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Control of Immunity by the Microbiota

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Abstract

The immune system has coevolved with extensive microbial communities living on barrier sites that are collectively known as the microbiota. It is increasingly clear that microbial antigens and metabolites engage in a constant dialogue with the immune system, leading to microbiota-specific immune responses that occur in the absence of inflammation. This form of homeostatic immunity encompasses many arms of immunity, including B cell responses, innate-like T cells, and conventional T helper and T regulatory responses. In this review we summarize known examples of innate-like T cell and adaptive immunity to the microbiota, focusing on fundamental aspects of commensal immune recognition across different barrier sites. Furthermore, we explore how this cross talk is established during development, emphasizing critical temporal windows that establish long-term immune function. Finally, we highlight how dysregulation of immunity to the microbiota can lead to inflammation and disease, and we pinpoint outstanding questions and controversies regarding immune system–microbiota interactions.

1. INTRODUCTION

Multicellular organisms exist as meta-organisms, comprising both the macroscopic host and its symbiotic microbiota (1). These complex communities of microbes that include bacteria, fungi, viruses, and other microbial and eukaryotic species play a fundamental role in controlling all aspects of host physiology, including the immune system (2).

The immune system is composed of a complex network of innate and adaptive components endowed with an extraordinary capacity to adapt and respond to highly diverse challenges. Collectively this cellular network acts as a formidable regulator of host homeostasis able to sustain and restore tissue function in the context of microbial and environmental encounters. The development of specific arms of the immune system, and in particular those associated with adaptive immunity, coincided with the acquisition of a complex microbiota, supporting the idea that a large fraction of this complex system evolved to maintain the symbiotic relationship with these microbes. In turn the microbiota promote and calibrate all aspects of the immune system, ranging from hematopoiesis to the induction of cognate responses. The impact of the microbiota begins during development, and early encounters with microbes are believed to set the stage for the immune system for the long term. Sustained calibration of the immune threshold of activation by the microbiota plays a fundamental role in the promotion of protective immunity against infectious agents. This adjuvant property has also been proposed to account for the impact of the microbiota in vaccine responses and cancer immune checkpoint therapy (3, 4).

The vast majority of immune system–microbial encounters and associated immune responses result from the symbiotic relationship of the host with its microbiota. Far from being ignored, as originally postulated, microbes at all barrier surfaces promote the induction of immune responses, including those directed at the microbiota itself. What differentiates these responses from those resulting from encounters with pathogenic microbes is that both initiation of immune responses to the microbiota and accumulation of commensal-specific lymphocytes within tissues occur in the absence of inflammation, a process that has been referred to as homeostatic immunity (5). As further discussed in this review, homeostatic immunity to the microbiota represents a distinct and likely dominant class of immune responses able to control numerous aspects of host physiology, including tissue repair.

Acquisition of a complex immune system and its reliance on the microbiota also came at a price. Pathologies that increasingly affect humans such as allergies and autoimmune and inflammatory disorders all arise from a failure to control misdirected immune responses against self, environmental antigens, and/or the microbiota (6, 7). Microbiota alteration resulting from antibiotic usage, diet variation, and elimination of constitutive partners such as helminthic worms is believed to have transformed our microbial allies into potential liabilities. Genetic predisposition or infection can promote dominance by proinflammatory microbes, expression of virulence genes, and/or acquisition of new invasive microorganisms. This, in turn, can trigger aberrant immune responses to the microbiota, an inflammatory loop that eventually leads to a breakdown of tissue homeostasis. However, an important point to consider is how, in most cases, microbe-induced pathogenicity strongly depends on context. As such traditional reliance on Koch's postulates (8) has often proven counterproductive in exploring the impact of the microbiota, and we should consider microbial causation in a broader systems biology context in which host genetic variability, health status, exposure history, and microbial strains and communities are all integrated.

In this review, we focus on the known and proposed mechanisms underlying early-life and long-term imprinting of the immune system by the microbiota, with a particular focus on adaptive immunity induced by the microbiota and the broad impact of these responses on host homeostasis.

2. ESTABLISHMENT OF MICROBIOTA–IMMUNE SYSTEM HOMEOSTASIS DURING A SPECIFIC DEVELOPMENTAL WINDOW

When operating properly, early host-microbiota interactions lead to the establishment of durable and mutualistic relationships, a dialog that sets the lifelong host immune threshold. On the other hand, early-life stressors such as malnutrition, infection, and mode of delivery are emerging as important factors altering long-term immune function, and human epidemiological studies have confirmed that this critical time of microbial exposure can have lasting consequences for disease susceptibility (9, 10). The immune system is uniquely susceptible to long-term programming by the microbiota and maternal-derived factors in the postnatal period. This form of developmental plasticity has been hypothesized to provide greater adaptability: Alternative homeostatic set points of immunity would be programmed in early life based on perinatal exposures in anticipation of the adult environment for greater adaptability. However in the context of maladapted microbial encounters, such flexibility could also contribute to increased disease susceptibility (11).

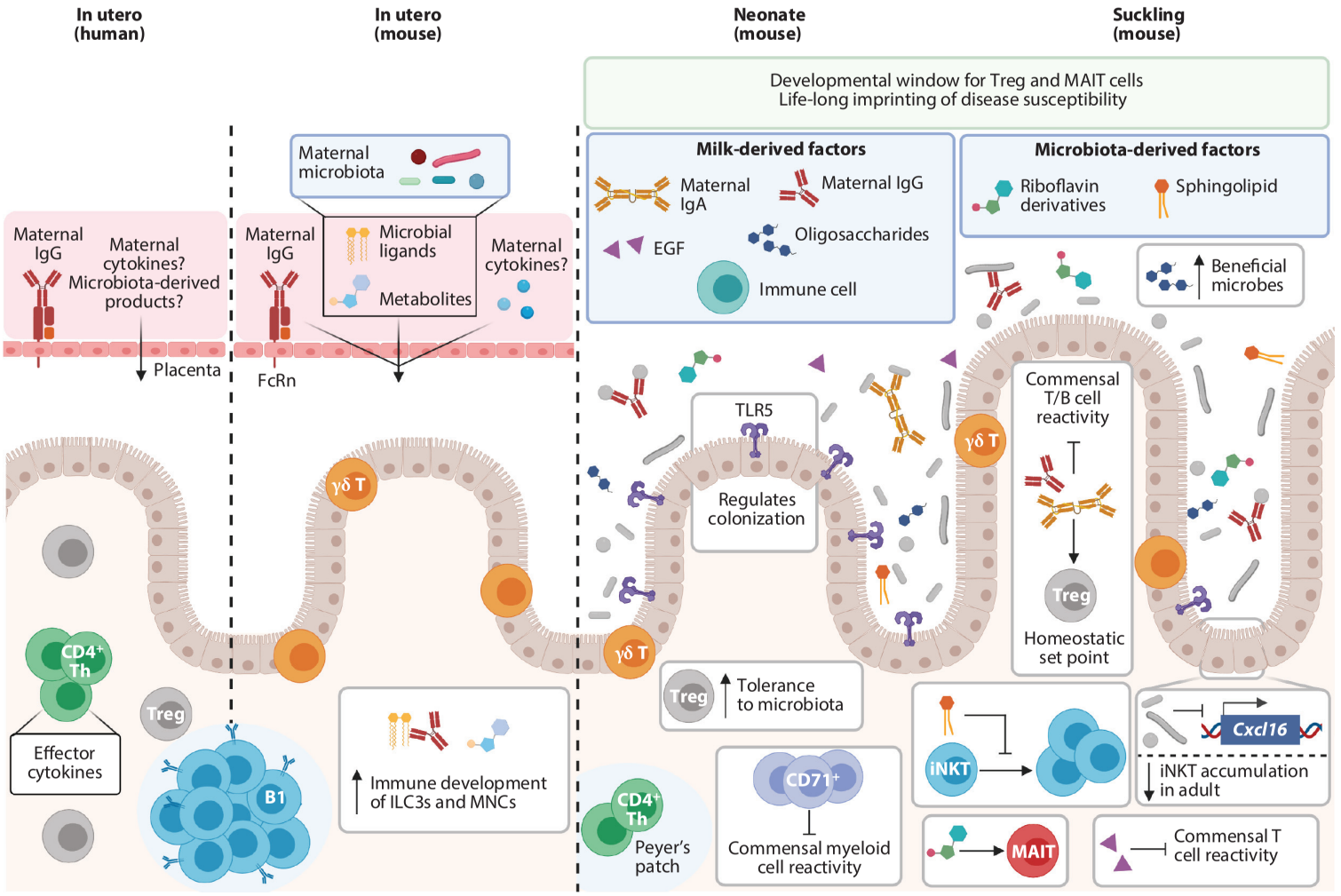
Birth is a pivotal step in development of the organism. Upon delivery, neonates face tissue-specific colonization of barrier sites by microbes derived from the mother and the environment. During early life, introduction of solid foods and cessation of breastfeeding bring additional challenges in the form of expansion and restructuring of fluctuating microbial communities (12). Microbiota–immune system interactions during this critical developmental window are thought to pave the way for homeostasis and disease susceptibility in the adult, yet the mechanisms that coordinate these interactions remain poorly understood. Experimental evidence has started to uncover some of the mechanisms and sequences of events mediating host-commensal mutualism in early life and long-term consequences of these early encounters. Notably, maternal factors and nutrients present in breast milk shape the microbiota and responses to these microbes. Further, temporally regulated processes guide early-life interactions with the microbiota and dictate homeostatic set points of host immunity. Finally, specific subsets of cells enriched at an early stage, including innate-like lymphocytes and unconventional T cells as well as regulatory T (Treg) cells, guide microbiota-immune dialog in early life.

In this section we summarize our understanding of microbial exposures in early life and their effects on host immunity (**Figure 1**), and we discuss known and proposed mechanisms of immune education in early life. This discussion focuses on innate-like and adaptive lymphocyte cross talk with the microbiota but also briefly discusses additional maternal, fetal, and environmental factors of early life with the potential to affect immune interactions with the microbiota in the long term.

2.1. Microbial Education Begins In Utero

While the fetal environment has traditionally been considered sterile, with microbial exposure starting only upon birth, the existence of commensal microbes resembling human oral and skin microbiota in the placenta and amniotic fluid has been reported (13, 14). However, these findings have been called into question (15, 16). Regardless of true colonization, in utero influence of the maternal microbiota on the developing fetus can be mediated directly via microbiota-derived products or metabolites or indirectly via the ability of the microbiota to control the tone of the maternal immune system.

The placenta allows for transport of gases and small molecules into the fetus, while it is considered to be largely impermeable to diffusion of large macromolecules (17). Receptor-mediated transcytosis via the neonatal Fc receptor (FcRn) allows for the transfer of antibodies into the fetus (18), but to what extent additional macromolecules use similar transport mechanisms remains poorly understood. Recent animal studies have revealed that maternal microbiota-derived



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

Early-life microbiota–immune system interactions establish homeostatic set points of immunity. Education of the immune system begins in utero, where immune populations are developing and seeding peripheral tissues, including B1 B cells and γδ T cells in both mice and humans and $CD4^+$ T effector (Th) and Treg cells in humans. Maternal microbiota-derived metabolites, antibody-bound microbial ligands, and cytokines can reach the developing fetus and impact immune development. After birth, maternal factors transmitted through milk, including maternal antibodies, EGF, and milk oligosaccharides, further modulate immune development and early encounters with the microbiota. Maternal antibodies prevent aberrant T cell reactivity to the microbiota and help establish the long-term homeostatic set point of Treg cells. Microbiota-derived factors impact the development and long-term function of unconventional T cells. For example, the microbiota induce hypermethylation upstream of the *Cxcl16* locus in epithelial cells, preventing aberrant iNKT cell accumulation in adulthood. Furthermore, during this window, microbiota-derived sphingolipids inhibit iNKT cell proliferation, and microbiota-derived riboflavin derivatives promote the development of MAIT cells in the thymus and their accumulation in peripheral tissues. Developmental programs also impact immune reactivity to the microbiota: $CD71^+$ erythroid cells inhibit proinflammatory responses to the microbiota, and an increased frequency of Treg cells promotes tolerance to the colonizing microbiota. Finally, broad expression of TLR5 by epithelial cells in utero is downregulated after birth and confined to Paneth cells in the adult small intestine, thereby impacting microbiota assembly. Abbreviations: EGF, epidermal growth factor; FcRn, neonatal Fc receptor; ILC3, group 3 innate lymphoid cell; iNKT, invariant natural killer T; MAIT, mucosal-associated invariant T; MNC, mononuclear cell; Th, T helper; TLR5, Toll-like receptor 5; Treg, regulatory T. Figure adapted from image created with BioRender.com.

metabolites [e.g., short-chain fatty acids (SCFAs)] and ligands are able to reach the fetus and impact tissue development (19). A reversible colonization model has been used in which germ-free pregnant dams are colonized with an auxotrophic *Escherichia coli* strain that does not persist such that the dams give birth to germ-free pups. This study revealed that microbial metabolites and antibody-bound microbial fragments derived from the maternal microbiota can reach the fetus and impact intestinal group 3 innate lymphoid cell (ILC3) and mononuclear cell populations in the offspring (20) (**Figure 1**).

The indirect impact of the maternal microbiota on the offspring's physiology has been proposed to contribute to the etiology of neurodevelopmental disorders (21). For instance, specific gut commensal bacteria can increase abnormalities consistent with autism spectrum disorder in the offspring of pregnant mothers undergoing immune system activation (22). In this model maternal IL-17A promoted abnormal cortical development in the fetus (23), and while transplacental transport of IL-17A was not conclusively shown, this and previous work suggest that specific maternal cytokines may be able to reach the fetus and impact its development (23, 24).

