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Microbiome Dependent Regulation of T_{regs} and Th17 Cells in Mucosa

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Mammals co-exist with resident microbial ecosystem that is composed of an incredible number and diversity of bacteria, viruses and fungi. Owing to direct contact between resident microbes and mucosal surfaces, both parties are in continuous and complex interactions resulting in important functional consequences. These interactions govern immune homeostasis, host response to infection, vaccination and cancer, as well as predisposition to metabolic, inflammatory and neurological disorders. Here, we discuss recent studies on direct and indirect effects of resident microbiota on regulatory T cells (T_{regs}) and Th17 cells at the cellular and molecular level. We review mechanisms by which commensal microbes influence mucosa in the context of bioactive molecules derived from resident bacteria, immune senescence, chronic inflammation and cancer. Lastly, we discuss potential therapeutic applications of microbiota alterations and microbial derivatives, for improving resilience of mucosal immunity and combating immunopathology.

Keywords: microbiome, mucosa, T_{reg}, mucosal immunity, inflammation, Th17, antibiotics, resident microbes

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INTRODUCTION

Mammals harbor a highly diverse microbiome of at least 1000 species, and an astounding number of 10–100 trillion microbial cells, co-existing in a remarkable balance with the host immune system. Healthy human microbiome is mostly bacteria although other microbial domains such as archaea, viruses, and eukaryotes (principally fungi and protists) are also present (1). While these microbes are distributed in skin, and mucosa of ocular, nasal, oral, eye, and reproductive organs, gastrointestinal (GI) tract mucosa is the major reservoir of resident microbes in terms of abundance and species diversity (2, 3). The human colon harbors approximately 3.8×10^{13} microorganisms, followed by skin in the range of $\sim 10^{11}$ (4). Since the resurgence of microbiome research in recent years, there has been a sharp increase in understanding of how resident microbiome shapes immunity, health and disease of humans. Only a perennial holiday on a lonely island could excuse an immunologist's incognizance on intimate interrelationships between intestinal microbiota and immune balance. Direct crosstalk between resident microbes and host immune cells in mucosa emerges as a pivotal determinant of such an immune balance. Dysbiosis of resident microbes has strong association with a number of immunological disorders, including opportunistic and pathogenic infections (5–13).

Mucosal immune system has not only evolved to protect the mucosal barrier surface against external insults, it has also co-evolved with resident microbes in an interdependent harmonious relationship with them (14–21). The resulting immune balance is crucial to drive optimal immune responses without causing an over-exuberant inflammation (22–25). Past few decades have seen that an increase in hyper-hygiene mentality, mindless use of antibiotics and diet changes, have led to reduced diversity and impaired resilience in resident microbiota (26). Consequently, a disruption in aforementioned immune balance leads to rise in autoimmune and inflammatory disorders. Therefore, understanding the mechanisms of these mutualistic relationships between resident microbiota and different components of innate and adaptive immunity is vital to our understanding of immune diseases. Although gut microbiota in laboratory mice and humans differ significantly, murine models have provided a powerful tool to explore host-microbiota-pathogen interactions in mucosa (27, 28). Here we review the effects of resident microbiota on T_{regs} and Th17 cells, important players in determining immune balance, mucosal barrier integrity and host protective functions in mucosa. These cells mucosa can develop in mucosa independent of commensal microbiota. For example, there is evidence in germ free mice that T_{reg} cells can be induced by dietary antigens from solid food (29). These T_{reg} cells are of limited life span, but are distinguishable from microbiota-induced T_{reg} cells and capable of repressing inadvertent immune responses to ingested protein antigens. Similarly, in oral mucosa, mechanical damage from mastication of food induces barrier protective Th17 cells, independent of oral commensal microbiota under homeostatic conditions (30). However, dysbiosis can lead excessive Th17 cells and lead to periodontal inflammation (31). Thus, while it is known that these cells can develop independent of microbiota, resident bacterial dysbiosis is strongly associated with alterations in these cells, causing mucosal inflammation seen in many diseases including HIV immunopathogenesis (32–41). Although other cells also play important roles in mucosal tolerance and immunity, we will not review them here.

T_{REGS} AND TH17 CELLS IN MUCOSA UNDER STEADY STATE-CONDITIONS

Majority of the studies on mucosa-microbiota interactions discuss GI tract. Indeed, GI mucosa harbors by far the largest and most diverse microbiota, as well as abundant and dynamic population of T_{regs} and Th17 cells. T_{regs} are defined by the expression of CD25 and Foxp3, and are predominantly known for their immunosuppressive properties. These cells also express other molecules such as Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), PD-1, interleukin 10 (IL-10), transforming growth factor beta 1(TGF-β1), and amphiregulin. Each of the aforementioned proteins has been shown to be either important, or dispensable for different mechanisms of T_{reg}-mediated immunosuppression. Divergent conclusions derived from various T_{reg} mechanism investigations have been strikingly similar to those in the popular parable of the "Blind men and an elephant." It is now increasingly clear that suppressive and non-suppressive functions of $Foxp3^+$ cells are largely variable, depending on local tissues, disease phenotypes, responding effector cells, and cytokine milieu (42–49).

While $CD4^+$ effector T cell responses contribute to overt intestinal inflammation, T_{regs} are associated with controlling immunopathology (42, 43, 50). It is well known that T_{regs} are also pivotal for commensal tolerance (51–53). There have been contentions regarding the T_{regs} found in colon mucosa (colon T_{regs}; cT_{regs}); whether they develop in thymus (thymic $T_{regs;}$ tT_{regs}), or periphery (peripheral T_{regs}; pT_{regs}). The usage of Nrp-1 and Helios as markers of tT_{regs}, and the extent to which the TCR repertoire of cT_{reg} overlaps with that of tT_{regs} have been debated (54, 55). Nevertheless, it is well established that cT_{regs} require the presence of microbiota for their development, sustenance and function (56–58). There is also evidence that mucosal sites are the primary sites of development and maintenance of pT_{regs} (59–61). First formal proof for the requirement of microbiota for the induction and maintenance of intestinal T_{regs} was provided by studies using germ-free (GF) animal models. GF mice show a several-fold reduction in the frequency of $Helios^-$ T_{regs}, when compared with conventionally housed specific pathogen free (SPF) mice. Association of individual bacterial isolates or defined consortia in GF mice is sufficient to induce intestinal T_{regs} (56, 57). Even antibiotic treated mice, which show depletion in resident microbiota correlating with a drastic reduction in the frequency of T_{regs}, lend further credence to the positive role of microbiota in sustenance of T_{regs} (53, 55, 62). In addition to commensal tolerance, mucosal T_{regs} have been shown to regulate excessive immune responses during infections (43, 63–65). Recently, they are also shown to accumulate in other tissues and provide functions such as non-suppressive tissue repair functions in muscle (66). While T_{regs} play diverse and often opposite roles in mucosal infections (**Table 1**), effects of microbiome on T_{regs} during these infections are largely ignored in many studies.

