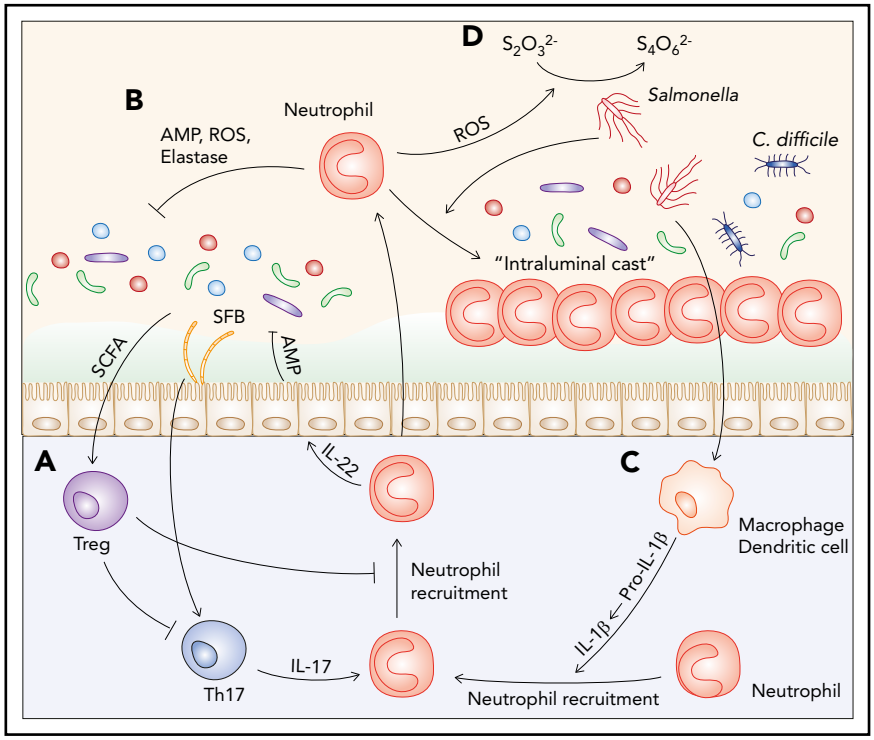

Figure 2. Neutrophil response toward the microbiota. (A) Under steady state, the microbiota induces a regulatory network that suppresses neutrophil recruitment to prevent inflammatory responses toward the epithelium and commensals. (B) SFB and other commensals can induce Th17 cells, which secrete IL-17 to recruit neutrophils to the intestinal epithelium, resulting in a negative feedback control of the microbiota by neutrophils. Neutrophils also produce IL-22, which can elicit AMP secretion from the epithelium and IgA production from intestinal B cells. (C) Macrophages and dendritic cells in the mucosal system constitutively produce large amounts of pro-IL1β, the inactive form of the pleiotropic cytokine IL-1β. Signals from pathogenic microorganisms trigger the conversion through the NLRC4 inflammasome pathway, which induces robust neutrophil recruitment. Recruited neutrophils can migrate into the lumen of intestine to form an organized intraluminal structure that prevents translocation and expansion of both commensal and pathogenic species. (D) Certain pathogenic species, such as *Salmonella*, can take advantage of neutrophil-mediated defense mechanisms to acquire growth advantages by using $S_4O_6^{2-}$ as an electron acceptor. $S_2O_3^{2-}$, thiosulfate; $S_4O_6^{2-}$, tetrathionate; Th, T helper.

IgA production.[5,8] The induction of IL-22 in neutrophils is most likely mediated by local stimulation by IL-23 via the mTOR pathway.[99] Consequently, IL-17–induced neutrophil recruitment significantly limits the expansion of SFB,[94] a major Th17–inducing species, suggesting neutrophils acting as a key component of the feedback mechanisms that precisely control commensal species.

Neutrophil-derived AMPs fine tunes the microbiota

That the host can select for commensal species suggests that the immune system can recognize its microbial allies. This recognition is at least partially dependent on adaptive immune responses because mice carrying distinct major histocompatibility complex alleles exhibit individualized microbial communities in their intestine.[100] AMPs also play an important role in this process. In the intestine, the microbiota is compartmentalized by layers of mucus mixed with IgA and AMPs, leading to constitutive exposure of commensal species to these molecules. Similar to the effect of long-term antibiotic treatment, the commensal species develop strategies, such as modification of their membrane structures, to acquire resistance to high levels of AMPs, establishing a mechanism that distinguishes commensals from pathogens and maintaining stability and resilience of a healthy microbiota.[101] AMPs include 2 major groups, defensins and cathelicidins, both of which are small molecules that suppress the growth of microorganisms by membrane permeabilization.[13] Neutrophils store large quantities of AMPs in their granules and rapidly release them on pathogen encounter. In mouse models that are deficient in the mouse ortholog of cathelicidin (LL-37), cathelin-related antimicrobial peptide (CRAMP), the deficiency leads to significant dysbiosis and increased susceptibility to dextran sulfate sodium–elicited colitis, suggesting loss of microbiota protection of the mucosal epithelium.[102] Notably, when CRAMP is deleted from neutrophils and macrophages using *LysM-Cre*, or from intestinal epithelial cells using *Villin-Cre*, *LysM-Cre/Cramp*$^{f/f}$ mice exhibit the most significant defects in their microbiota.[102] In addition, neutrophil elastase, another major neutrophil granular protein that can convert inactive cathelicidins into functional LL-37, has been suggested to mediate shifts in microbiota composition during infection.[103] These findings thus support the idea that neutrophils play a fundamental role in the regulation of microbiota composition by secreting AMPs.