The human immune system and tissue architecture reach greater maturity in utero compared to that of mice (**Figure 1**): T and B cell development in the mouse fetus encompasses innate-like B1 B cells and γδ T cells (25, 26), with αβ T cells first appearing around birth and accumulating in secondary lymphoid structures and peripheral tissues postnatally (27, 28). In human fetal development, incomplete fetal B cell tolerance leads to the accumulation of polyreactive naive B cells with commensal specificities (29). Furthermore, mature αβ T cells including memory $CD4^+$ T cells with the capacity for cytokine secretion and evidence of clonal expansion seed peripheral human tissues in utero (30, 31). The role of this preset and potentially microbiota-reactive T and B cell repertoire in shaping early life encounters with the microbiota remains to be determined. For a more comprehensive review on human prenatal immune development see Reference 32. More generally, researchers are at an early stage of investigating the impact of the microbiota on both maternal and fetal immune function and the impact of the temporal windows associated with these defining encounters.

2.2. Maternal Factors Instruct Host-Microbiota Homeostasis in the Offspring

In addition to seeding the primordial microbiota in the infant's tissues (12, 33, 34), the mother is also a source of immunomodulatory factors that guide the offspring's initial response to the microbiota (**Figure 1**). These factors include maternal antibodies, transferred in utero and through

breastfeeding, as well as other components enriched in maternal milk: growth hormones, and nutrients such as oligosaccharides that are able to promote the expansion of specific constituents of the microbiota (35).

Maternal antibodies have long been recognized to provide the offspring with passive immunity against infection (36, 37). Furthermore, maternal antibodies are emerging as a key player in shaping initial interactions with the microbiota. A study in preterm infants revealed that necrotizing enterocolitis, an inflammatory disease driven by the microbiota, is associated with a decrease in IgA binding to the microbiota and a bloom of IgA-unbound *Enterobacteriaceae*, a family of commensals with proinflammatory potential. Mouse models confirmed a protective role for maternal IgA against necrotizing enterocolitis (38). Furthermore, maternal antibodies, which have broad commensal-reactive specificities, limit adaptive immune reactivity to the microbiota in early life (39) and impact microbiota composition into adulthood (40) (**Figure 1**). In the absence of maternal antibodies, suckling mice have reduced body weight and develop compensatory T-dependent immune responses in early life (39). Further, the homeostatic set point of colonic RORγt$^+$ Tregs is vertically transmitted during a short postnatal window by maternal IgA. RORγt$^+$ Tregs, in turn, control intestinal and milk IgA levels, thus transmitting this set point throughout multiple generations (41). In summary, maternal antibodies orchestrate early interactions with the microbiota, although the mechanisms remain incompletely understood.

Additional maternal factors present in breast milk also control initial interactions with the offspring's microbiota. Epidermal growth factor (EGF) is highly abundant in breast milk at birth and coordinates commensal antigen uptake and induction of commensal T cell reactivity in the gut through modulation of goblet-associated passages (42). Furthermore, milk-derived EGF controls the timing of a programmed inflammatory reaction to the microbiota around the time of weaning (42, 43). How alterations in reactivity to the microbiota during this window stably imprint disease susceptibility remains to be addressed, but these studies may identify mechanisms that explain epidemiological observations revealing that perinatal exposures have long-term impacts on disease susceptibility (9).

Milk is also rich in maternal leukocytes with an activated phenotype that express gut- and lung-homing receptors (44). While the functional relevance of maternal milk–derived leukocytes in the offspring in early life remains unclear, evidence supports the idea that specific subsets including CD8$^+$ T cells transiently engraft in offspring Peyer's patches and present an activated phenotype in mice (45). Milk is also the earliest substrate upon which the neonatal microbiota grow. In turn, it has profound impacts on microbial composition and downstream immunological effects. Milk is rich in oligosaccharides, specifically sialylated oligosaccharides, which act as prebiotics for beneficial microbes, many belonging to the phylum *Actinobacteria* (35). *Bifidobacterium* species in particular play many beneficial roles for the developing offspring, including improved responses to vaccines (46, 47), enhanced epithelial barrier function (48), and protection from enteropathogenic infection (49). Additionally, in studies of breast milk from mothers with healthy or severely stunted offspring, sialylated oligosaccharides were sufficient to induce beneficial, long-term metabolic shifts in gnotobiotic animals by modulating the gene expression profiles of some microbiota members (35).

2.3. Early-Life Developmental Programs Guide Initial Interactions with the Microbiome

At birth, the neonate is tasked with the formidable challenge to interact with microbiota acquired via both the mother and the environment and to develop mutualistic interactions with microbial communities at all barrier sites. In physiological settings, these phenomena can in part be

explained by the relative immaturity of the neonatal immune system at birth and the regulatory environment that defines early mammalian life (30) (**Figure 1**). For instance, $CD71^+$ erythroid cells, which contribute to immune cell–extrinsic immunosuppression during this period through arginine depletion, can blunt inflammatory responses of myeloid cells to the intestinal microbiota (50). In contrast to adult T cells, neonatal T cells are poised for rapid differentiation with a bias toward becoming FOXP3-expressing Treg or Th2 cells (51). Furthermore, the frequency of Treg cells in secondary lymphoid organs and mucosal tissues is greater during early life than in adulthood (52, 53).

Experimental studies have revealed that early life presents a unique window for the generation of Treg responses to the microbiota: In mouse skin, Treg cells abruptly accumulate in neonatal skin by day 13, and Treg cell responses to *Staphylococcus epidermidis*, a skin commensal, are preferentially induced during this early period (54). Impaired Treg cell responses to *S. epidermidis* during neonatal exposure lead to increased inflammation upon secondary exposure to this commensal in the context of barrier disruption (54). Early life also presents a unique window for antigen sampling and Treg cell responses to the microbiota in the intestine (42), and a transient defect in Treg cells during this period leads to susceptibility to inflammatory pathology later in life (42, 43). Within the lung, early-life exposure to the microbiota promotes local accumulation of Treg cells, a phenomenon that limits susceptibility to allergic airway inflammation (55). Importantly, ablating early-life Treg cell responses does not alter total adult Treg frequencies in the tissue in any of these models, as Treg cells can still occupy their niche in adulthood (42, 43). However, disease susceptibility is sustained, suggesting that antigen specificity, functional capacity of neonatal Treg cells induced by the microbiota, or both are critical to maintain tissue homeostasis later in life. Similarly, B1 B cell repertoire specificities are uniquely programmed during early life. For instance, the repertoire of antibody responses to *Streptococcus pyogenes* induced by neonatal colonization is distinct from responses to the same microbe seen later in life (56).

Taken together, these studies show that during the neonatal period multiple and still poorly understood developmental programs are deployed to establish homeostatic responses to the microbiota. These early responses are now believed to set up the host immune threshold for the long term. As the study of early-life immunity progresses, additional temporally regulated, tissue-specific mechanisms coordinating interactions with the microbiota remain to be uncovered.

2.4. Unconventional T Cells and Early-Life Responses to the Microbiota

A growing body of evidence supports a role for innate-like/unconventional T cells, an evolutionarily ancient arm of the immune system, in the control of early-life interactions with the microbiota (**Figure 1**). In contrast to conventional T cells, which have the potential to recognize an almost limitless diversity of peptides, unconventional T cells recognize a set of conserved antigens, such as modified peptides and small molecules including lipids and other metabolites (57). A large fraction of unconventional T cells are restricted by monomorphic major histocompatibility class Ib (MHC-Ib) molecules, and many express semi-invariant T cell receptors (TCRs), including invariant natural killer T (iNKT) cells, mucosal-associated invariant T (MAIT) cells, and γδ T cells (57, 58). As such, unconventional T cell recognition of microbial ligands can be considered as bridging germ line–encoded innate immune receptors and randomly generated adaptive TCRs and B cell receptors. These cell subsets typically acquire the capacity for cytokine release and tissue tropism during development, prior to thymic egress, and accumulate in tissues prior to the arrival of conventional effector cells (26, 59). The ability of MHC-Ib molecules to present antigens with specific chemical or amino acid sequence motifs, which can be derived from a large constituency of the microbiota, places unconventional T cells as ideal candidates for the constitutive sensing

and recognition of microbiota-derived antigens and metabolites. For example, MAIT cells recognize riboflavin derivatives from microbiota metabolism that are presented on MR1 (60), and iNKT cells can respond to microbe-derived lipid antigens bound by CD1d (61). As such, unconventional T cells may be optimally positioned to control early-life communication between the microbiota and the immune system.

Adequate seeding of tissues with unconventional T cells depends on exposure to specific microbes during key developmental windows. For example, iNKT cell accumulation in mucosal tissues is regulated by the microbiota in the postnatal period. Increased CXCL16 expression in the colon and lungs of adult germ-free mice leads to aberrant accumulation of iNKT cells and greater susceptibility to asthma and colitis (62). These effects can only be rescued by neonatal exposure to the microbiota. Furthermore, neonatal but not adult exposure to sphingolipids produced by *Bacteroides fragilis* can prevent aberrant iNKT cell accumulation in the colon by locally inhibiting iNKT cell proliferation (63) (**Figure 1**). The development of MAIT cells is also dependent on early-life exposure to microbes that produce riboflavin derivatives during an early-life window (64), and impaired microbiota-derived antigen exposure in early life leads to irreversible defects in MAIT cells (64–66). Microbes belonging to the order *Enterobacterales*, *Proteus mirabilis* and *Klebsiella oxytoca*, were sufficient for MAIT cell development, and perhaps uncoincidentally, members of this order are highly enriched in the neonatal gut and decrease in abundance with age (67). Taken together, these studies argue that the microbiota establish homeostatic set points of nonconventional T cell populations in early life. These fundamental interactions between unconventional T cells and early-life microbiota also support the idea that innate-like immune responses may have been sustained throughout evolution as a means to promote early responses to the microbiota prior to the establishment of adaptive immune responses.

3. IMMUNE RECOGNITION OF THE MICROBIOTA

As mentioned above, the vast majority of microbial encounters and associated immune responses result from the symbiotic relationship of the host with its microbiota. Notably, the microbiota express an extraordinary number of putative antigens for both conventional and unconventional lymphocytes (**Table 1**). As such, the vast majority of antigens seen by the immune system both in the context of homeostasis and during inflammatory states are expected to be microbiota derived. The corollary of this is that the vast majority of effector and memory B and T cells may be microbiota specific. A remarkable property of these responses is that, in contrast to those induced by infections, initiation of T and B cell responses to the microbiota and accumulation of these cells in tissues occur in the absence of inflammation, a process referred to as homeostatic immunity.

Emerging evidence supports the idea that a major aspect of the microbiota's effect on the immune system, including the induction of cognate noninflammatory responses to the microbiota, is reinforcement of barrier function and integrity so as to constrain the microbiota's ecological niche. Homeostatic immunity to the microbiota is a distinct and likely dominant class of immune responses, regulated by specific processes and able to control numerous aspects of host physiology. In this section, we review our understanding of immune recognition of the microbiota by innate-like T cells and adaptive lymphocytes, focusing on the ontogeny, specificity, and function of commensal-specific responses at barrier sites (for recent reviews on innate immunity and the microbiota, see References 68 and 69).

3.1. Humoral Immunity to the Microbiota

Antibodies play a major role not only in the establishment of early-life dialog with the microbiota but also in the maintenance of life-long interaction with microbial partners via impacts on

Table 1 Examples of defined interactions between the immune system and members of the microbiota

Microbe	Microbial product	Known antigens	Dominant immune impact	Downstream consequences	References
Humoral responses					
Enterobacteriaceae spp.	NA	NA	IgG	Protection against related pathogens	101
Akkermansia muciniphila	NA	NA	Cognate Tfh cell		90
			T-dependent IgG1 and IgA		
Mucispirillum spp.	NA	NA	T-dependent IgA		88
SFB	NA	NA	T-dependent IgA		88
Unconventional T cell responses					
Staphylococcus epidermidis	NA	*N*-Formylated peptides (fMIIINA)	Tc17 cell	Wound healing	119–121
Numerous microbes, e.g., *Proteus mirabilis*, *Klebsiella oxytoca*, *Staphylococcus epidermidis*	NA	Riboflavin derivatives (5-OP-RU)	MAIT cell	Wound healing	64
Corynebacterium spp.	Mycolic acid	NA	IL-17-producing γδ T cell	Protective immunity/inflammation	126, 127
T cell responses					
SFB	NA	QFSGAVPNKTD	Antigen-specific Th17 cell	Protective immunity	88, 132–138
				Enhanced systemic Th17 responses	
Clostridium spp.	NA	Flagellin (DMATEMVKY-SNANILSQAGQ)	Antigen-specific IgA and Treg cell (homeostasis)	Immunoregulation	42
			Th1 or Th17 cell (inflammation)		110
Numerous microbes, e.g., *Clostridium* spp.	Short-chain fatty acids	NA	Treg cell	Immunoregulation	187, 188
Helicobacter hepaticus	NA	GNAYISVLAHYGKNG	Antigen-specific Treg and Tfh cell (homeostasis)	Immunoregulatory response to *Helicobacter hepaticus*	158
			Antigen-specific Th17 and Th1 cell (inflammation)		158–162
Akkermansia muciniphila	NA	TLYIGSGAILS	Antigen-specific Tfh, Th1, Th2, Treg, and Th17 cell (context dependent)		90
		LIFESSNALGLGR			
Bacteroides fragilis	Capsular factor polysaccharide A	NA	Treg cell	Suppression of inflammation	194, 195
Bifidobacterium bifidum	Surface B-glucan/galactan	NA	Treg cell		193
Staphylococcus epidermidis	NA	NA	Tc17 and Th17 cell (homeostasis)	Protective immunity/wound healing	119, 120
			Th17 cell with poised Th2 transcriptome	Th2 cytokine-mediated inflammation	121
			Treg cell (early life)	Immunoregulatory response to *Staphylococcus epidermidis*	54

Abbreviations: MAIT, mucosal-associated invariant T; NA, not applicable (microbial products or known antigens have not yet been described); SFB, segmented filamentous bacteria; Tc17, H2-M3-restricted $CD8^+$ T; Tfh, T follicular helper; Th17, T helper 17; Treg, regulatory T.

microbial localization and function. While IgAs have long been recognized for this fundamental role, more recent work revealed that other isotypes, including IgGs, are also involved in microbiota-immune cross talk (**Figure 2**).