Th17 cells are $ROR\gamma t^+$, $CCR6^+$, $IL\text{-}17A^+$, $IL\text{-}17F^+$, with some cells expressing IL-21 and IL-22, and have been implicated both in mucosal barrier functions. Th17 cells are an important subset of effector T cells that are protective during extracellular bacterial and fungal invasion (83, 88–91). However, excessive Th17 responses are also associated with a variety of pathogenic conditions, depending on the pro-inflammatory cytokines they co-produce (30, 91–95). Littman and colleagues showed for the first time that commensal microbiota play important roles in the development of intestinal Th17 cells (22, 53, 96–100). Th17 development and differentiation is controlled by cytokine and epigenetic regulation (91, 92, 101, 102), but the mechanistic details of microbiome dependent control of Th17 development during mucosal infection is largely unclear.

IMPACT OF MICROBIOME ON T_{REGS} AND TH17 CELLS DURING GI INFECTION AND INFLAMMATION

"Healthy" GI microbiota is mainly composed of the phyla Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria,

TABLE 1 | $Foxp3^+T_{reg}$ functions in mucosal infections.

Pathogen	T_{reg} manipulation	Outcome
BACTERIA		
Listeria monocytogenes	T_{regs} cause increased pathogen burden (67)	Detrimental
Salmonella enterica	Foxp3+ cell ablation accelerates bacterial clearance (68)	Detrimental
Aggregatibacter actinomycetemcomitans	T_{regs} attenuate experimental periodontitis progression (69)	Protective
Yersinia Enterocolitica	T_{regs} reduce pathogenic burden and attenuate inflammation (70)	Protective
VIRUSES		
HIV	Early interference with the T_{reg}'s suppressive function worsened infection and inflammation (71, 72)	Protective
	T_{regs} are preserved in elite controllers in humans (73)	Protective
	T_{regs} suppress anti-viral CD8 responses (74)	Detrimental
	Foxp3+ cell ablation accelerates mortality and increases viral load (197)	Protective
Herpes simplex virus 2	Foxp3+ cell ablation increases mortality (75)	Protective
West Nile virus		
PARASITES		
Toxoplasma gondii	Loss of Foxp3+ T_{reg} cells results in fatal pathology (76)	Protective
Toxoplasma gondii	Loss of Foxp3+ T_{reg} cells results in pathology (77)	Protective
Toxoplasma gondii	Loss of Foxp3+ T_{reg} cells results in pathology (78)	Protective
Heligmosomoides polygyrus	No changes in pathogen burden with T_{reg} ablation (79)	No effect
Leishmania major	T_{regs} promote increased pathogen burden (80).	Detrimental
Schistosoma mansoni	$CD4^+CD25^+$ depletion increases inflammation (81)	Protective
FUNGUS		
Candida albicans	CD4+CD25+T_{regs} regulate immunopathology in Th1 mediated gastrointestinal/disseminated Candidiasis (82)	Protective
	CD4+CD25+Foxp3+T_{regs} promote Th17 antifungal immunity and dampen immunopathology (41, 83)	Protective
	T_{regs} regulate immunopathology (84)	
	T_{regs} suppress pulmonary hyperinflammation (85)	
Aspergillus fumigatus		Protective
Pneumocystis carinii		Protective
MYCOBACTERIA		
Mycobacterium tuberculosis	Selective depletion of T_{regs} reduces pathogen burden (86).	Detrimental
	Foxp3+ cells induce resistance to TB lesions (87)	Protective

Proteobacteria, and Verrucomicrobia. Small intestine is dominated by Enterobacteriaceae and Lactobacillaceae, whereas colon contains the members of Bacteroidaceae, Lachnospiraceae, Prevotellaceae, Rikenellaceae, and Ruminococcaceae respectively (3). A number of factors including diverse environmental conditions, intake of diet and medication, as well as host genetic factors determine the dynamic composition of gut microbiota in individuals (103–107). Gut microbiota are capable of restraining the mucosal colonization by enteric pathogens, a process defined as colonization resistance (108). Thus, administration of antibiotics, and altering the resident microbiota during a mucosal infection is known to lead to post-antibiotic expansion of the pathogens. Loss of overall diversity, or even deficit in single group of bacteria can alter the susceptibility to gastrointestinal infections. For example, Clostridium difficile (C. difficile) infection, the most common cause of nosocomial diarrhea is often preceded by antibiotic usage. Colonization of C. difficile in healthy mice in fact requires a pre-exposure to a cocktail of antibiotics to alter the microbiota composition (109). However, mono-colonization of GF mice with a murine isolate from the family Lachnospiraceae could limit the colonization of C. difficile, suggesting that individual bacterial species are sufficient to confer colonization resistance to C. difficile (110). Enhanced susceptibility toward other infections after antibiotic-mediated disruption of the intestinal microbiota composition has also been reported for vancomycin-resistant Enterococcus Spp and Salmonella enterica serovar typhimurium (*S. typhimurium*) (108, 111). Mechanistically, mucosal carbohydrates such as fucose and sialic acid liberated by resident microbiota have been shown to control the growth of enteric pathogens. Antibiotics cause spikes in sugars that can worsen *S. typhimurium* and C. difficile infections (112). Microbiota alterations reduce the numbers of germinal centers in IL21-receptor knockout mice, resulting in diminished IgA^+ B cells and reduced activation-induced cytidine deaminase in Peyer's patches. These events lead to the expansion of T_{regs} and Th17 cells, and higher bacterial burdens, but dampening of Citrobacter rodentium-induced immunopathology (113). Resident microbiota at mucosal