Pathogen strategies to hijack neutrophils for their growth advantage

In the turf war between neutrophils and pathogens, certain pathogen species have evolved strategies to use neutrophil-mediated inflammation to gain growth advantage over the commensals. One example is *Salmonella*, a diarrheal pathogen that can cause acute gut inflammation by its virulence factors. *Salmonella*-induced inflammation results in robust neutrophil recruitment into the intestinal lumen, where they produce ROS that can react with an endogenous luminal sulfur compound, thiosulfate, to form tetrathionate.[104,105] The normal microbiota cannot use tetrathionate, or ethanolamine, a complex carbon compound abundant in the intestine. However, *Salmonella* has the *ttrSR ttrBCA* gene cluster that allows it to use tetrathionate as a respiratory electron acceptor and thus consumes ethanolamine as a nutrient resource. Consequently, *Salmonella* sidesteps nutritional competition and gains growth advantage over the commensals, leading to its overgrowth during intestinal inflammation.[104,105] Similarly, pathogenic *E coli* can also benefit from intestinal inflammation. During inflammation, recruited leukocytes and the epithelium upregulate inducible nitric oxide synthetase, leading to the production of nitrate.[106] The pathogenic *E. coli* can use nitrate as an electron acceptor to generate energy, whereas the majority of gut microbiota species cannot, leading to a growth advantage for these pathogens.[106]

Interplay between neutrophils and the microbiota in chronic diseases

Neutrophils provide essential immune protection against pathogens, but also promote chronic inflammatory diseases and cancer by producing organ damage or suppressing adaptive immune reactions.[13,15] The microbiota has also been shown to profoundly influences the pathophysiology of inflammatory diseases and cancer.[6-8]

IBDs and airway disorders

Inflammatory diseases at the barrier sites, such as the intestinal and pulmonary epithelium, arise from a complex confluence of genetic factors, the host immune system, and the microbiota. Inflammatory bowel diseases (IBDs), such as Crohn disease and ulcerative colitis, are often associated with variants of genes involved in barrier maintenance, such as *NOD2*, *IL23R*, *CARD9*, and *IL18RAP*.[107] Following a triggering factor (eg, infection), genetic predispositions can lead to a compromised intestinal immune system, which may break down the regulatory network that prevents immune activation in the epithelium and may induce dysbiosis. Disrupted microbial communities, in turn, promote robust neutrophil recruitment and activation, leading to chronic inflammation and tissue injury.[108] Consistent with this theory, *T-bet*$^{-/-}$*Rag2*$^{-/-}$ mice (TRUC mice) exhibit severe defects in both adaptive and innate immunity, resulting in a spontaneous transferrable form of ulcerative colitis.[109] The deficiencies also transform the microbiota to become colitogenic, which promotes TNF-α secretion from intestinal dendritic cells, leading to severe infiltration of neutrophils, epithelial injury, and colorectal cancer.[109,110] The development of colitis in these animals largely depends on the microbiota, as demonstrated by transmission of the disease between TRUC mothers and wild-type offspring, and between TRUC and wild-type animals upon cohousing.[109] Further, several "proinflammatory" pathogen and microbiota species have been pinpointed to induce the disease in IBD-susceptible mice, including adherent-invasive *E coli*, *Clostridium difficile*, and commensal *Bacteroides*.[111-113] Conversely, IBDs are also associated with reductions in the commensal species that mediate immune regulations. For example, patients with IBDs exhibit depletion of Firmicutes species,[114] which are known to produce SCFAs and induce Tregs to suppress immune activation.[115]

Similar with IBDs, the interplay between neutrophils and the microbiota is also implicated in airway inflammatory disorders. In cystic fibrosis, inactivating mutation in the cystic fibrosis transmembrane conductance regulator gene leads to accumulation of mucus on the surface of pulmonary epithelium, resulting in outgrowth of several commensal species, with *Pseudomonas* and *Staphylococcus* most relevant for disease progression.[116] The expansion of microbes drives robust neutrophil infiltration and activation, which have been shown to be the best markers for disease severity in cystic fibrosis.[117] Neutrophils, in turn, contribute to persistent expansion of lung microbes by suppressing T-cell recruitment and activation[118] and promoting anaerobic respiration in these microbes.[119] Close interactions between neutrophils and the lung microbiome have also been reported for other airway disorders such as chronic obstructive pulmonary disease and asthma.[120,121]