IgA antibodies represent a key aspect of immunity at mucosal barriers. The J chain polypeptide mediates the dimerization of IgA and pentamerization of IgM, enabling their secretion into the lumen through polymeric immunoglobulin receptors. Here, secretory IgA and IgM (SIgA and SIgM) provide protection against toxins and invading pathogens but also control host-commensal interactions by controlling microbiota composition, localization, and function (70) and limiting aberrant adhesion to epithelial surfaces (71) (**Figure 2**). As further discussed below, some of these IgAs can express broad reactivity toward diverse members of the microbiota, supporting the idea that together with unconventional T cells, IgAs may contribute to a canonical form of immunity to overcome the extraordinary antigenic diversity contained within the microbiota.

IgA accounts for a dominant fraction of antibody production, with intestinal IgA^+ plasma cells (PCs) constituting 80% of all PCs in humans (72). Part of the intestinal microbiota is coated with IgA antibodies (73, 74), and observations of germ-free mice revealed that intestinal IgA^+ PCs are largely dependent on microbial colonization (75). Early seminal studies uncovered a T cell-independent arm of the IgA response to commensal bacteria (76) and revealed that lack of activation-induced cytidine deaminase (AID)-dependent antibodies leads to aberrant expansion of anaerobic bacteria and isolated lymphoid follicle (ILF) hyperplasia (77). Since then, much work has been devoted to characterizing the ontogeny, specificity, and function of microbiota-reactive antibodies (also reviewed in References 57 and 58) (**Figure 2**).

Mucosal IgA responses to the microbiota are thought to be induced locally by B1 and B2 precursors, via both T-independent (TI) and T-dependent (TD) pathways (78–81). However, the exact contribution of each of these pathways in the control of host-microbiota interactions remains an object of active investigation. TI, in situ class-switch recombination (CSR) to IgA can occur in the lamina propria and ILFs and involves interactions with the epithelial cell– or dendritic cell–derived ligands BAFF and APRIL (79, 82, 83). Additional factors present in the intestinal microenvironment, such as retinoic acid and TGF-β, have also been implicated in gut homing and IgA CSR (80, 84, 85). Furthermore, intestinal epithelial cell endoplasmic reticulum stress induces the activation and proliferation of peritoneal B1 cells and recruitment of microbiota-reactive, TI PCs to the intestine (86). TD IgA responses in Peyer's patches, including those of microbiota-reactive IgA, require B cell CD40-CD40L interactions with T cells, which lead to upregulation of CCR6 in B cells, subsequent migration to the subepithelial dome by the activated B cell, and local dendritic cell–mediated activation of TGF-β and IgA class-switching (80) (**Figure 2**).

Sequencing of members of the microbiota bound to IgA has enabled the exploration of the landscape of IgA responses to commensal bacteria. This revealed that IgA predominantly targets bacteria that reside in the small intestine (87, 88). T cells appear to be dispensable for most commensal IgA binding, while select taxa such as segmented filamentous bacteria (SFB) and *Mucispirillum* spp. require TD responses for IgA coating (88) (**Figure 2**). Indeed, a large fraction of IgA antibodies have natural polyreactive specificities and bind multiple structurally diverse antigens and commensal bacteria with low affinity (81). Furthermore, the presence of somatic hypermutation (SHM) in some of these sequences does not confer increased affinity, arguing that TD affinity maturation may not play a major role in some microbiota-reactive IgA (81, 89). However, TD examples of high-affinity antibody responses to commensal microbiota do occur (87, 90, 91), and the extent to which TI and TD responses contribute to commensal-specific IgA is still debated (87, 89). Regardless, in the absence of T cells, the levels of intestinal IgA PCs and luminal IgA are severely reduced (76, 88), supporting the idea that T cells do play important roles in enabling IgA

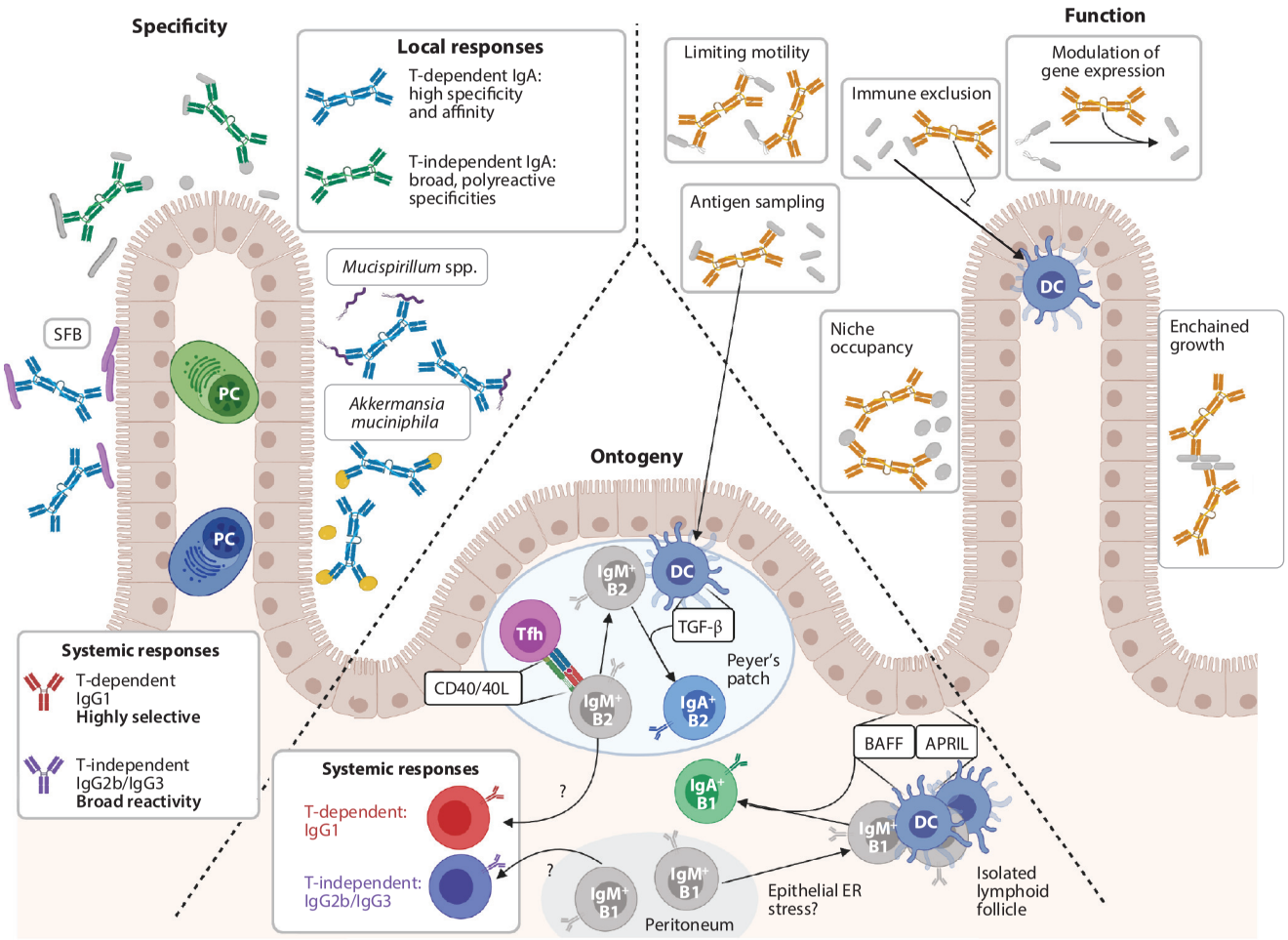


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Figure 2 (*Figure appears on preceding page*)

Commensal-reactive T-dependent and T-independent antibody responses control host-microbiota mutualism. Specificity: T-dependent and -independent IgAs coat a large and diverse fraction of the microbiota at homeostasis. Systemic, T-independent IgG2b and IgG3 responses also target a diverse and overlapping fraction of commensal bacteria, whereas IgG1 and IgA responses that are highly selective to immunostimulatory members of the microbiota are induced in a T-dependent manner. Ontogeny: B1 cells are a major contributor to T-independent IgA, and potentially to IgG2b and IgG3. B1 cells are thought to migrate from the peritoneum to the intestine in response to poorly characterized signals, including intestinal epithelial ER stress, and undergo class-switch recombination in response to local factors including epithelial- and dendritic cell–derived BAFF and APRIL. T-dependent IgA responses occur locally in lymphoid structures such as the Peyer's patches from naive B2 cells. Interactions with antigen-specific Tfh cells induce migration of B cells to the subepithelial dome, where interactions with dendritic cells induce TGF-β-mediated class-switch recombination to IgA. The ontogeny of systemic IgG1 and IgG2b/3 remains unclear but is thought to originate from similar B cell precursors as IgA responses. Function: Limiting bacterial motility, enabling occupancy of mucosal-associated niches, preventing antigen uptake and immune activation, enchainment of growing bacteria limiting invasion, and modulation of bacterial gene expression have all been proposed as functions of microbiota-reactive antibodies. Abbreviations: DC, dendritic cell; ER, endoplasmic reticulum; PC, plasma cell; Tfh, T follicular helper. Figure adapted from image created with BioRender.com.

responses to the microbiota. Furthermore, mice carrying a mutation in AID that abrogates SHM but allows CSR revealed that SHM, not just IgA class-switching, is required to prevent aberrant expansion of small-intestine microbiota and germinal center hyperplasia (92). Finally, abundant IgA coating in the context of inflammatory bowel disease identifies specific members of the microbiota with the potential to drive intestinal inflammation (91), and IgA binding also identifies microbial taxa capable of inducing enteropathy in undernourished children (93). Previous work supported the idea that mucosal IgA responses may lack classical memory characteristics and are able to change according to fluctuations in microbiota composition. Indeed, established mucosal IgA specificities are outcompeted by novel antibacterial responses allowing the mucosal immune system to respond to a constantly changing microbiota (94, 95). Whether this phenomenon is true for TI and TD responses remains to be addressed. Thus, the current model is that IgA responses to the microbiota include both TI and TD specificities that play complementary roles in the control of host-microbiota responses.

Selective IgA deficiency is the most common primary immunodeficiency in humans (96). However, a large percentage of individuals are asymptomatic, and compensatory mechanisms such as increased secretory IgM have been reported (96). Indeed, IgM and IgG antibodies also play important roles in protection of many barrier sites (97), and while IgM^+ and IgG^+ PCs are mostly absent from the murine intestine, they are abundant in the human gut (89, 98, 99). This observation may reflect the fact that mice are raised in pathogen-free settings and in the absence of natural stressors. Early seminal work suggested that IgG antibodies do not recognize most commensal bacteria during homeostasis and that commensal IgG recognition is predominantly induced in the context of intestinal barrier breach (100). Indeed, invasive members of the *Enterobacteriaceae* family that are able to reach systemic sites induce IgG antibodies that can protect against related pathogens (101). However, during homeostasis, mice mount systemic IgG antibody responses to a large, phylogenetically diverse fraction of the intestinal microbiota (39). The bulk of this response consists of TI, Toll-like receptor (TLR)-dependent IgG2b and IgG3 antibodies, and the large overlap between IgG2b/3- and IgA-targeted commensals suggests that they may share similar specificities and ontogeny (39). Additionally, a small subset of commensal bacteria, including *Akkermansia muciniphila*, are targeted by high-affinity TD IgG1 and induce cognate T follicular helper (Tfh) responses (90). Thus, similarly to the case of IgA, both TI and TD IgG responses to the microbiota are induced and target different subsets of commensal bacteria (**Figure 2**).