interfaces can govern transmission and progress of parasitic protozoan infections such as Toxoplasmosis and Amoebiasis (114). In the case of Toxoplama gondii infection in mice, reduction of microbiota in the gut by prolonged antibiotic treatment leads to impaired Toll like receptor (TLR)-11 and Myeloid differentiation response 88 (MyD88) signaling and subsequent deficit in Th1 immunity, substantiating that gut commensals serve as natural molecular adjuvants during T. gondii infection (115). In a mouse model of Giardia duodenalis infection, antibiotic induced alteration of the microbiome prevents CD8 T cell activation by G. duodenalis. Conversely, GI infection can also modulate microbiota specific adaptive immunity (116). For example, a pathogenic GI infection, in parallel to specific immune reactions against the pathogen, induces immune responses to commensals and generates long-lived commensal-specific T cells. Thus an adaptive response against commensals is an integral component of mucosal immunity. However, such a commensal specific-adaptive response in a dysbiosis setting can also contribute to excessive inadvertent inflammation. In the context of HIV-1 infection, damages in GI tract and gut microbial translocation (Proteobacterial species) are associated with reduction of systemic and gut/rectal mucosal Th17 cells and T_{regs} (despite increased T_{reg}/Th17 ratio) (36, 71, 72, 117, 118). A large body of evidence suggests that increased T_{regs} in circulation correlate to reduced immune activation in HIV+ patients, underlining the anti-inflammatory protective roles of T_{regs} in patients (71–73, 118–125). While combined anti-retroviral (cART) therapy in HIV^+ patients generally ensures immune reconstitution in the peripheral blood, dysbiosis and T_{reg}/Th17 abnormalities persist in gut and other mucosae (41, 126–132). This can present residual inflammation and heightened morbidities in cART treated HIV^+ patients. However, in cART-treated HIV^+ patients with elevated levels of immune activation, it is not clear whether altered levels and function of mucosal T_{regs}/Th17 cells are associated with local microbial dysbiosis (131), and if these alterations contribute to residual inflammation in HIV disease. Collectively, these findings highlight the role of microbiota in restraining pathogens and inflammation by having significant impact on T_{regs} and Th17 cells.

Alterations in resident microbiota and host immune cells, caused by host genetic makeup also play a role in the pathogenesis of inflammatory bowel diseases (IBD). One of the adaptive arms of immunity that is impacted by such changes is T_{regs} (133). *Bacteroides fragilis* for example, has been found to invade mucosa and cause excessive activation of the host intestinal immune response in genetically susceptible patients (134), while under steady-state conditions the same bacterium can enhance T_{reg} differentiation and ensure intestinal homeostasis. Loss of autophagy protein ATG16L1 in T_{regs} results in aberrant type 2 responses and spontaneous intestinal inflammation (135). It is unclear whether microbiota directly induce the expression of ATG16L1 in T_{regs}, but it is evident that ATG16L1 and autophagic process directly promote T_{reg} survival and metabolic adaptation in the intestine. Similarly, other genetic risk variants associated with IBD such as: *NOD2, CARD9, ATG16L1, IRGM and FUT2* significantly influence the gut microbiota changes (136). For example, a decrease in *Roseburia* spp (known acetate to butyrate converters), *Clostridiaceae* family, the genera *Bifidobacterium*, *Ruminococcus* and *Faecalibacterium* has been observed in patients with IBD. Although many of these communities are strongly implicated in T_{reg} maintenance, direct mechanisms of T_{reg} regulation in the context of these genetic variants and IBD are unclear. Combined deficiency of MyD88 and JH gene, which disrupts innate interactions of immune cells with intestinal microbiota and IgA responses respectively, causes overt inflammation, highlighting the requirement of T_{reg}-IgA mediated mechanism in tolerance (51, 137). It has also been shown that microbiota-specific $Foxp3^+$ T_{reg} cells can convert to interferon-γ-producing $Foxp3^+$ T cells that have a potential to establish mucosal tolerance (138). Disruption of TLR/MyD88 signaling in Foxp3-deficient mice protect them from excessive inflammation at the environmental interfaces of skin, lungs, and intestine, showing that T_{regs} normally also restrain commensal dependent tonic MyD88-dependent pro-inflammatory signals (139). Mice lacking *CLEC7A* gene (Dectin-1), thus having dys-regulated interactions with fungal microbiome (mycobiome) show an increased susceptibility to dextran sulfate sodium (DSS) induced colitis (140). The role of Th17 cells and T_{regs} in this model is unknown. Certain proportion of intestinal T_{regs} co-expresses RORγt, the master transcription factor of the Th17 lineage, with up to 35 % in small intestine and 65 % in colon (141–143). Some of these $ROR\gamma t^+$ T_{reg} co-produce IL-17A ($T_{reg}17$), and are substantially diminished in GF or antibiotics-treated mice. Mono-association of GF mice with a panel of 22 bacterial species from the human gastrointestinal tract shows that a number of microbes, not only *Clostridiales*, are capable of induce colonic $ROR\gamma t^+$ T_{regs} (142). Segmented filamentous bacteria (SFB) were only mediocre inducers of $ROR\gamma t^+$ T_{regs} in that study (142). These studies demonstrate that intestinal $ROR\gamma t^+$ T_{regs} are highly microbiota-dependent and have functions in promoting host immunity (62). Yet, RORγt is not a perfect marker for pT_{regs}, because recent reports show the existence of $ROR\gamma t^+$ tT_{regs}, particularly developing under inflammatory conditions (143–145).

While most studies have focused on in-depth characterization of mechanisms by which microbiota engage to counter-regulate their immunostimulatory properties, the reciprocal effect of T_{regs} on the composition and function of the intestinal microbiota was largely ignored (53, 56, 99, 146, 147). Very recently, analysis of mice harboring a reduced number of TGF-β-dependent pT_{regs} demonstrated numerous underrepresented metabolic processes and a limited overall diversity of the microbiome, including a significant reduction of *Lactobacillus johnsonii* and *Mucispirillum schaedleri* (148). Mechanistically, it was confirmed that the impaired pT_{reg} generation could adversely affect the microbiota niche by elevating type 2 immune responses in the host, thereby declining the microbiota abundance during the process of community assembly. In conclusion, the presence of pT_{regs} in the intestinal immune system has a strong impact on the composition and function of the intestinal microbiota. Similarly, IL-17F deficiency induces T_{reg} cells in the colon and modifies the composition of the intestinal microbiota and mediates protection against colitis

(149). Taken together, two-way interactions between resident microbiota and host intestinal immunity confer intestinal tolerance and immunomodulation.