Autoimmune and vascular diseases

As a major cell type recruited during the effector phase, neutrophils play important roles in autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis, by promoting inflammation, producing tissue damage, and facilitating autoantibody production and disposition.[122-125] In vascular diseases, heterotypic interactions between neutrophils and red blood cells or platelets promote vaso-occlusion in sickle cell disease,[126] and produce vascular damage in transfusion-related acute lung injury.[126,127] These neutrophil-mediated inflammatory events occur far from the mucosal epithelium and thus do not involve direct microbe-neutrophil interactions. However, the microbiota can influence disease progression by regulating neutrophil production and activation via systemically diffused microbial products. For example, the germ-free condition has been found to strongly alleviate arthritis in *K/BxN* and *IL1rn*$^{-/-}$ mouse models, which results from a reduction of Th17 cells in the small intestinal lamina propria.[128,129] Th17 cells produce IL-17, which can directly act on B cells to induce autoantibodies[128] and promote neutrophil production by inducing G-CSF.[21,26] Interestingly, expansion of *Prevotella*, a specific intestinal commensal species, is reported to correlate with enhanced susceptibility to arthritis, and reduction of *Prevotella* by dietary modulation leads to reduced pro-IL-1β secretion in distal neutrophils.[130,131] Similarly, mice maintained in germ-free conditions are fully protected from multiple sclerosis,[132] although the specific effects on neutrophils remain unclear. Further, the heterotypic interactions between neutrophils and other blood cell types are mediated by activated $\alpha_M\beta_2$ integrin,[126] a function enhanced by the time that neutrophils spend in the circulation. Neutrophil aging is driven by the microbiota through TLR/Myd88 pathways; microbiota depletion protects mice from vaso-occlusion and chronic organ damage in sickle cell disease.[19] In addition, depletion of commensal bacteria also improves neutrophil-mediated thrombosis in endotoxin-induced sepsis and prevents transfusion-related acute lung injury by reducing neutrophil recruitment to the lung.[19,133]

Cancer

Neutrophils are abundant in malignant lesions and play an important role in modulating tumor progression. Tumor-associated neutrophils can promote tumorigenesis by producing angiogenic factors, enhancing metastasis, and suppressing antitumor immune responses.[13] Under certain circumstances, such as invariant NKT activation or TGF-β inhibition, tumor-associated neutrophils can also switch to antitumor phenotypes[134,135] to suppress tumor growth. The interactions between neutrophils and the microbiota have been shown to affect tumor progression. For example, in a mouse model of serrated polyps (SPs), a premalignant lesion of the colon, the expression of the endothelial growth factor receptor ligand throughout the intestine promotes the development of SPs only in the cecum. The development of SPs requires a specific microbial niche in the cecal mucosa, and is associated with barrier dysfunction, bacteria invasion, neutrophil infiltration, and inflammatory cytokine secretion. Antibiotic treatment or neutrophil depletion both abrogates SP development, suggesting an important role for neutrophils and the microbiota in this disease.[136] In addition, specific intestinal commensal or pathogen species, such as *Lactobacillus johnsonii* or *Helicobacter hepaticus*, can modulate

the progression of various cancer types by regulating systemic inflammatory tone.[137-140] Similarly, the lung microbiota can also promote pulmonary tumor progression by inducing IL-17 secretion from the epithelium, which enhances neutrophil recruitment by cancer cells.[141] Importantly, the microbiota also modulates host response to cancer therapy by regulating drug metabolism and the innate and adaptive responses following treatment.[142] An efficient cancer therapy requires robust activation and ROS production from neutrophils, which is significantly impaired in microbiota-depleted or TLR/Myd88-deficient mice.[143]

Concluding remarks

Neutrophils are involved in close interactions with the microbiota in both normal and pathological conditions; however, the mechanisms mediating the communications between neutrophils and the microbiota remain incompletely understood. For example, it is not clear whether other components of the microbiota, including commensal protozoa, viruses, and fungi, play an important role in neutrophil regulation. In addition, only a small subset of metabolites and nutrients from the microbiota has been studied with regards to their regulation of neutrophil functions. Different metabolites and microbial components have been shown to produce distinct effects on neutrophils by either enhancing or suppressing their functions. How these microbiota-derived signals are orchestrated remains unclear. In chronic diseases, the roles of microbiota and neutrophils are highly contextual and can both improve or worsen disease activity. Dissection of their interactions in different disease conditions is still in the early stage. Although much remains to be learned about the interplay between neutrophils and the microbiota, these studies hold enormous potential for clinical application. Understanding of the molecular mechanisms mediating the cross talk and identification of specific "proinflammatory" or "anti-inflammatory" commensal species may allow the development of novel therapeutic approaches for the treatment of chronic inflammatory diseases and cancers.

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