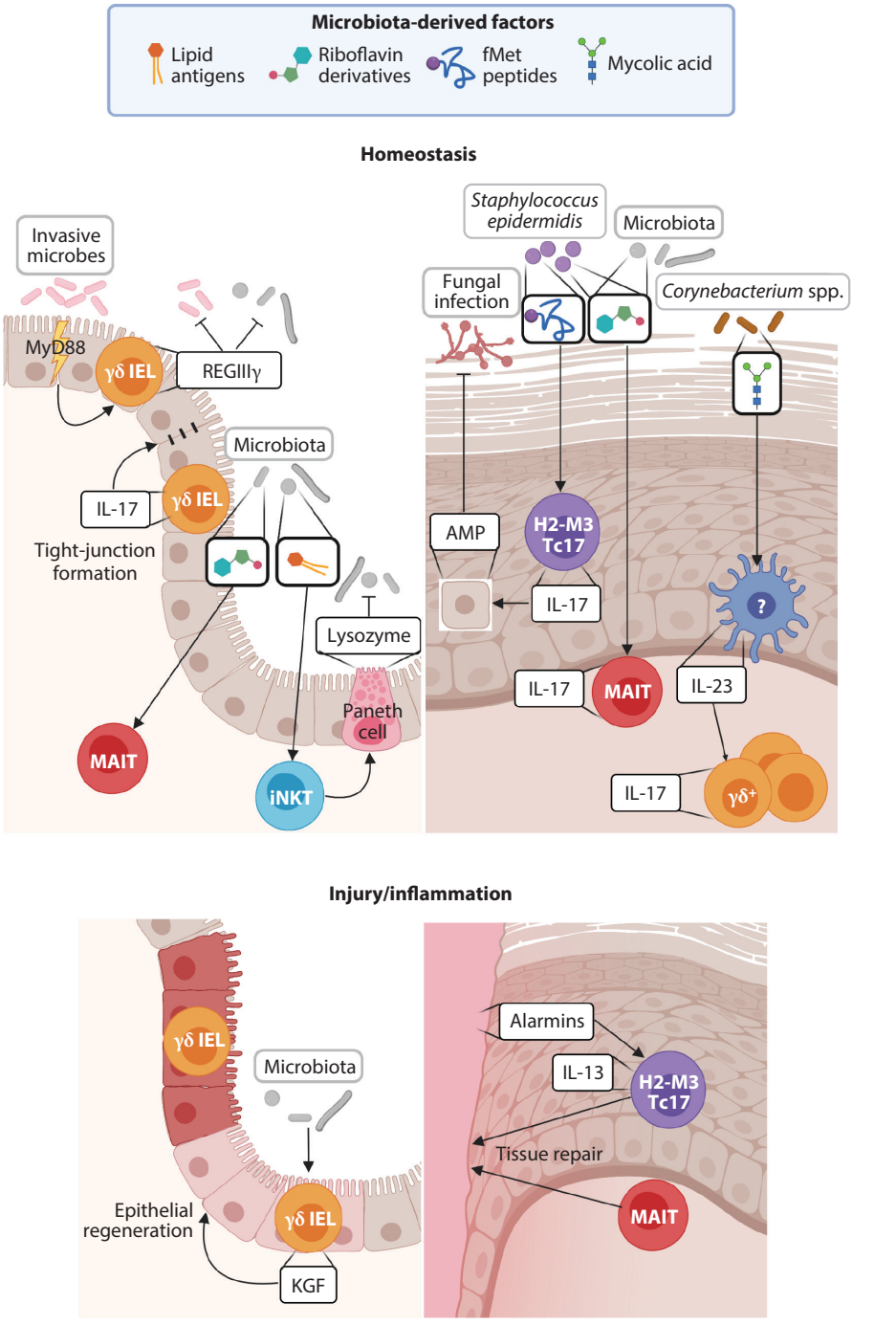
Recent efforts have been devoted to elucidating the role of antibodies, and in particular IgA, in the control of host-microbiota mutualism. Study of the divergent adaptive immune system of teleosts revealed that their secretory immunoglobulin (SIgT) is involved in both protection

from mucosal infections and establishment of homeostasis with the microbiota (102); similar observations have been made of other adaptive immune systems. Like SIgA in mammals, SIgT in rainbow trout coats a significant fraction of the microbiota during homeostasis, and IgT depletion leads to marked dysbiosis, loss of SIgT-targeted taxa, microbial translocation, and tissue damage (102). Consequently, this study argues that mediating mutualistic interactions with the microbiota is an ancestral function of secretory antibodies arising early in the evolution of the adaptive immune system. A plethora of functions have been suggested for microbiota-reactive IgA antibodies (**Figure 2**). Attributing differences in microbiota composition to a specific factor such as IgA requires rigorous experimental design to avoid caveats with microbiome variability and drift (103). Still, many studies argue that IgA antibodies regulate the colonization, diversity, and composition of bacterial communities in the intestine (40, 104, 105). A variety of mechanisms have been invoked (**Figure 2**). For example, IgA can enable colonization of a privileged mucus-associated niche for some commensal species (106), and enchainment of dividing bacteria may limit invasiveness (107). Furthermore, TLR5-dependent, flagellin-binding IgA can limit bacterial motility and overall flagellin expression by the microbiota (108). Additionally, use of a *Bacteroides thetaiotaomicron* monocolonization system in *Rag1*$^{-/-}$ mice carrying a hybridoma that produced *B. thetaiotaomicron*–specific IgA suggested both direct and indirect in vivo modulation of bacterial gene expression by IgA (70). Microbiota-specific IgA can mediate immune exclusion of its targets, retaining antigen in the intestinal lumen and preventing further priming of immune responses: Flagellin-specific, TD IgA prevents systemic activation of CBir1 TCR transgenic T cells, which are specific for a commensal flagellin (109). This observation, coupled with programmed early-life antigen uptake of CBir1 flagellin (42), may help explain why CBir1 flagellin-specific T cells remain naive during homeostasis and are only activated during a specific early-life window (42) or in the context of barrier breach (110). Conversely, polyreactive IgA antibodies may instead enhance bacterial delivery to the Peyer's patches in the small intestine (105), which could increase immune priming. Finally, commensal-reactive antibodies can also positively and negatively impact disease outcomes: Systemic IgA, which unlike mucosal IgA is mostly TD, can protect against sepsis after barrier breach and bacteremia (111). On the other hand, microbiota-specific IgG antibodies, which are increased in ulcerative colitis patients, can contribute to pathogenesis by activating intestinal macrophages through Fc receptors and fueling inflammation (112). While most of what we understand today about the role of antibodies in host-microbiota interactions derives from the exploration of the gut environment, it is known that in humans, IgA is secreted at other mucosal surfaces as well as within the skin surface by eccrine and sebaceous glands (113). The impact of these responses on microbiota composition and function remains to be addressed.

In conclusion, the evolution of antibodies is tightly linked to their role in maintaining homeostatic relationships with the microbiota. In mammals, TD and TI commensal-reactive specificities have unique ontogenies and appear to target functionally distinct subsets of commensal bacteria while exhibiting a range of potential homeostatic functions. However, we have yet to address how distinct subsets or pathways contribute to the control of host-microbiota homeostasis at distinct developmental stages or in specific contexts.

3.2. Unconventional Immunity to the Microbiota

In addition to their role in early life, unconventional and innate-like lymphocytes contribute to the long-term maintenance of homeostatic responses to the microbiota. Indeed, as discussed above, in Section 2.4, the ability of unconventional T cells to respond to canonical antigens derived from a large fraction of the microbiota makes these cells powerful regulators of tissue physiology, with an increasingly appreciated role in tissue repair (**Figure 3**).



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Unconventional T cells engage in a lifelong dialog with the microbiota and control tissue homeostasis. Unconventional T cells, including γδ T cells, MAIT cells, iNKT cells, and Tc17 cells, seed barrier tissues such as the skin and gut, where they control numerous physiological tissue responses. These include regulation of the microbiota composition and promotion of tissue repair. In response to epithelial MyD88 signaling, gut epithelium–resident γδ T cells produce the AMP REGIIIγ, which in turn regulates microbial composition and localization. Colonization can also be regulated indirectly in response to microbial antigens: Microbiota-derived lipids induce iNKT-dependent Paneth cell secretion of lysozyme. Within the skin, IL-17-producing H2-M3-restricted $CD8^+$ T cells accumulate in the epidermis in response to *Staphylococcus epidermidis*–derived *N*-formylated peptides. These T cells promote the production of AMPs in an IL-17A-dependent manner, thereby enhancing protection against subsequent infection. MAIT cells and γδ T cells are also enriched within the skin and respond to microbiota-derived metabolites, microbiota-induced cytokines, or both. In the gut, γδ T cell–derived IL-17A contributes to tight-junction formation between enterocytes, and following tissue injury these cells are potent producers of the epithelial mitogen KGF. Skin-resident unconventional T cells, including MAIT cells and H2-M3-restricted $CD8^+$ T cells, also contribute to wound healing in an IL-13-dependent manner. Abbreviations: AMP, antimicrobial peptide; fMet, N-formylated; IEL, intraepithelial lymphocyte; iNKT, invariant natural killer T; KGF, keratinocyte growth factor; MAIT, mucosal-associated invariant T; Tc17, H2-M3-restricted $CD8^+$ T. Figure adapted from image created with BioRender.com.

Throughout life, barrier tissues are marred by frequent inflammatory insults requiring rapid induction of repair mechanisms that are under the control of several redundant cell subsets and pathways. One of the first indications of a role for the microbiota in tissue repair was the observation that, in the gut, TLR activation by commensals is required to protect the host from acute injury (114). Dendritic epidermal T cells were the first subset of unconventional T cells linked to tissue repair, a property that has since been attributed to numerous other subsets of innate-like or nonclassically restricted T cells (115). Within the gut, intraepithelial γδ T cells are the majority of innate-like T cells within the epithelium and contribute to mucosal repair via the production of keratinocyte growth factor (KGF) (116) (**Figure 3**). MAIT cells from both humans and mice constitutively express a wound healing program (64, 117). Within the skin, MAIT cells can make up a significant portion of lymphocytes: up to 40% of the αβ T cells in mice and approximately 2% of $CD3^+$ lymphocytes in humans (64, 118). The enrichment within this tissue could be explained by the fact that a significant portion of the skin microbiota express the machinery associated with the production of MAIT cell antigens (64). Following skin colonization with *S. epidermidis*, a commensal capable of synthesizing riboflavin derivatives, MAIT cells significantly expand and contribute to wound repair (64) (**Figure 3**). Similarly, *S. epidermidis* promotes the induction of nonclassical MHC-Ib-restricted $CD8^+$ T cells (119–121). Topical exposure to a particular clade of *S. epidermidis* also promotes the induction and tissue accumulation of H2-M3-restricted $CD8^+$ T cells specific for commensal-derived *N*-formyl-containing peptides (120). Within the epidermis these commensal-specific IL-17-producing $CD8^+$ T cells can promote tissue repair by releasing IL-13 in response to tissue alarmins (119–121) (**Figure 3**). While H2-M3 is absent in humans (58), we could postulate that alternate mechanisms of antigen presentation may be in place to recognize these highly abundant microbiota-derived *N*-formylated peptides.

Unconventional T cells also regulate microbiota composition and protect against microbial invasion. For instance, γδ T cells from the intraepithelial lymphocyte (IEL) compartment promote production of the antimicrobial peptide REGIIIγ at steady state and constrain the microbiota in the context of mucosal damage (122, 123). The immunomodulatory, antibacterial program expressed by γδ T cells is controlled by the microbiota itself, and microbial sensing via epithelial MyD88 governs γδ T cell metabolism and dynamics of epithelial surveillance (122, 124) (**Figure 3**). Responses of iNKT cells to microbe-derived lipid antigens presented by CD1d (58)

regulate intestinal Paneth cell function and production of lysozyme, an enzyme that catalyzes the destruction of bacterial cell walls (125). As such, iNKT cells can indirectly limit bacterial translocation across the intestinal barrier (125) (**Figure 3**). Further, nonclassical MHC-Ib-restricted $CD8^+$ T cells induced by skin commensals can promote the production of antimicrobial peptides by keratinocytes, thereby promoting heterologous protection against subsequent fungal infection (119) (**Figure 3**). Similarly, within the eye, IL-17-producing γδ T cells elicited in response to the commensal microbe *Corynebacterium mastitidis* provide heterologous protection against ocular fungal infections via local increase in antimicrobial peptides (126). Within the skin, IL-17-producing γδ T cells also respond to colonization with *Corynebacterium* in a mycolic acid/IL-23-dependent manner (127) (**Figure 3**). The nature of the antigens recognized by γδT cells remains largely unknown, but these cells are believed to also recognize lipid and phosphoantigens (128), a point of particular relevance for potential recognition of commensal-derived products. MAIT cells have also been proposed to contribute to antimicrobial defense, and a large number of bacteria and yeast, including pathogens, are prolific producers of riboflavin derivatives (60, 129).

Emerging evidence supports the idea that the fundamental role of innate-like T cells may result from their action as a network of cells with overlapping and potentially synergistic functions rather than as individual subsets. For instance, H2-M3-restricted $CD8^+$ T cells, MAIT cells, and γδ T cells all produce type 17 cytokines involved in barrier function and immune surveillance (64, 119, 126, 127) (**Figure 3**). These cell subsets also compete for a shared physiological niche (64, 66). This phenomenon was recently confirmed in a patient harboring a homozygous point mutation in MR1 associated with the absence of MAIT cells and concomitant expansion of γδ T cells (130). The redundancy of innate-like T cells and ability to compete for similar survival factors or niches may have been maintained through evolution as a way to increase tissue resilience and long-term protection of host homeostasis.

3.3. T Cell Responses to the Microbiota

As discussed above, microbes at all barrier surfaces constitutively engage the immune system and promote the induction of cognate responses, including noninflammatory responses directed at the microbiota itself (131). Together with unconventional responses, adaptive T cell responses to the microbiota converge to broadly and contextually control tissue homeostasis.

While the microbiota as a whole can promote all aspects of host immunity, individual species or groups of bacteria can be the predominant influence on the immune system under steady state (**Figure 4**). SFB are a prototype of such keystone species in the gastrointestinal tract. These spore-forming, gram-positive anaerobic bacteria colonize the terminal ileum of mice and have a dominant effect on the mucosal immune system by promoting the accumulation of Th17 cells as well as driving the production of IgA (132–136). Notably, SFB-specific Th17 cells not only promote local antimicrobial defenses but also impact local and systemic inflammatory responses (133, 136–138). In contrast to most members of the microbiota that reside outside of the gut demilitarized zone (139), SFB interact closely with their host via tight adhesion to Peyer's patches and epithelial cells (140, 141). Indeed, intimate contact with epithelial cells, a property shared by a minority of commensal organisms, is proposed to account for the ability of specific microbes to preferentially induce immune responses and heighten tissue immunity (133, 142).