IMPACT OF MICROBIOTA ON T_{REGS} AND TH17 CELLS IN ORAL MUCOSA

Oral microbiome is vital to maintaining both oral and systemic immune homeostasis because oral mucosa is the primary gateway for the GI tract, the biggest component of the immune system (150). While a vast majority of microbiota studies has focused on intestinal mucosae and their interactions with gut microbiota, little is known about oral mucosal microenvironment colonized with a large array of resident microbes, which is structurally and functionally distinct from the GI tract (151–160). *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria* are the major phyla accounting for ∼96–99% of the oral microbiome, while SR1, TM7, *Cyanobacteria, Spirochaetes, Synergistetes,* and *Tenericutes,* are also found (<1% distribution). It is well established that oral-resident microbiota in poly-microbial interactions and soft-tissue biofilms avert oral diseases, but direct effect of such interactions on host oral immune cells is less clear (161–166). Oral mucosa maintains subsets of dendritic cells (DC), which produce immunomodulatory cytokines such as IL-10, TGF-β1 and Prostaglandin E2, and are predominantly tolerogenic (89, 167–169). These cells may be in intimate cross-talk with oral mucosal T_{regs} (58, 62, 170, 171), albeit details of such interactions between these cells are unexplored in oral mucosa. However, alterations in T_{regs} and Th17 functions have been implicated in human oral *Candida* infections and periodontitis (36, 38, 40, 69, 172–176). We and others have shown the presence of oral mucosal $Foxp3^+$ T_{regs} with protective functions during local infection (89, 158, 169, 170). The interrelationship between these cells and oral commensals during an oral infection was also explored (58, 170). In the context of oropharyngeal candidiasis (OPC) infection, T_{reg} cells play a critical role in reducing fungal burden and establishing homeostasis during post anti-fungal response (177). T_{regs} play rather an unconventional role of enhancing the Th17 cell response and neutrophil infiltration during early acute response, but are associated with reduced TNF-α expression in CD4 T cells at resolution phase (83, 91, 178). *Candida* infection in mice by itself increases the proportion of $Foxp3^+T_{regs}$, in a TLR2/MyD88 dependent manner in oral mucosal tissues and draining cervical lymph nodes (58, 83, 91). A small proportion of those $Foxp3^+$ cells co-express RORγt and IL-17A ($T_{reg}17$). Antibiotic mediated depletion of resident bacteria significantly diminishes the frequency of $Foxp3^+T_{reg}$ $IL\text{-}17A^-$ and $T_{reg}17$ cells, as well as conventional Th17 cells not expressing Foxp3. Reduction of these cells is concomitant with an increase in tissue pathology and fungal burden in oral mucosa, demonstrating that resident bacteria are important for controlling $Foxp3^+$ cells and Th17 cells, as well as mucosal immunity (**Figure 1**). Interestingly, *Candida* can also promote Th17 and T_{reg} responses in oral mucosa (83, 179, 180). The impact of oral resident microbiome in periodontal inflammation, which is now considered a "resident microbial perturbation" rather than a disease caused by a single pathogen, is well known (181). Resident bacterium *P. gingivalis*, the keystone pathogen contributes to altering the abundance and composition of other normal microbiota. Shift and accumulation of gram-positive aerobes to gram-negative anaerobes such as *P. gingivalis, T. denticola, F. nucleatum, and Prevotella sp*. are strongly associated with damage in gingival barrier, loss of immune balance and destruction of oral tissue in periodontal disease (150). During this process, bacterial antigens from skewed microbiota can access connective tissues causing abnormal activation and expansion of inflammatory $CD4^+CD69^+CD103^-$ memory T cells and Th17 cells (182). Another recent study showed that periodontitis-associated expansion of Th17 cells required both IL-6 and IL-23, and was dependent on the local dysbiotic microbiome (31). Shift in resident microbiota can also include increase in *C. albicans*, a part of resident mycobiome in ∼50–70% of healthy humans, which can rapidly transition to a pathogen and cause infections in immune-compromised and cancer patients. *C. albicans* is also shown to heighten *P. gingivalis* accumulation, worsening the series of inflammatory events associated with periodontitis severity (183, 184). It is known that $T_{reg}17$ cells exist in periodontitis lesions and could be involved in inflammatory responses against periodontopathic bacteria (185). While there may be only small changes in oral microbiome in HIV+ individuals, underlying mechanisms causing dysbiosis and its association with HIV associated periodontitis during SIV/HIV infection are unclear (117, 186, 187). Precise events defining Th17 and T_{reg} dysfunctions in the context of underlying dysbiosis and aggravating oral inflammation in HIV disease and periodontitis remain to be seen.

MICROBIOME IN MUCOSAL IMMUNITY AND INFLAMMATION IN OTHER MUCOSAE

Lung, previously thought to be sterile, is now known to harbor a complex and dynamic microbial community of ∼500 species, with a high resemblance to oral microbiome (188, 189). Lung microbiome strongly influences the development and progression of allergic responses and asthma (190). Disrupting the normal microbiome with childhood antibiotic exposure increases the risk of childhood asthma. *Proteobacteria* abundance in lower airway secretions correlates with pro-inflammatory Th17 cell proportions in asthmatic individuals (191, 192). Similarly, in cystic fibrosis patients, alterations of some groups in the polymicrobial community significantly affect the disease progression. Also, in chronic obstructive pulmonary disease (COPD) patients, microbial dysbiosis associated with mucus hyper-secretion and reduced airway clearance results in chronic aberrant inflammation and airway damage (193). Lung microbiota alterations are also associated with differences in pneumococcal clearance (194).

Multiple genera of microbiota exist in vaginal mucosa, often dominated by species of *Lactobacillus*, and a diverse array of anaerobic microorganisms, including *Atopobium,*

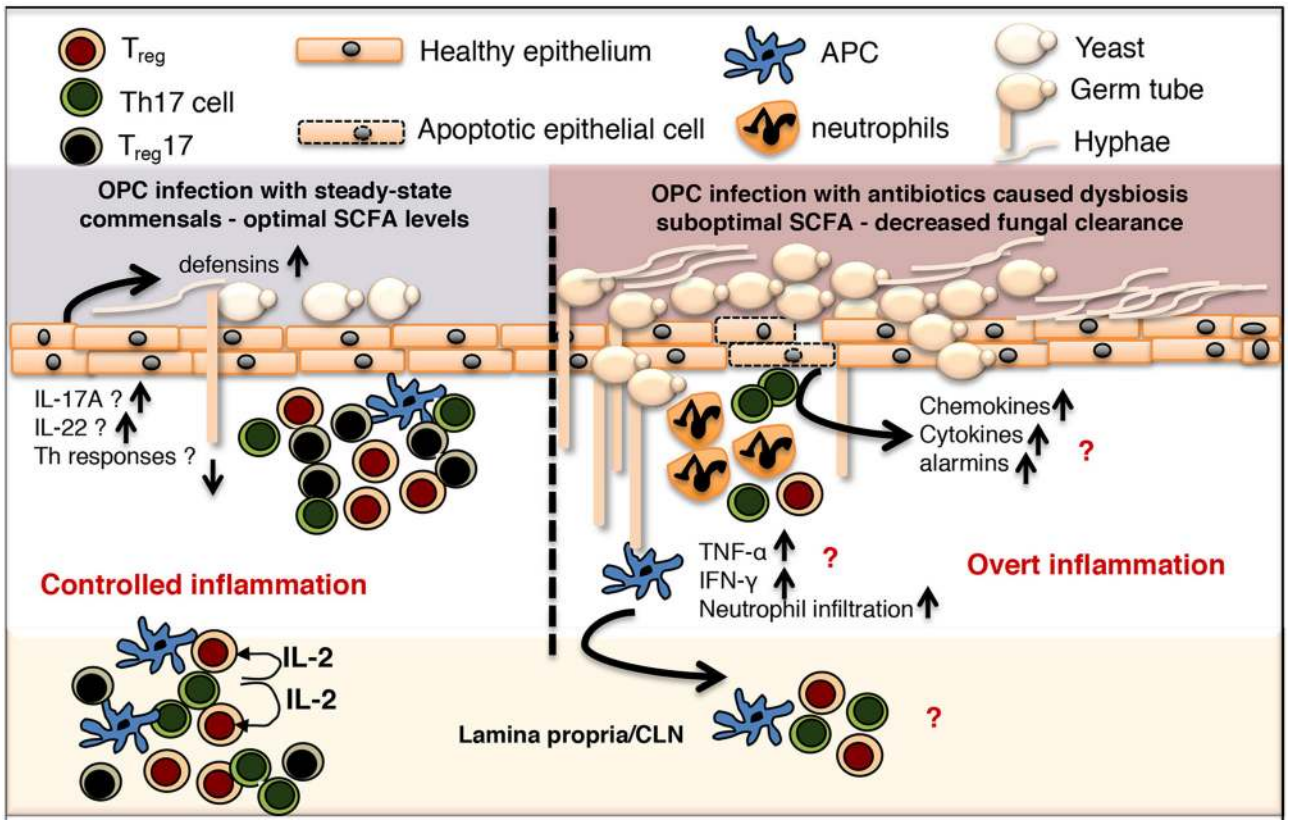

FIGURE 1 | Controlled commensal bacteria/T_{reg}/T_{reg}17/Th17 cell interplay functions as a switch between protective immunity and overt inflammation in oral mucosa. OPC, Oropharyngeal candidiasis; SCFA, short chain fatty acid; CLN, cervical lymph node; APC, antigen presenting cells.

Anaerococcus, Corynebacterium, Eggerthella, Gardnerella, Mobiluncus, Peptoniphilus, Prevotella, Sneathia, and *Finegoldia* genera (195). *Lactobacilli* largely impact the susceptibility to *T. vaginalis* infection in women. Although mechanisms are still under investigation, there is precedence that Th17 cells and T_{regs} can have protective and anti-inflammatory effects during *T. vaginalis* infection (196). During a vaginal herpes simplex virus-2 (HSV-2) infection, mice lacking T_{regs} fail to timely accumulate HSV-2-specific CD4 T cells and control the infection. This finding underscores the protective role of T_{regs} in facilitating productive mucosal immunity in vaginal mucosa (197, 198). However, mechanisms of direct control of vaginal microbiome on T_{regs} and Th17 cells and infection responses remain to be seen. In ocular mucosa, *Corynebacterium mastiditis* induces commensal specific IL-17 response γδ T cells, recruiting neutrophils and protecting the ocular mucosa from pathogenic infections (199). In nasal mucosa, on the one hand there is evidence that butyric acid-producing microorganisms associate with an impaired olfactory function (200–202). On the other, nasal microbiome is structured by IL-17 Signaling that that supports resistance to *S. pneumoniae* colonization in the nasal mucosa of mice (203). Collectively, while mcrobial dysbiosis and T_{regs}/Th17 changes are associated with many of these infections, detailed mechanisms remain to be investigated.

MOLECULAR MECHANISMS OF MICROBIOTA-ASSOCIATED ALTERATIONS OF T_{REG}/TH17 CELLS IN MUCOSAE

Resident microbes have a variety of mechanisms for conferring mucosal colonization resistance (17, 204–207). They include: (1) directly competing for shared metabolites, (2) expression of inhibitory bacteriocins, (3) induction of protective mucus layer, and (4) priming of protective immune responses (208, 209). Some of the examples include commensal dependent metabolism of secondary bile acids to deoxycholate, production of organic acids, induction of antimicrobial peptides in Paneth cells, and promoting elevated antibacterial T cell responses preventing colonization and dissemination of pathogens (210–213). Although resident bacteria are known to modulate energy metabolism producing pyruvic acid, citric acid, fumaric acid and malic acid (214), how pH changes determine the mucosal immunity and T cells warrants further investigation. Resident microbiota employ multiple mechanisms that contribute to coordination of T_{reg}/Th17 axis and safeguarding of mucosa (**Figure 2**). For example, microbiota dependent TLR signaling in host is one of the important mechanisms by which microbiota control inflammation and tolerance. TLR2/MyD88signaling is required for generation and expansion of $Nrp1^{low}$ Foxp3+