In vivo screening of phylogenetically distinct bacteria in monocolonized germ-free mice supports the idea that only a handful of microbes can promote and enhance accumulation of effector T cells. For example, *Coprobacillus* induces IL-10-expressing T cells, *Bifidobacterium longum* induces Th1 cells, and *Bifidobacterium adolescentis* promotes Th17 cells (143, 144). However, it is important to acknowledge that while informative, studies using monocolonized germ-free mice

have obvious limitations since physiological responses depend on the preexistence of a complex microbiota. Studies with defined bacteria consortia in gnotobiotic animals also reveal that the ability to stimulate effector T cell responses is microbe and consortium dependent (145). Early studies examining T cell immunity to a clostridial flagellin (CBir1) revealed that CBir1-specific T cells remain naive during homeostasis in the context of a complex microbiota (109), arguing that some commensal antigens may indeed be ignored (**Figure 4**). However, lack of CBir1-specific T cell activation during homeostasis depends on preexisting antigen-specific IgA (109), and subsequent studies revealed that CBir1 antigen is sampled and induces antigen-specific Treg cell responses during an early-life window via temporally regulated goblet-associated passages (42). This suggests that while continuous antigen sampling and T cell activation may not occur, initial encounters with this antigen can lead to immune recognition and T cell activation (143, 144). Whether induction of cognate immunity to all gut commensals is the norm as opposed to being

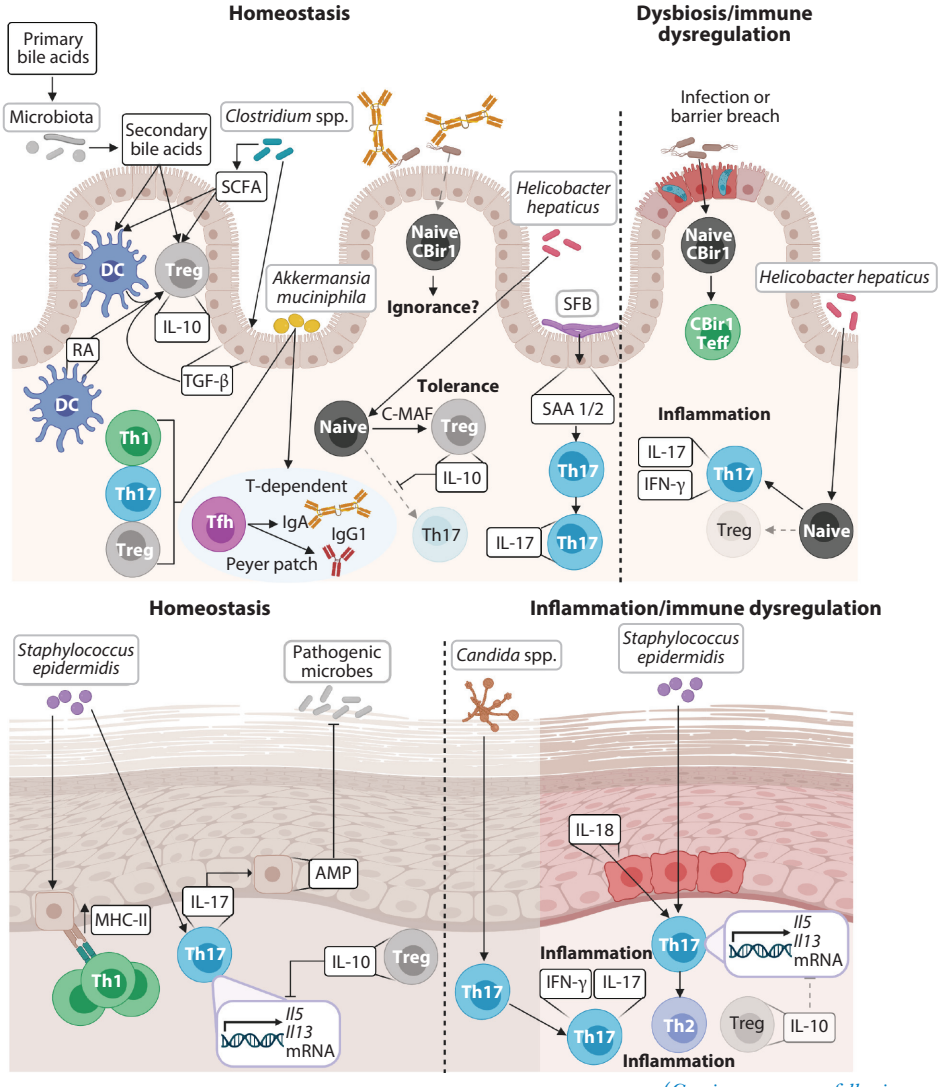


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Figure 4 (*Figure appears on preceding page*)

Microbiota-specific T cell immunity in homeostasis and disease. Treg cells are induced in response to microbiota colonization. Microbiota-derived metabolites, including SCFAs and secondary bile acids, impact Treg cell development and tissue accumulation via action on Treg cells or via DCs. SFB induce homeostatic Th17 cells in the small intestine, and SFB adhesion to epithelial cells leads to production of SAA1 and SAA2, which act on local Th17 cells to license IL-17 production. *Akkermansia muciniphila* induces a strong Tfh response in Peyer's patches in gnotobiotic settings, but in the context of a complex microbiota, *A. muciniphila* promotes various cell subsets, including Th17, Th1, and Treg cells. *Helicobacter hepaticus* induces RORγt$^+$ Treg cells during homeostasis. However, in the context of immune dysregulation, such as in the absence of IL-10, *Helicobacter hepaticus*–specific T cells adopt a proinflammatory Th17 program with coexpression of IL-17 and IFN-γ, leading to tissue inflammation. CBir1-specific T cells remain naive during homeostasis but adopt proinflammatory fates during infection or intestinal inflammation. Within the skin, *Staphylococcus epidermidis* induces classical Th1 and Th17 responses. Th1 cell accumulation within the skin is controlled by keratinocyte MHC-II expression. Tc17 (**Figure 3**) and Th17 responses to *S. epidermidis* enhance protection from subsequent infections by inducing AMP expression. *S. epidermidis*–specific Th17 cells also express a poised type II transcriptome, leading to the production of type 2 cytokines in the context of tissue injury and exposure to local alarmins such as IL-18. Type 2 cytokine production by commensal-specific T cells promotes tissue repair in an IL-13-dependent manner but can also contribute to tissue inflammation in the context of defects in local regulation. Preexisting fungus-specific Th17 cells can contribute to inflammation in the context of psoriasis. Abbreviations: AMP, antimicrobial peptide; DC, dendritic cell; MHC, major histocompatibility complex; RA, retinoic acid; SAA, serum amyloid A; SCFA, short-chain fatty acid; SFB, segmented filamentous bacteria; Tc17, H2-M3-restricted CD8$^+$ T; Teff, T effector; Tfh, T follicular helper; Th, T helper; Treg, regulatory T. Figure adapted from image created with BioRender.com.

restricted to specific microbes remains difficult to confirm at this stage, and further development of tools allowing us to track commensal-specific T cells in both mice and humans is required to address this fundamental question.

Most bacteria and commensal fungi colonizing the skin can promote commensal-specific T cell responses (119, 146, 147) (**Figure 4**). The ability of the skin to rapidly respond to new microbes could be, in part, explained by the low density of resident microbes within the skin and their localization in highly specialized appendages such as hair follicles and sebaceous glands (148). As discussed in Section 3.2, commensal *S. epidermidis*–specific T cells (classical and nonclassical) accumulate within the epidermis, where they constitutively act on keratinocytes in an IL-17-dependent manner to promote antimicrobial peptide production and enhanced responses against subsequent infections (119). This ability of commensal-specific T cells to broadly heighten tissue immunity and promote heterologous immunity is conserved across barrier tissues (133).

The microbiota play a dominant role in the control of tissue repair via numerous mechanisms, including promotion of T cell responses that remodel or repair tissue (64, 120, 121, 149, 150). Commensal-specific T cells promote immunity and tissue repair through distinct molecular mechanisms. Indeed, within the skin, commensal-specific, RORγt-expressing CD4$^+$ and CD8$^+$ T cells (but not those induced by infection) coexpress a poised type 2 transcriptome that can be unleashed in the context of tissue injury and exposure to alarmins such as IL-18 (121). As such, upon tissue damage, release of type 2 cytokines and in particular IL-13 by commensal-specific T cells directly contributes to their ability to promote tissue repair (121). Thus, commensal-specific T cells can co-opt tissue residency and cell-intrinsic flexibility as a means to promote both local immunity and tissue adaptation to injury.

Tissues not only dictate the induction of responses directed toward the microbiota but also provide specific checkpoints allowing the functional licensing of commensal-specific T cells (**Figure 4**). Indeed, upon accumulation in tissues, commensal-specific T cells exert their local function via the release of cytokines such as IL-17A and IL-22, the production of which is tightly regulated by tissue-specific cues. For instance, within the skin, local production of IL-1α is

required to license cutaneous T cells to release IL-17A (151), while the vast majority of Th17 responses to the gut microbiota can develop in the absence of this cytokine (140). On the other hand, within the gut, serum amyloid A (SAA) proteins play a central role in the control of homeostatic Th17 responses. Notably, colonization of mice with microbes able to adhere to the epithelium, such as SFB, triggers the local secretion of SAA1 and SAA2 by epithelial cells that remain confined to the ileum and locally license IL-17A production by SFB-specific RORγt-expressing T cells (133, 152). SAAs are also carriers of high-density lipoprotein and retinol (153), and as such they can potentially deliver these molecules to antigen-presenting cells, providing another amplification mechanism for the induction of IL-17 responses within tissues. The microbiota also promote epithelial cell release of cytokines such as IL-18, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) that can promote innate lymphoid cell responses that in turn can contribute to the induction and control of commensal-specific T cells (68, 152). Epithelial cells can also coordinate responses to the microbiota in both the skin and the gastrointestinal tract via local release of chemokines involved in T cell tropism, such as CXCL9, CXCL10, and CCL20 (145, 154). Within the skin, accumulation and licensing of commensal-induced Th1 cells, but not Th17 cells, were controlled by MHC-II-expressing keratinocytes (154). Similarly, studies of the gut barrier demonstrated a role for epithelial cell expression of MHC-II in the accumulation of $CD4^+$ $CD8\alpha\alpha^+$ IELs (155). Much remains to be learned about how structural cells, including epithelial and stromal cells from distinct compartments, respond to commensals in a way that promotes the induction and function of commensal-specific responses.

While the default program of immune responses directed toward the microbiota is associated with the preservation of tissue homeostasis, the fate of commensal-specific T cells is highly contextual and can be affected by host activation status, the microenvironment, and the composition of the microbiota itself. This latter point can be illustrated by the contextual fate of responses to *A. muciniphila* (90). *A. muciniphila* is a member of the microbiota of both mice and humans that impacts a large array of biological outcomes, ranging from host metabolism to response to checkpoint therapy (156, 157). *A. muciniphila* exclusively promotes the generation of Tfh cells in a gnotobiotic, nine-member bacterial community (90). However, in the context of a complex microbiota, *A. muciniphila*–specific T cells can adopt highly diverse fates, including differentiation into Th1, Th17, and Treg cells (90). Thus, microbiota composition and function can contribute to the quality of responses directed toward the microbiota, illustrating once again the limitation of monoassociation studies.

Infections are a highly volatile setting for barrier tissues. During infection, commensal antigens are recognized in an inflammatory context. Furthermore, breach of the intestinal or skin barrier due to infection allows commensal antigen translocation to ectopic sites. In this context, commensal-specific T cells can differentiate to an inflammatory phenotype and form memory cells that are phenotypically and functionally indistinguishable from pathogen-specific T cells (110). Indeed, CBir1-specific T cells, which remain naive during homeostasis, are activated and adopt T cell fates consistent with the inflammatory challenge: *Toxoplasma gondii* infection leads to CBir1-specific Th1 cell differentiation, while chemically induced colitis induces Th17 differentiation (110). Barrier tissues are also involved in numerous inflammatory disorders, a phenomenon that can trigger the induction of aberrant and inflammatory responses to the microbiota. For instance, under steady-state conditions, *Helicobacter hepaticus* colonizes the gut without causing inflammation, a process associated with the induction of both $ROR\gamma t^+$ Treg cells and Tfh cells (158). On the other hand, in the context of defects in tissue regulation, including in Treg cells, the same microbe can also trigger severe colitis (158–161). Mechanistically, a heightened level of IL-23 together with systemic production of SAAs, as opposed to discrete production in steady state,

promotes *H. hepaticus*–specific, pathogenic Th17 responses and expression of a proinflammatory program, including coexpression of T-bet/RORγt and IFN-γ/IL-17 (152, 162).

It is unknown whether commensal-specific T cells are maintained independent of continuous cognate antigen stimulation in a manner similar to that of virus-induced tissue-resident memory T cells or IELs (155, 163). Commensal-specific memory T cells decline steadily over time (110, 164), and the ability of the microbiota to promote true immunological T cell memory at steady state or in the context of inflammation remains an open question. Perhaps an evolving pool of specificities within the regulatory and effector T cell compartment allows for the maintenance of both tissue immunity and tolerance to the microbiota in the face of microbiota shifts and intermittent infections. Nonetheless, based on the scope of antigenic load within the microbiota, a significant fraction of effector and memory cells, including tissue-resident memory T cells, may be microbiota specific. The implication of this phenomenon is that barrier breach or infection at barrier sites is likely to occur in the context of broader recall responses against microbiota-derived antigens. For instance, within the skin, preexistence of homeostatic Th17 responses to fungal commensals can promote inflammatory responses in the context of subsequent experimental psoriasis (147). The long-term fate of microbiota-specific T cells as well as the relative contribution of memory responses or neo-inflammatory responses to the microbiota in the etiology of inflammatory disorders remains to be addressed.

3.4. Immunoregulatory T Cells

Maintenance of tissue homeostasis in the face of the ability of the microbiota to engage the immune system requires both constitutive (e.g., barrier structures, mucus) and induced immunoregulatory responses. Indeed, the ability of the microbiota to promote immunity is coupled with its ability to promote numerous immunoregulatory mechanisms. The balance of these responses defines the tissue threshold of activation. Failure to regulate responses to the microbiota can have catastrophic consequences for the host, as exemplified in the context of numerous inflammatory disorders, including inflammatory bowel diseases (165). While regulation of responses to the microbiota encompasses multiple structural and cellular components, we focus our present discussion on the role of T cells, and more specifically Treg cells, in these processes.