cells and $T_{reg}17$ cells in oral and gut mucosa (58). In gut mucosa the capsular polysaccharide A of the *Bacteroides fragilis* stimulates production of IL-10 by $Foxp3^+$ cells in a TLR2 dependent manner, thus facilitating mucosal tolerance (215). Recently it was found that this commensal also delivers immunomodulatory molecules to immune cells via secretion of outer membrane vesicles through a non-canonical autophagy pathway for inducing IL-10 expressing $Foxp3^+$ cells. This mechanism requires the expression of host genes *ATG16L1* and *NOD2,* whose polymorphisms are known to be associated with IBD (216). Selective deletion of *Atg16l1* in T cells in mice also results in loss of $Foxp3^+$ T_{reg} cells and spontaneous intestinal inflammation characterized by aberrant Th2 responses. These data indicate microbiota-host interactions intimately involve the processes of autophagy and T_{reg} differentiation. Moreover, loss of MyD88-STAT3 signaling in T_{regs} causes loss of mucosal T_{regs} and impaired T follicular regulatory cell interactions, resulting in poor IL-21 and anti-microbial IgA responses (217). Failure of this pathway results in over-growth of pathobionts, overt Th17 cell expansion and intestinal inflammation. However, the requirement of resident microbiome induced MyD88 signaling specifically in T_{regs}, to promote T_{reg} sustenance and intestinal tolerance is still debated (217–219). Similar to *B.fragilis*, colonic *Clostridium rhamnosus* also potently induces IL-10^+T_{regs} in a TGF-β1 dependent manner, which is correlated to increase in systemic IgE and resistance to colonic inflammation (56, 99). Similarly, microbiota and immune cell networks are known to control the production of IgA, which is central for mucosal barrier and intestinal tolerance. For example, *Mucispirillum* spp. and SFB have been directly implicated in production of intestinal IgA (137, 220, 221). T_{regs} are also known to promote IgA secretion, and maintenance of diversified and balanced microbiota, which in turn facilitates their expansion through a symbiotic regulatory loop, and prevent overt inflammation (222, 223). Moreover, $ROR\gamma t^+$ Th17 cells, as well as IL-17A from other cells also promote epithelial polymeric Ig receptor and intestinal IgA expression, further contributing to intestinal homeostasis (224, 225). SFB also control commensal tolerance and anti-microbial host responses through intestinal epithelial cell fucosyl tranferase 2 expression and fucosylation, a process that is dependent on $ROR\gamma t^+$ group 3 innate lymphoid cells (ILC3s) and IL-22 expression (226, 227). Loss of intestinal fucosylation results in increased susceptibility to infection by *Salmonella typhimurium*. ILC3s can also express major histocompatibility complex class II (MHCII) and mediate intestinal selection of $CD4^+$ T cells in order to limit commensal bacteria-specific CD4 T-cell responses (228). Although IL-6, induction of $T_{regs,}$ or Th17 cells were shown to be not required for ILC-mediated tolerance, alterations in $T_{reg}17$ and Th17 cells in the context of fucosylation remain to be studied. T_{reg}/Th17 cell differentiation and expansion are also independently controlled by specific members of anaerobic bacteria producing short chain fatty acids (SCFAs), such as acetate, propionate and butyrate (229, 230). Some of these bacteria include *Bacteroides, Bifidobacterium, Feacalibacterium* genera, *and Enterobacteriaceae family, Porphyromonas gingivalis, Fusobacterium nucleatum* (mouth), *Clostridium cochlearium, Eubacterium multiforme* (intestine), and *Anaerococcus tetradius* (vagina). These bacteria ferment indigestible oligosaccharides and cell surface fucosylated proteins by anaerobic glycolysis, resulting in SCFA production. SCFAs are present in the intestinal lumen at a total concentration of ∼100 mM at a ratio of ∼6:3:1, for acetate, propionate and butyrate respectively. Although this ratio hinges on carbohydrate availability, microbiota composition and intestinal transit time, acetate and butyrate appear to be the highest and least in abundance respectively (231). Emerging data show that SCFAs contribute to immune homeostasis in mucosa, although excessive and suboptimal levels of SCFAs are often associated with inflammation and cancer. Intestinal SCFAs have been shown to potentiate $Foxp3^+$ cell differentiation and immunomodulatory activity in the colon (53, 99, 147, 232). Mechanistically, in addition to direct histone deacetylase (HDAC) inhibition, SCFAs can induce the expression of retinal aldehyde dehydrogenase 1 family member 1a (Aldh1a) and TGF-β1 in intestinal epithelial cells and DCs (100, 221, 233, 234). Aldh1a could further convert vitamin A into its metabolite retinoic acid in G protein–coupled receptor43 (GPCR43) and Gpr109a manner, which is capable of facilitating T_{reg} induction. These tolerogenic DCs express CD103, sample antigens in the intestinal lamina propria, and migrate to the draining mesenteric lymph node (MLN) to induce immunomodulatory T cells (235–237). Whether SCFA mediated induction and or sustenance of mucosal T_{regs} require these aforementioned processes is unclear and remain to be studied. However, antibiotics precipitously decrease the oral SCFAs in saliva, showing that in the oral resident bacteria-derived-SCFA is functionally involved in controlling oral mucosal immunity and inflammation (62). Lending credence to this tenet, antibiotics treated mice show not only increased oral inflammation, but also intestinal immunopathology, when infected with oral *Candida*. Mechanistically, antibiotic treatment results in reduced T_{regs}, Th17 and $T_{reg}17$ cells in oral mucosa and tissue draining cervical and axillary lymph nodes in infected mice. Intestinal inflammation in oral *Candida* infected mice is characterized by an increase in IFN-γ producing Th1 cells and co-producers of IFN-γ and IL-17A (Th1*) cells. Although the exact mechanism of antibiotic mediated reduction of T_{regs}, Th17 cells and $T_{reg}17$ cells is unclear, administration of SCFA partially restored these populations and reduces oral immunopathology during the infection. SCFA administration however, only moderately ameliorates the intestinal inflammation. Therefore, the mechanism of Th1-mediated gut inflammation during oral *Candida* infection in the context of altered microbiota remains to be addressed. Recently, Atarashi et al. showed that oral bacterium *Klebsiella* spp. isolated from the salivary microbiota elicits a severe Th1 gut inflammation in the context of intestinal dysbiosis, in a genetically susceptible host (238). This finding underscores the role of oral resident microbes such as *Klebsiella* spp. and *C. albicans* in modulating T cells, possibly translocating to gut and causing overt inflammation in the gut in the context of resident microbial dysbiosis. Supporting this tenet, post oral gavage of *C. albicans*-infected mice pre-treated with antibiotics showed significantly altered composition of intestinal microbiota as well as $CD4^+$ T cell mediated lung inflammation, following

aerosol introduction of an allergen. However, mice without any antibiotics pre-treatment did not develop an allergic response in the airways (239, 240). Whether changes in SCFA, or T_{reg} and Th17 cells in the lung contribute to the inflammation is unknown.

Mechanistically, SCFAs also cause acetylation of p70 S6 kinase and phosphorylation rS6, promoting the mTOR activity. mTOR activity was shown to be required for generation of Th17 (T helper type 17), Th1, and IL-10^+ T cells (241). Moreover phosphoinositide 3-kinase and mTOR pathways play pivotal roles in integrating growth signals in $CD4^+$ T cell differentiation (242–249). Multiple studies support the role of mTORC1 and mTORC2 proteins in regulating Th17 and T_{reg} fate decisions (247, 250, 251). mTORC1 signaling is constitutively active in T_{reg} cells, and disruption of mTOR protein as well as unrestrained mTOR hyper-activation, both have been shown to cause autoimmunity by impairing Foxp3 expression and T_{reg} functions (252–260). Another study has also shown that mTORC1 and its downstream target hypoxia-inducible factor-1α (HIF-1α) are needed for Foxp3 induction, T_{reg} lipid and cholesterol biosynthesis from glucose, and proliferation and suppressive function *in vivo* (244, 254). Taken together, while direct role of SCFA in mediating mTOR activation and subsequent T_{reg} induction in mucosa is unclear, these studies highlight the importance of how immunologically relevant microbiome can control T_{regs} and mucosal homeostasis through multiple mechanisms.

MICROBIOTA AND T_{REG}/TH17 CELL REGULATION OF IMMUNE SENESCENCE AND CHRONIC INFLAMMATION

While resident microbes have aforementioned protective functions in mucosa, they can also trigger and sustain inflammation during aging and other chronic inflammatory conditions. Some studies demonstrate direct relationship between aging and changes in microbiota, albeit the mechanisms remain largely unstudied. Aging causes increased accumulation of gut *Enterobacteria, Streptococci, and yeasts* but declining levels of *Akkermansia muciniphila*, *Bifidobacteria* and *Bacteroides* (261–266). Reduced *Akkermansia muciniphila* is associated with reduced butyrate and impaired intestinal barrier. Consequently, aged mice display endotoxin leakage, and triggering of 4-1BB receptor signaling and insulin resistance. In oral mucosa, aging causes higher levels of $RANKL^+$ cells, and increased inflammatory Th17 cell accumulation, with concomitant loss of alveolar bone, which are dependent on the presence of commensal microbiota (30, 267, 268). In contrast, these events do not occur in in germfree mice periodontium, showing potentially pathogenic roles of commensal microbiota in aging associated dysbiosis setting. Similarly, resident microbiota have been implicated in the onset and progression of experimental autoimmune encephalomyelitis (EAE) (269). GF mice exhibit lower levels of the pro-inflammatory cytokine IFN-γ and IL-17A producing cells, and a reciprocal increase in T_{regs} in the intestine and spinal cord. These changes in GF mice correlate with a significantly attenuated EAE, compared with conventionally raised mice. Remarkably, intestinal colonization with SFB alone can promote Th17 cells in the gut and in the central nervous system (CNS), enhancing disease progression (270). Furthermore, partial elimination of intestinal microbiota ameliorates established collagen-induced arthritis by dampening Th17 responses in mice (271). Some bacteria also provide inflammatory signals resulting in chronic inflammation and tumorigenesis, likely by inducing genetic and epigenetic changes in host cells. For example, *Fusobabacteria* spp. has been implicated in increased risk of IBD and colorectal cancer (272–275). Also, in oral mucosa, the abundance of *Fusobacterium* increases, while the number of *Streptococcus, Haemophilus, Porphyromonas, and Actinomyces* decreases with cancer progression in oral squamous cell carcinoma (276). Interestingly, *Fusobabacteria,* and several other bacteria of oral mucosal origin, including genera of *Streptococcus, Staphylococcus, Peptostreptococcus* may translocate to intestine in the context of gut inflammation and carcinogenesis (277–279), similar to *Klebsiella* spp *and C. albicans* in susceptible host (62, 238). It is tempting to speculate that loss of T_{reg} functions in the context of dybiosis, excessive SCFA and oral microbial translocation may have contributed to exuberant intestinal inflammation and predisposition to carcinogenesis in these studies (**Figure 2**). However, whether the mouth- to -gut translocation is a cause, or consequence of dysbiosis and intestinal inflammation, and the underlying mechanisms still remain to be understood and warrant further investigation.

THERAPEUTIC APPLICATIONS OF MICROBIOTA ALTERATIONS AND MICROBIOTA DERIVED METABOLITES

As we discussed above, studies on patient cohorts, mechanistic studies on mice and epidemiological studies have led to a better understanding of how microbiota changes impact mucosal immunity, and *vice versa.* Mechanistic "proof-of principle" studies using disease models have opened ways to manipulate these processes, providing therapeutic approaches. Some of the widely used approaches include administration of sodium butyrate and pre- and pro-biotics, and transplantation of fecal microbiota (280–283). However, there are hurdles in pro-biotic and microbiota transplantation approaches. Existing microbiota, whether it is healthy or dysbiotic is largely stable over time in an individual. Without profound perturbation of the existing microbiota, it is challenging to introduce microbiota exogenously. The effects of exogenous bacteria introduced by probiotic and transplant approaches are greatly influenced by existing microbiota in a competitive niche, and are inconsistent. Therefore, approaches to target these niches in favor of exogenous bacteria are being studied (283, 284). Direct administration of microbial derivatives appears to be a promising venue. Butyrate has been shown to alleviate high-fat-diet induced non-alcoholic fatty liver disease. It potently down modulates peroxisome proliferator-activated receptor α-mediated activation of β oxidation, causing reduced inflammation (285). For cART