FOXP3$^+$ Treg cells play a nonredundant role in the maintenance of immunological homeostasis (166). Within the gut, induced Treg cells constrain responses to orally acquired antigens, including antigens derived from the microbiota. This phenomenon is promoted by a complex network of cells, including specialized antigen-presenting cells, as well as a milieu enriched in TGF-β, retinoic acid, and, as further discussed, microbiota-derived metabolites (167–170) (**Figure 4**). It has been long recognized that oral tolerance, a phenomenon associated with the induction of FOXP3$^+$ Treg cells (169), cannot be induced in the absence of the microbiota (171). Indeed, subsets of Treg cells are reduced under germ-free conditions or in the context of antibiotic treatment (172–175). Although tools to track commensal-specific responses remain limited, the use of microbe-specific transgenic T cells supports the idea that within the gut, and in particular the colon, Treg cells are enriched for TCRs specific for microbiota-derived antigens (54, 158, 176, 177). While in germ-free settings only a few individual microbes promote the induction of effector responses, a large number of isolates or microbiota-derived consortia are able to promote Treg cell induction and function (90, 143, 172, 178). This supports the ideas that Treg cell induction is a dominant feature of microbes associated with health and that these responses are promoted by microbiota-derived, canonical, and redundant factors. In contrast, microbes that dominate in the context of inflammatory states such as IBD fail to promote Treg cells when transferred to mice (91, 179). While IL-10 production by Treg cells plays a dominant role in the gastrointestinal

tract, Treg cells use several redundant and complementary mechanisms to constrain reactivity to the microbiota (166, 180).

The capacity of Treg cells to adapt to specific environments and to respond to tissue-specific cues contributes to their ability to regulate tissues colonized by the microbiota (166). For instance, upon migration into the gut epithelial layer, Treg cells lose FOXP3 expression and convert to $CD4^+$ IELs in a microbiota-dependent manner, a phenomenon that may contribute to the ability of these cells to regulate this compartment (181). The microbiota also specifically induce a subset of Treg cells expressing RORγt with a preferential tropism for the colon lamina propria (173, 174, 182). The accumulation of RORγt-expressing Treg cells occurs between two and three weeks after birth (173, 174), which coincides with weaning associated with a profound immune reprograming and a shift in microbiota composition (43). This subset of Treg cells has been associated with the control of aberrant immune responses to specific commensals. For instance, the ability of *H. hepaticus* to promote the induction of RORγt-expressing Treg cells and associated IL-10 constrains inflammatory responses to the very same microbe (158). The induction and function of this subset depend on the expression of the transcription factor C-MAF (158, 183). Of note, in the context of *H. hepaticus* colonization and C-MAF but not RORγt deletion, Treg cell function is impaired (158). On the other hand, RORγt deletion in FOXP3-expressing cells is associated with either enhanced Th2 responses and worm-induced, Th2-mediated pathology (173) or enhanced type 1/17 responses and enhanced susceptibility to experimental colitis (174). Based on the fundamental role of the microbiota in setting the host immune set point, we could speculate that these differential outcomes are related to distinct microbiota-associated immune imprinting.

Numerous microbiota-associated factors converge to promote optimal Treg cell induction and function. For instance, SCFAs, metabolites produced in the colon by bacterial fermentation of dietary fibers and resistant starch, can promote numerous biological functions, including Treg cell induction (173, 184–186). As such, microbes endowed with the ability to generate large amounts of SCFAs, such as consortia of *Clostridium* strains, can preferentially induce Treg cells (187, 188). Mechanistically, SCFAs have been proposed to act directly on T cells and indirectly on macrophages or dendritic cells via histone deacetylase inhibition, G protein–coupled receptors such as GPR43, or both (173, 184–186). Promotion of Treg cells by the microbiota is also associated with the ability of specific microbes such as *Clostridium* to promote a TGF-β-rich environment (187, 189).

Other canonical products resulting from microbiota activity have been recently shown to promote Treg cell induction. Bile acids are highly abundant in the gut, where they undergo bacterium-mediated transformation leading to the generation of a large pool of bioactive molecules. Recent work revealed that diet alteration impacts the level of colonic $ROR\gamma t^+$ Treg cells, and a role for microbial bile metabolites in this phenomenon was proposed (190–192). For instance, isoalloLCA, a secondary bile acid, directly promotes Treg cell induction via mitochondrial reactive oxygen responses in T cells (191). Secondary bile acids, which are the products of metabolism of primary bile acids by the microbiota, can also enhance the ability of dendritic cells to promote Treg cells, as in the case of isoDCA (190). Because of the fundamental importance of Treg cells in constraining reactivity to the microbiota, each of these factors is likely to act in a synergistic and redundant manner. Further, it is likely that a large fraction of microbiota-derived products at all barrier sites have coevolved with the immune system to favor regulatory responses. For instance, specific microbe-associated molecules such as the *B. fragilis*–derived capsular factor polysaccharide A can impact dendritic cell function in a manner that promotes Treg cell induction, and cell surface polysaccharides of *Bifidobacterium bifidum* promote the induction of IL-10-producing Treg cells in a TLR2-dependent manner (193–195).

In contrast to gut microbes, skin microbes are not essential for the optimal seeding of dermal Treg cells (151). As shown within the gut, the continuous action of Treg cells within the skin controls reactivity to commensals acquired later in life. For instance, in mice with a specific defect in Treg fitness within the skin, RORγt-expressing, commensal-specific T cells produce aberrant type 2 cytokines, a phenomenon that can have consequences in the etiology of skin inflammatory disorders (121). As discussed in Section 2.3, neonatal colonization of the skin with *S. epidermidis* can promote accumulation of commensal-specific FOXP3$^+$ Treg cells, thereby preventing inflammatory reactivity to the same microbe later in life (54). Whether this phenomenon applies only to specific microbes encountered early in life remains to be addressed. A recent study also supported the idea that microbes potentially able to cause disease escape such regulatory imprinting (196). For instance, induction of IL-1β in response to *Staphylococcus aureus*–associated α-toxin actively inhibits the induction of Treg cells in neonates (196), which may contribute to the maintenance of immune awareness of these microbes later in life. Importantly, many other cell types involved in immune surveillance also help enforce immune tolerance in response to the microbiota, either indirectly by supporting Treg cell induction and function (197) or directly by secreting immunomodulatory factors (198–200).

Altogether these findings support major roles for the microbiota in shaping the repertoire, number, and activation of Treg cells and in maintenance of host-microbe mutualism at barrier sites. However, much remains to be learned about the molecular basis of host-microbe interactions within individual barrier sites and the overall dynamic of effector versus regulatory responses required to maintain or restore host-microbiota homeostasis.

4. CONCLUSIONS AND PERSPECTIVES

Our increasing integration of the microbiome in our understanding of host physiology has transformed our understanding of the immune system and its relationship with its symbionts. Not only do immune cells and the microbiota help control infections and malignancy but the extraordinary plasticity and motility of immune cells and their reliance on the highly dynamic microbiota also bridge virtually all physiological systems, making the immune system and microbiota central regulators of host homeostasis.

This novel understanding also brings unique challenges. Exploration of the relationship between the microbiota and the immune system in health and disease requires the integration of numerous other branches of biology, including biochemistry, cellular biology, genetics, and microbiology. Emerging evidence reveals that harnessing the microbiota for therapeutic purposes may be a valid and promising approach. For instance, in the gut, microbe-mediated therapy in the form of fecal transplantation has been successful for *Clostridium difficile* infections (201), and *Lactobacillus plantarum* has been successful in preventing neonatal sepsis (202). Harnessing the immunomodulatory or antimicrobial properties of particular members of the microbiota also has great potential. Furthermore, prebiotic intervention or local alterations in specific nutrients may have a marked impact on the composition or function of microbiota at all barrier surfaces and—when rationally designed—could promote the expansion of microbes endowed with regulatory or protective properties. However, it is important to remember that mechanistic exploration of immune system–microbiota interactions is relatively recent, and despite the extraordinary diversity of microbial products and antigens, only a few instances of immune recognition of microbiota-derived antigens or metabolites have been identified. Further exploration of host-microbiota interactions is groundwork for the formidable task of developing novel approaches and unique tools for the next generation of therapeutics.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Sender R, Fuchs S, Milo R. 2016. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164(3):337–40
2. Dethlefsen L, McFall-Ngai M, Relman DA. 2007. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* 449:811–18
3. Collins N, Belkaid Y. 2018. Do the microbiota influence vaccines and protective immunity to pathogens? Engaging our endogenous adjuvants. *Cold Spring Harb. Perspect. Biol.* 10(2):a028860
4. Kroemer G, Zitvogel L. 2018. Cancer immunotherapy in 2017: the breakthrough of the microbiota. *Nat. Rev. Immunol.* 18(2):87–88
5. Belkaid Y, Harrison OJ. 2017. Homeostatic immunity and the microbiota. *Immunity* 46(4):562–76
6. Blander JM, Longman RS, Iliev ID, Sonnenberg GF, Artis D. 2017. Regulation of inflammation by microbiota interactions with the host. *Nat. Immunol.* 18(8):851–60
7. Tilg H, Zmora N, Adolph TE, Elinav E. 2020. The intestinal microbiota fuelling metabolic inflammation. *Nat. Rev. Immunol.* 20(1):40–54
8. Byrd AL, Segre JA. 2016. Adapting Koch's postulates: Criteria for disease causation must take microbial interactions into account. *Science* 351(6270):224–26
9. Gollwitzer ES, Marsland BJ. 2015. Impact of early-life exposures on immune maturation and susceptibility to disease. *Trends Immunol.* 36(11):684–96
10. Reynolds LA, Finlay BB. 2017. Early life factors that affect allergy development. *Nat. Rev. Immunol.* 17(8):518–28
11. Kotas ME, Medzhitov R. 2015. Homeostasis, inflammation, and disease susceptibility. *Cell* 165(5):816–27
12. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, et al. 2015. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 17(5):690–703
13. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. 2014. The placenta harbors a unique microbiome. *Sci. Transl. Med.* 6(237):237ra65
14. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. 2016. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Sci. Rep.* 6:23129
15. Hornef M, Penders J. 2017. Does a prenatal bacterial microbiota exist? *Mucosal Immunol.* 10(3):598–601
16. de Goffau MC, Lager S, Sovio U, Gaccioli F, Cook E, et al. 2019. Human placenta has no microbiome but can contain potential pathogens. *Nature* 572(7769):329–34
17. Sibley CP, Brownbill P, Glazier JD, Greenwood SL. 2018. Knowledge needed about the exchange physiology of the placenta. *Placenta* 64:S9–15
18. Roopenian DC, Akilesh S. 2007. FcRn: The neonatal Fc receptor comes of age. *Nat. Rev. Immunol.* 7(9):715–25
19. Kimura I, Miyamoto J, Ohue-Kitano R, Watanabe K, Yamada T, et al. 2020. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* 367(6481):eaaw8429
20. Gomez de Aguero M, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, et al. 2016. The maternal microbiota drives early postnatal innate immune development. *Science* 351(6279):1296–302

21. Atladóttir HÓ, Thorsen P, Østergaard L, Schendel DE, Lemcke S, et al. 2010. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *J. Autism Dev. Disord.* 40(12):1423–30
22. Kim S, Kim H, Yim YS, Ha S, Atarashi K, et al. 2017. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* 549(7673):528–32
23. Choi GB, Yim YS, Wong H, Kim S, Kim H, et al. 2016. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* 351(6276):933–39
24. Zaretsky MV, Alexander JM, Byrd W, Bawdon RE. 2004. Transfer of inflammatory cytokines across the placenta. *Obstet. Gynecol.* 103(3):546–50
25. Baumgarth N. 2017. A Hard(y) look at B-1 cell development and function. *J. Immunol.* 199(10):3387–94
26. Havran WL, Allison JP. 1988. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature* 335(6189):443–45
27. Randall T, Carragher D, Rangel-Moreno J. 2008. Development of secondary lymphoid organs. *Annu. Rev. Immunol.* 26:627–50
28. Torow N, Yu K, Hassani K, Freitag J, Schulz O, et al. 2015. Active suppression of intestinal $CD4^+TCR\alpha\beta^+$ T-lymphocyte maturation during the postnatal period. *Nat. Commun.* 6:7725
29. Chen JW, Rice TA, Bannock JM, Bielecka AA, Strauss JD, et al. 2020. Autoreactivity in naïve human fetal B cells is associated with commensal bacteria recognition. *Science* 369(6501):320–25
30. Zhang X, Zhivaki D, Lo-Man R. 2017. Unique aspects of the perinatal immune system. *Nat. Rev. Immunol.* 17(8):495–507
31. Li N, van Unen V, Abdelaal T, Guo N, Kasatskaya SA, et al. 2019. Memory $CD4^+$ T cells are generated in the human fetal intestine. *Nat. Immunol.* 20(3):301–12
32. Park JE, Jardine L, Gottgens B, Teichmann SA, Haniffa M. 2020. Prenatal development of human immunity. *Science* 368(6491):600–3. **https://doi.org/10.1126/science.aaz9330**
33. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, et al. 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *PNAS* 107(26):11971–75
34. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, et al. 2019. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature* 574:117–21
35. Charbonneau MR, O'Donnell D, Blanton LV, Totten SM, Davis JCC, et al. 2016. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* 164(5):859–71
36. Niewiesk S. 2014. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Front. Immunol.* 5:446
37. Caballero-Flores G, Sakamoto K, Zeng MY, Wang Y, Hakim J, et al. 2019. Maternal immunization confers protection to the offspring against an attaching and effacing pathogen through delivery of IgG in breast milk. *Cell Host Microbe* 25(2):313–23.e4
38. Gopalakrishna KP, Macadangdang BR, Rogers MB, Tometich JT, Firek BA, et al. 2019. Maternal IgA protects against the development of necrotizing enterocolitis in preterm infants. *Nat. Med.* 25(7):1110–15
39. Koch MA, Reiner GL, Lugo KA, Kreuk LSM, Stanbery AG, et al. 2016. Maternal IgG and IgA antibodies dampen mucosal T helper cell responses in early life. *Cell* 165(4):827–41
40. Rogier EW, Frantz AL, Bruno MEC, Wedlund L, Cohen DA, et al. 2014. Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. *PNAS* 111(8):3074–79
41. Ramanan D, Sefik E, Galván-Peña S, Wu M, Yang L, et al. 2020. An immunologic mode of multigenerational transmission governs a gut Treg setpoint. *Cell* 181(6):1276–90.e13
42. Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Coughlin PE, et al. 2017. Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria. *Sci. Immunol.* 2(18):eaao1314
43. Al Nabhani Z, Dulauroy S, Marques R, Cousu C, Al Bounny S, et al. 2019. A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity* 50(5):1276–88.e5