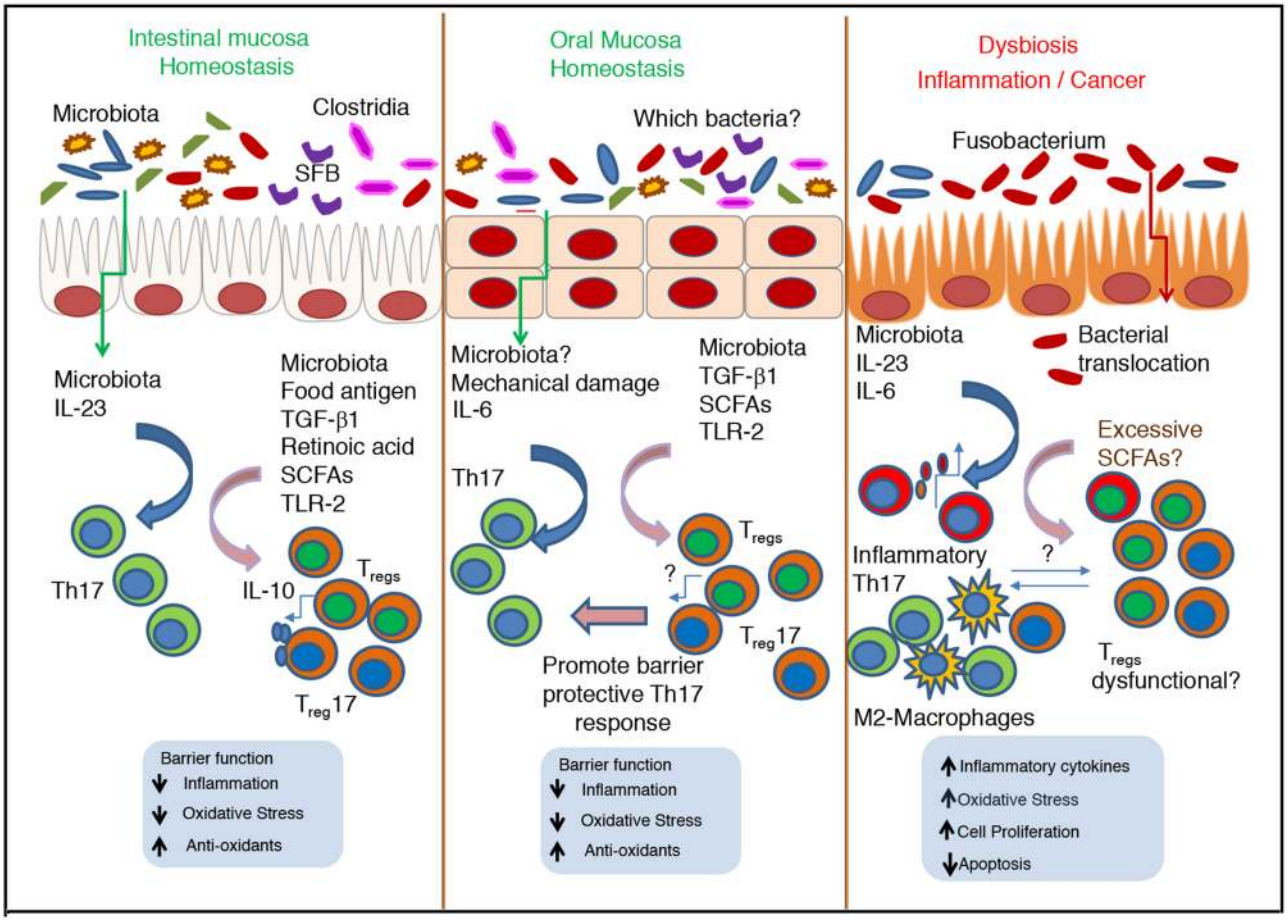

FIGURE 2 | Cross talk between microbiota and immune cells during homeostasis and dysbiosis–Role of Th17 cells and T_{regs} in oral and intestinal mucosa. During homeostatic conditions, normal microbiota promote the stimulation of epithelial cells, Th17 cells and T_{regs}, and maintain barrier function and commensal tolerance. In oral mucosa, Th17 cells are induced by mastication induced mechanical damage, independent of commensals. However, in both mucosae SCFA mediated induction of T_{regs} is key for mucosal barrier function and immunomodulation. During inflammation and cancer, excessive SCFAs can increase inflammatory Th17 cells and T_{reg} population that may be dysfunctional. The nature of their interaction with Th17 cells, tumor associated M2-type macrophages and other cells remain unclear.

treated HIV^+ individuals, aside from cART treatment, probiotics have been studied to combat persistent systemic inflammation. This approach in the context of cART may lead to improved and holistic management of inflammatory events and higher cancer susceptibility in HIV+ patients. Application of probiotics has also shown positive effect on the course of pneumonia, acute exacerbation of bronchial asthma and COPD in mice models, but warrants further studies in humans (286). SCFA has been shown to have therapeutic potential in microbiome-targeted interventions in anti-aging medicine. Butyrate and dietary fibers have been shown to promote anti-inflammatory effects in the context of aging associated neuro-inflammation in mice (287). Adult and aged mice fed with 5% inulin (high fiber) diet for 4 weeks show an altered gut microbiome and increased butyrate, acetate, and total SCFA production, coinciding with a reduction in neuro-inflammation. High fiber supplementation in aging is a non-invasive strategy to increase butyrate levels, and these data suggest that an increase in butyrate through added soluble fiber such as inulin could counterbalance the age-related microbiota dysbiosis, potentially leading to neurological benefits (287, 288). Similarly, dietary fiber also suppresses colon carcinogenesis in polyposis mice (289). Mechanistically it has been shown to inhibit colorectal cancer cell migration through micro-RNA regulation (290). In summary, alterations of mechanisms of microbiota-host interactions are proving to hold promise for treating a variety of disorders in humans.

CONCLUSION

It is now well established that resident microbes provide enormous advantages to the host, while dysbiosis can trigger acute and chronic inflammatory conditions. One of the mechanisms by which these microbes regulate immunity id through controlling T_{regs} and Th17 cells. These cells present in various mucosal locations and share various signaling pathways for their development and sustenance, as stated above. However,

signals modulating these subsets unique to each mucosal environment in different epithelial cell contexts are unclear. Most mechanistic studies showing T_{reg}/Th17 developmental regulation were performed using the *in vitro* cultures using cells isolated from blood (human), spleen and lymph nodes (mice). While there is enough evidence to show that these cells could be regulated by overlapping signaling mechanisms, cells from these mucosae were not directly compared for similarities and differences in their development and functions. Such studies are warranted to get further insights in to homeostatic and dysbiotic conditions in different mucosae. Such studies in the context of microbial manipulation approach will offer new avenues to manipulate their interactions with the host for treating immune-mediated and metabolic disorders. While mono-association of certain genera in GF mice have proven to alter mucosal T_{regs} and Th17 cells and offer some beneficial effects in some experimental settings (98), from a therapeutic perspective, the field is still at its infancy and warrants intense mechanistic investigations. Taken together, further research in microbiota targeted approaches will enable the field to take the center stage in the management of health and disease in humans.

AUTHOR CONTRIBUTIONS

PP and JH wrote the manuscript. NB, ES, MZ, and SJ contributed to the discussion.

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