44. Laouar A. 2020. Maternal leukocytes and infant immune programming during breastfeeding. *Trends Immunol.* 41(3):225–39
45. Cabinian A, Sinsimer D, Tang M, Zumba O, Mehta H, et al. 2016. Transfer of maternal immune cells by breastfeeding: Maternal cytotoxic T lymphocytes present in breast milk localize in the Peyer's patches of the nursed infant. *PLOS ONE* 11(6):e0156762
46. Huda MN, Lewis Z, Kalanetra KM, Rashid M, Ahmad SM, et al. 2014. Stool microbiota and vaccine responses of infants. *Pediatrics* 134(2):e362–72
47. Huda MN, Ahmad SM, Alam MJ, Khanam A, Kalanetra KM, et al. 2019. *Bifidobacterium* abundance in early infancy and vaccine response at 2 years of age. *Pediatrics* 143(2):e20181489
48. Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, et al. 2008. Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. *Am. J. Physiol. Gastrointest. Liver Physiol.* 295(5):1025–34
49. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, et al. 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature* 469(7331):543–49
50. Elahi S, Ertelt JM, Kinder JM, Jiang TT, Zhang X, et al. 2013. Immunosuppressive CD71 1 erythroid cells compromise neonatal host defence against infection. *Nature* 504(7478):158–62
51. Rudd BD. 2020. Neonatal T cells: a reinterpretation. *Annu. Rev. Immunol.* 38:229–47
52. Thome JJC, Bickham KL, Ohmura Y, Kubota M, Matsuoka N, et al. 2016. Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. *Nat. Med.* 22(1):72–77
53. McGovern N, Shin A, Low G, Low D, Duan K, et al. 2017. Human fetal dendritic cells promote prenatal T-cell immune suppression through arginase-2. *Nature* 546(7660):662–66
54. Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, Pauli ML, et al. 2015. A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity* 43(5):1011–21
55. Gollwitzer ES, Saglani S, Trompette A, Yadava K, Sherburn R, et al. 2014. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat. Med.* 20(6):642–47
56. New JS, Dizon BLP, Fucile CF, Rosenberg AF, Kearney JF, King RG. 2020. Neonatal exposure to commensal-bacteria-derived antigens directs polysaccharide-specific B-1 B cell repertoire development. *Immunity* 53(1):172–86.e6
57. Godfrey DI, Uldrich AP, Mccluskey J, Rossjohn J, Moody DB. 2015. The burgeoning family of unconventional T cells. *Nat. Immunol.* 16(11):1114–23
58. Adams EJ, Luoma AM. 2013. The adaptable major histocompatibility complex (MHC) fold: structure and function of nonclassical and MHC class I–like molecules. *Annu. Rev. Immunol.* 31:529–61
59. McVay LD, Carding SR. 1996. Extrathymic origin of human γδ T cells during fetal development. *J. Immunol.* 157(7):2873–82
60. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, et al. 2012. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 491(7426):717–23
61. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, et al. 1997. CD1d-restricted and TCR-mediated activation of V_α14 NKT cells by glycosylceramides. *Science* 278(5343):1626–29
62. Olszak T, An D, Zeissig S, Vera MP, Richter J, et al. 2012. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 336(6080):489–93
63. An D, Oh SF, Olszak T, Neves JF, Avci FY, et al. 2014. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* 156(1–2):123–33
64. Constantinides MG, Link VM, Tamoutounour S, Wong AC, Perez-Chaparro PJ, et al. 2019. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. *Science* 366(6464):eaax6624
65. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, et al. 2003. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 422(6928):164–69
66. Koay HF, Gherardin NA, Enders A, Loh L, Mackay LK, et al. 2016. A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. *Nat. Immunol.* 17(11):1300–11
67. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. 2017. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat. Med.* 23(3):314–26

68. Thaiss CA, Zmora N, Levy M, Elinav E. 2016. The microbiome and innate immunity. *Nature* 535(7610):65–74
69. Robertson SJ, Goethel A, Girardin SE, Philpott DJ. 2018. Innate immune influences on the gut microbiome: lessons from mouse models. *Trends Immunol.* 39(12):992–1004
70. Peterson DA, McNulty NP, Guruge JL, Gordon JI. 2007. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe* 2(5):328–39
71. Fernandez MI, Pedron T, Tournebize R, Olivo-Marin JC, Sansonetti PJ, Phalipon A. 2003. Anti-inflammatory role for intracellular dimeric immunoglobulin A by neutralization of lipopolysaccharide in epithelial cells. *Immunity* 18(6):739–49
72. Fagarasan S, Kawamoto S, Kanagawa O, Suzuki K. 2010. Adaptive immune regulation in the gut: T cell–dependent and T cell–independent IgA synthesis. *Annu. Rev. Immunol.* 28:243–73
73. Van Der Waaij LA, Limburg PC, Mesander G, Van Der Waaij D. 1996. In vivo IgA coating of anaerobic bacteria in human faeces. *Gut* 38:348–54
74. Kramer DR, Cebra JJ. 1995. Early appearance of "natural" mucosal IgA responses and germinal centers in suckling mice developing in the absence of maternal antibodies. *J. Immunol.* 154(5):2051–62
75. Macpherson AJ, Hunziker L, McCoy K, Lamarre A. 2001. IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes Infect.* 3(12):1021–35
76. Macpherson AJ, Gatto D, Sainsbury E, Harriman GR, Hengartner H, Zinkernagel RM. 2000. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* 288(5474):2222–26
77. Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T. 2002. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* 298(5597):1424–27
78. Macpherson AJ, Uhr T. 2004. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 303(5664):1662–65
79. Fagarasan S, Kinoshita K, Muramatsu M, Ikuta K, Honjo T. 2001. In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* 413(6856):639–43
80. Reboldi A, Arnon TI, Rodda LB, Atakilit A, Sheppard D, Cyster JG. 2016. IgA production requires B cell interaction with subepithelial dendritic cells in Peyer's patches. *Science* 352(6287):aaf4822
81. Bunker JJ, Erickson SA, Flynn TM, Henry C, Koval JC, et al. 2017. Natural polyreactive IgA antibodies coat the intestinal microbiota. *Science* 358(6361):eaan6619
82. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, et al. 2002. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat. Immunol.* 3(9):822–29
83. He B, Xu W, Santini PA, Polydorides AD, Chiu A, et al. 2007. Intestinal bacteria trigger T cell-independent immunoglobulin A_2 class switching by inducing epithelial-cell secretion of the cytokine APRIL. *Immunity* 26(6):812–26
84. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, et al. 2006. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 314(5802):1157–60
85. Cazac BB, Roes J. 2000. TGF-β receptor controls B cell responsiveness and induction of IgA in vivo. *Immunity* 13(4):443–51
86. Grootjans J, Krupka N, Hosomi S, Matute JD, Hanley T, et al. 2019. Epithelial endoplasmic reticulum stress orchestrates a protective IgA response. *Science* 363(6430):993–98
87. Macpherson AJ, Yilmaz B, Limenitakis JP, Ganal-Vonarburg SC. 2018. IgA function in relation to the intestinal microbiota. *Annu. Rev. Immunol.* 36:359–81
88. Bunker JJ, Flynn TM, Koval JC, Shaw DG, Meisel M, et al. 2015. Innate and adaptive humoral responses coat distinct commensal bacteria with immunoglobulin A. *Immunity* 43(3):541–53
89. Bunker JJ, Bendelac A. 2018. IgA responses to microbiota. *Immunity* 49(2):211–24
90. Ansaldo E, Slayden LC, Ching KL, Koch MA, Wolf NK, et al. 2019. *Akkermansia muciniphila* induces intestinal adaptive immune responses during homeostasis. *Science* 364(6446):1179–84
91. Palm NW, De Zoete MR, Cullen TW, Barry NA, Stefanowski J, et al. 2014. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 158(5):1000–10
92. Wei M, Shinkura R, Doi Y, Maruya M, Fagarasan S, Honjo T. 2011. Mice carrying a knock-in mutation of *Aicda* resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense. *Nat. Immunol.* 12(3):264–70

93. Kau AL, Planer JD, Liu J, Rao S, Yatsunenko T, et al. 2015. Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. *Sci. Transl. Med.* 7(276):276ra24
94. Hapfelmeier S, Lawson MAE, Slack E, Kirundi JK, Stoel M, et al. 2010. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science* 328(5986):1705–9
95. Li H, Limenitakis JP, Greiff V, Yilmaz B, Schären O, et al. 2020. Mucosal or systemic microbiota exposures shape the B cell repertoire. *Nature* 584(7820):274–78
96. Yel L. 2010. Selective IgA deficiency. *J. Clin. Immunol.* 30:10–16
97. Chen K, Magri G, Grasset EK, Cerutti A. 2020. Rethinking mucosal antibody responses: IgM, IgG and IgD join IgA. *Nat. Rev. Immunol.* 20(7):427–41
98. Benckert J, Schmolka N, Kreschel C, Zoller MJ, Sturm A, et al. 2011. The majority of intestinal IgA^+ and IgG^+ plasmablasts in the human gut are antigen-specific. *J. Clin. Investig.* 121(5):1946–55
99. Magri G, Comerma L, Pybus M, Sintes J, Lligé D, et al. 2017. Human secretory IgM emerges from plasma cells clonally related to gut memory B cells and targets highly diverse commensals. *Immunity* 47(1):118–34.e8
100. Slack E, Hapfelmeier S, Stecher B, Velykoredko Y, Stoel M, et al. 2009. Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* 325(5940):617–20
101. Zeng MY, Cisalpino D, Varadarajan S, Hellman J, Warren HS, et al. 2016. Gut microbiota-induced immunoglobulin G controls systemic infection by symbiotic bacteria and pathogens. *Immunity* 44(3):647–58
102. Xu Z, Takizawa F, Casadei E, Shibasaki Y, Ding Y, et al. 2020. Specialization of mucosal immunoglobulins in pathogen control and microbiota homeostasis occurred early in vertebrate evolution. *Sci. Immunol.* 5(44):eaay3254
103. Stappenbeck TS, Virgin HW. 2016. Accounting for reciprocal host-microbiome interactions in experimental science. *Nature* 534(7606):191–99
104. Kawamoto S, Tran TH, Maruya M, Suzuki K, Doi Y, et al. 2012. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. *Science* 336(6080):485–89
105. Fransen F, Zagato E, Mazzini E, Fosso B, Manzari C, et al. 2015. BALB/c and C57BL/6 mice differ in polyreactive IgA abundance, which impacts the generation of antigen-specific IgA and microbiota diversity. *Immunity* 43(3):527–40
106. Donaldson GP, Ladinsky MS, Yu KB, Sanders JG, Yoo BB, et al. 2018. Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* 360(6390):795–800
107. Moor K, Diard M, Sellin ME, Felmy B, Wotzka SY, et al. 2017. High-avidity IgA protects the intestine by enchaining growing bacteria. *Nature* 544(7651):498–502
108. Cullender TC, Chassaing B, Janzon A, Kumar K, Muller CE, et al. 2013. Innate and adaptive immunity interact to quench microbiome flagellar motility in the gut. *Cell Host Microbe* 14(5):571–81
109. Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. 2009. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *PNAS* 106(46):19256–61
110. Hand TW, Dos Santos LM, Bouladoux N, Molloy MJ, Pagán AJ, et al. 2012. Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses. *Science* 337(6101):1553–56
111. Wilmore JR, Gaudette BT, Gomez Atria D, Hashemi T, Jones DD, et al. 2018. Commensal microbes induce serum IgA responses that protect against polymicrobial sepsis. *Cell Host Microbe* 23(3):302–11.e3
112. Castro-Dopico T, Dennison TW, Ferdinand JR, Mathews RJ, Fleming A, et al. 2019. Anti-commensal IgG drives intestinal inflammation and type 17 immunity in ulcerative colitis. *Immunity* 50(4):1099–114.e10
113. Metz D, Jurecka W, Gebhart W, Schmidt J, Mainitz M, Niebauer G. 1989. Immunohistochemical demonstration of immunoglobulin A in human sebaceous and sweat glands. *J. Investig. Dermatol.* 92(1):13–17
114. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. 2004. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 118(2):229–41
115. Macleod AS, Hemmers S, Garijo O, Chabod M, Mowen K, et al. 2013. Dendritic epidermal T cells regulate skin antimicrobial barrier function. *J. Clin. Investig.* 123(10):4364–74

116. Chen Y, Chou K, Fuchs E, Havran WL, Boismenu R. 2002. Protection of the intestinal mucosa by intraepithelial γδ T cells. *PNAS* 99(22):14338–43
117. Leng T, Akther HD, Hackstein CP, Powell K, King T, et al. 2019. TCR and inflammatory signals tune human MAIT cells to exert specific tissue repair and effector functions. *Cell Rep*. 28(12):3077–91.e5
118. Li J, Reantragoon R, Kostenko L, Corbett AJ, Varigos G, Carbone FR. 2017. The frequency of mucosal-associated invariant T cells is selectively increased in dermatitis herpetiformis. *Australas. J. Dermatol.* 58(3):200–4
119. Naik S, Bouladoux N, Linehan JL, Han S-J, Harrison OJ, et al. 2015. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* 520(7545):104–8
120. Linehan JL, Harrison OJ, Han SJ, Byrd AL, Vujkovic-Cvijin I, et al. 2018. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. *Cell* 172(4):784–96.e18
121. Harrison OJ, Linehan JL, Shih HY, Bouladoux N, Han SJ, et al. 2019. Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury. *Science* 363(6422):eaat6280
122. Ismail AS, Behrendt CL, Hooper LV. 2009. Reciprocal interactions between commensal bacteria and γδ intraepithelial lymphocytes during mucosal injury. *J. Immunol.* 182(5):3047–54
123. Ismail AS, Severson KM, Vaishnava S, Behrendt CL, Yu X, et al. 2011. γδ intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *PNAS* 108(21):8743–48
124. Hoytema van Konijnenburg DP, Reis BS, Pedicord VA, Farache J, Victora GD, Mucida D. 2017. Intestinal epithelial and intraepithelial T cell crosstalk mediates a dynamic response to infection. *Cell* 171(4):783–94.e13
125. Nieuwenhuis EES, Matsumoto T, Lindenbergh D, Willemsen R, Kaser A, et al. 2009. Cd1d-dependent regulation of bacterial colonization in the intestine of mice. *J. Clin. Investig.* 119(5):1241–50
126. St. Leger AJ, Desai JV, Drummond RA, Kugadas A, Almaghrabi F, et al. 2017. An ocular commensal protects against corneal infection by driving an interleukin-17 response from mucosal γδ T cells. *Immunity* 47(1):148–58.e5
127. Ridaura VK, Bouladoux N, Claesen J, Chen YE, Byrd AL, et al. 2018. Contextual control of skin immunity and inflammation by *Corynebacterium*. *J. Exp. Med.* 215(3):785–99
128. De Libero G, Lau S-Y, Mori L. 2015. Phosphoantigen presentation to TCR γδ cells, a conundrum getting less gray zones. *Front. Immunol.* 5:679
129. Dias J, Leeansyah E, Sandberg JK. 2017. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *PNAS* 114(27):E5434–43
130. Howson LJ, Awad W, von Borstel A, Jing Lim H, McWilliam HEG, et al. 2020. Absence of mucosal-associated invariant T cells in a person with a homozygous point mutation in *MR1*. *Sci. Immunol.* 5(49):eabc9492
131. Hegazy AN, West NR, Stubbington MJT, Wendt E, Suijker KIM, et al. 2017. Circulating and tissue-resident $CD4^+$ T cells with reactivity to intestinal microbiota are abundant in healthy individuals and function is altered during inflammation. *Gastroenterology* 153(5):1320–37.e16
132. Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, et al. 2009. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 31(4):677–89
133. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, et al. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139(3):485–98
134. Talham GL, Jiang HQ, Bos NA, Cebra JJ. 1999. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect. Immun.* 67(4):1992–2000
135. Umesaki Y, Setoyama H, Matsumoto S, Imaoka A, Itoh K. 1999. Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. *Infect. Immun.* 67(7):3504–11
136. Yang Y, Torchinsky MB, Gobert M, Xiong H, Xu M, et al. 2014. Focused specificity of intestinal T_H17 cells towards commensal bacterial antigens. *Nature* 510(7503):152–56
137. Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. 2011. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *PNAS* 108(Suppl. 1):4615–22

138. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, et al. 2010. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 32(6):815–27
139. Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, et al. 2011. The antibacterial lectin RegIIIγ promotes the spatial segregation of microbiota and host in the intestine. *Science* 334(6053):255–58
140. Ivanov II, Frutos RDL, Manel N, Yoshinaga K, Rifkin DB, et al. 2008. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 4(4):337–49
141. Sczesnak A, Segata N, Qin X, Gevers D, Petrosino JF, et al. 2011. The genome of Th17 cell-inducing segmented filamentous bacteria reveals extensive auxotrophy and adaptations to the intestinal environment. *Cell Host Microbe* 10(3):260–72
142. Atarashi K, Tanoue T, Ando M, Kamada N. 2015. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* 163(2):367–80
143. Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, et al. 2017. Mining the human gut microbiota for immunomodulatory organisms. *Cell* 168(5):928–43.e11
144. Tan TG, Sefik E, Geva-Zatorsky N, Kua L, Naskar D, et al. 2016. Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. *PNAS* 113(50):E8141–50
145. Tanoue T, Morita S, Plichta DR, Skelly AN, Suda W, et al. 2019. A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* 565(7741):600–5
146. Byrd AL, Deming C, Cassidy SKB, Harrison OJ, Ng WI, et al. 2017. *Staphylococcus aureus* and *Staphylococcus epidermidis* strain diversity underlying pediatric atopic dermatitis. *Sci. Transl. Med.* 9(397):eaal4651
147. Hurabielle C, Link VM, Bouladoux N, Han SJ, Merrill ED, et al. 2020. Immunity to commensal skin fungi promotes psoriasiform skin inflammation. *PNAS* 117(28):16465–74
148. Chen YE, Fischbach MA, Belkaid Y. 2018. Skin microbiota-host interactions. *Nature* 553(7689):427–36. Erratum. 2018. *Nature* 555(7697):543
149. Di Domizio J, Belkhodja C, Chenuet P, Fries A, Murray T, et al. 2020. The commensal skin microbiota triggers type I IFN-dependent innate repair responses in injured skin. *Nat. Immunol.* 21(9):1034–45
150. Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, et al. 2009. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat. Med.* 15(12):1377–82
151. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, et al. 2012. Compartmentalized control of skin immunity by resident commensals. *Science* 337(6098):1115–19
152. Sano T, Huang W, Hall JA, Yang Y, Chen A, et al. 2015. An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 responses. *Cell* 163(2):381–93
153. Derebe MG, Zlatkov CM, Gattu S, Ruhn KA, Vaishnava S, et al. 2014. Serum amyloid A is a retinol binding protein that transports retinol during bacterial infection. *eLife* 3:e03206
154. Tamoutounour S, Han SJ, Deckers J, Constantinides MG, Hurabielle C, et al. 2019. Keratinocyte-intrinsic MHCII expression controls microbiota-induced Th1 cell responses. *PNAS* 116(47):23643–52
155. Bilate AM, London M, Castro TBR, Mesin L, Bortolatto J, et al. 2020. T cell receptor is required for differentiation, but not maintenance, of intestinal $CD4^+$ intraepithelial lymphocytes. *Immunity* 53(5):1001–14.e20
156. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, et al. 2013. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *PNAS* 110(22):9066–71
157. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, et al. 2018. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* 359(6371):91–97
158. Xu M, Pokrovskii M, Ding Y, Yi R, Au C, et al. 2018. C-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont. *Nature* 554(7692):373–77
159. Hue S, Ahern P, Buonocore S, Kullberg MC, Cua DJ, et al. 2006. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J. Exp. Med.* 203(11):2473–83
160. Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, et al. 2006. IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J. Exp. Med.* 203(11):2485–94
161. Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. 2003. $CD4^+CD25^+$ T_R cells suppress innate immune pathology through cytokine-dependent mechanisms. *J. Exp. Med.* 197(1):111–19
162. Lee JY, Hall JA, Kroehling L, Wu L, Najar T, et al. 2020. Serum amyloid A proteins induce pathogenic Th17 cells and promote inflammatory disease. *Cell* 180(1):79–91.e16

163. Mackay LK, Stock AT, Ma JZ, Jones CM, Kent SJ, et al. 2012. Long-lived epithelial immunity by tissue-resident memory T (T_{RM}) cells in the absence of persisting local antigen presentation. *PNAS* 109(18):7037–42
164. Homann D, Teyton L, Oldstone MBA. 2001. Differential regulation of antiviral T-cell immunity results in stable $CD8^+$ but declining $CD4^+$ T-cell memory. *Nat. Med.* 7(8):913–19
165. Friedrich M, Pohin M, Powrie F. 2019. Cytokine networks in the pathophysiology of inflammatory bowel disease. *Immunity* 50(4):992–1006
166. Whibley N, Tucci A, Powrie F. 2019. Regulatory T cell adaptation in the intestine and skin. *Nat. Immunol.* 20(4):386–96
167. Coombes JL, Siddiqui KRR, Arancibia-Cárcamo CV, Hall J, Sun CM, et al. 2007. A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-β– and retinoic acid–dependent mechanism. *J. Exp. Med.* 204(8):1757–64
168. Esterházy D, Canesso MCC, Mesin L, Muller PA, de Castro TBR, et al. 2019. Compartmentalized gut lymph node drainage dictates adaptive immune responses. *Nature* 569(7754):126–30
169. Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto De Lafaille MA. 2005. Oral tolerance in the absence of naturally occurring Tregs. *J. Clin. Investig.* 115(7):1923–33
170. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, et al. 2007. Reciprocal T_H17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 317(5835):256–60
171. Wannemuehler MJ, Kiyono H, Babb JL, Michalek SM, McGhee JR. 1982. Lipopolysaccharide (LPS) regulation of the immune response: LPS converts germfree mice to sensitivity to oral tolerance induction. *J. Immunol.* 129(3):959–65
172. Geuking MB, Cahenzli J, Lawson MAE, Ng DCK, Slack E, et al. 2011. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 34(5):794–806
173. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, et al. 2015. The microbiota regulates type 2 immunity through $ROR\gamma t^+$ T cells. *Science* 349(6251):989–93
174. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, et al. 2015. Individual intestinal symbionts induce a distinct population of $ROR\gamma^+$ regulatory T cells. *Science* 349(6251):993–97
175. Yang BH, Hagemann S, Mamareli P, Lauer U, Hoffmann U, et al. 2016. $Foxp3^+$ T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol.* 9(2):444–57
176. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio C-W, et al. 2011. Peripheral education of the immune system by colonic commensal microbiota. *Nature* 478(7368):250–54
177. Nutsch K, Chai JN, Ai TL, Russler-Germain E, Feehley T, et al. 2016. Rapid and efficient generation of regulatory T cells to commensal antigens in the periphery. *Cell Rep.* 17(1):206–20
178. Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI. 2014. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. *Sci. Transl. Med.* 6(220):220ra11
179. Britton GJ, Contijoch EJ, Mogno I, Vennaro OH, Llewellyn SR, et al. 2019. Microbiotas from humans with inflammatory bowel disease alter the balance of gut Th17 and $ROR\gamma t^+$ regulatory T cells and exacerbate colitis in mice. *Immunity* 50(1):212–24.e4
180. Izcue A, Coombes JL, Powrie F. 2006. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. *Immunol. Rev.* 212:256–71
181. Sujino T, London M, Van Konijnenburg DPH, Rendon T, Buch T, et al. 2016. Tissue adaptation of regulatory and intraepithelial $CD4^+$ T cells controls gut inflammation. *Science* 352(6293):1581–86
182. Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, et al. 2011. GATA3 controls $Foxp3^+$ regulatory T cell fate during inflammation in mice. *J. Clin. Investig.* 121(11):4503–15
183. Neumann C, Blume J, Roy U, Teh PP, Vasanthakumar A, et al. 2019. c-Maf-dependent T_{reg} cell control of intestinal T_H17 cells and IgA establishes host-microbiota homeostasis. *Nat. Immunol.* 20(4):471–81
184. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, et al. 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504(7480):451–55
185. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, et al. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504:446–50
186. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, et al. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341:569–73

187. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, et al. 2011. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 331:337–41
188. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, et al. 2013. T_{reg} induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 500(7461):232–36
189. Narushima S, Sugiura Y, Oshima K, Atarashi K, Hattori M, et al. 2014. Characterization of the 17 strains of regulatory T cell-inducing human-derived Clostridia. *Gut Microbes* 5(3):333–39
190. Campbell C, McKenney PT, Konstantinovsky D, Isaeva OI, Schizas M, et al. 2020. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* 581(7809):475–79
191. Hang S, Paik D, Yao L, Kim E, Jamma T, et al. 2019. Bile acid metabolites control T_H17 and T_{reg} cell differentiation. *Nature* 576(7785):143–48. Erratum. 2020. *Nature* 579:E7
192. Song X, Sun X, Oh SF, Wu M, Zhang Y, et al. 2020. Microbial bile acid metabolites modulate gut RORγ^+ regulatory T cell homeostasis. *Nature* 577(7790):410–15
193. Verma R, Lee C, Jeun EJ, Yi J, Kim KS, et al. 2018. Cell surface polysaccharides of *Bifidobacterium bifidum* induce the generation of Foxp3$^+$ regulatory T cells. *Sci. Immunol.* 3(28):eaat6975
194. Mazmanian SK, Cui HL, Tzianabos AO, Kasper DL. 2005. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 122(1):107–18
195. Mazmanian SK, Round JL, Kasper DL. 2008. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* 453(7195):620–25
196. Leech JM, Dhariwala MO, Lowe MM, Chu K, Merana GR, et al. 2019. Toxin-triggered interleukin-1 receptor signaling enables early-life discrimination of pathogenic versus commensal skin bacteria. *Cell Host Microbe* 26(6):795–809.e5
197. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, et al. 2014. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science* 343(6178):1249288
198. Grainger JR, Wohlfert EA, Fuss IJ, Bouladoux N, Askenase MH, et al. 2013. Inflammatory monocytes regulate pathologic responses to commensals during acute gastrointestinal infection. *Nat. Med.* 19(6):713–21
199. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, et al. 2009. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461(7268):1282–86
200. Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, et al. 2012. Interactions between commensal fungi and the C-type lectin receptor dectin-1 influence colitis. *Science* 336(6086):1314–17
201. Rohlke F, Stollman N. 2012. Fecal microbiota transplantation in relapsing *Clostridium difficile* infection. *Therap. Adv. Gastroenterol.* 5(6):403–20
202. Panigrahi P, Parida S, Nanda NC, Satpathy R, Pradhan L, et al. 2017. A randomized synbiotic trial to prevent sepsis among infants in rural India. *Nature* 548(7668):407–12