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REVIEW ARTICLE

Microbial modulation of intestinal T helper cell responses and implications for disease and therapy

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Induction of intestinal T helper cell subsets by commensal members of the intestinal microbiota is an important component of the many immune adaptations required to establish host-microbial homeostasis. Importantly, altered intestinal T helper cell profiles can have pathological consequences that are not limited to intestinal sites. Therefore, microbial-mediated modulation of the intestinal T helper cell profile could have strong therapeutic potentials. However, in order to develop microbial therapies that specifically induce the desired alterations in the intestinal T helper cell compartment one has to first gain a detailed understanding of how microbial composition and the metabolites derived or induced by the microbiota impact on intestinal T helper cell responses. Here we summarize the milestone findings in the field of microbiota-intestinal T helper cell crosstalk with a focus on the role of specific commensal bacteria and their metabolites. We discuss mechanistic mouse studies and are linking these to human studies where possible. Moreover, we highlight recent advances in the field of microbial CD4 T cell epitope mimicry in autoimmune diseases and the role of microbially-induced CD4 T cells in cancer immune checkpoint blockade therapy.

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INTRODUCTION

Intestinal T helper cell adaptions

At birth, our intestinal tract is successively colonized by microbes, primarily commensal bacteria but also fungi, protozoa, parasites, and viruses. The intestinal microbiota has co-evolved with the immune system[1] to maintain a symbiotic relationship and aids in the digestion of inaccessible nutrients, provides colonization resistance to pathogens, and educates the immune system.[2] A dynamic crosstalk between the microbiota and the host immune system during compositional or metabolic changes in the microbiota is essential for maintaining local and systemic immune homeostasis. The induction of distinct CD4 T cell subsets in mucosal tissues represents a key adaption in response to bacterial colonization. The intestinal CD4 T cell compartment is composed of functionally diverse subsets with T helper type 1 (Th1), Th2, Th17, T follicular helper (T_{FH}), and regulatory T (Treg) cells being the most prominent and best characterized populations in the intestine. The modulation of T cell function by intestinal commensal bacteria and their metabolites and its consequences on health and disease is the main topic of this review. Other commensal microbes such as fungi have also been shown to modulate intestinal immunity during homeostasis or inflammation.[3–7] However, our review will focus on the effects mediated by commensal bacteria.

Bacterial metabolites

Immunomodulatory bacterial metabolites can travel systemically and ultimately influence health and disease at extra-intestinal sites.[8–10] The importance of bacterial metabolites on host biology was initially highlighted by the comparison of the metabolic profile of germ-free and conventionally housed animals.[8,9] These studies demonstrated drastic alterations in the plasma, urine, liver, kidney, and gut tissue metabolomes. More recently, Uchimura et al. utilized stable isotope tracing to demonstrate that microbial metabolites broadly penetrate host systemic organs and induce widespread host metabolic and immunological responses.[10]

Immune-mediated diseases

Autoimmune and inflammatory diseases that are driven by an imbalance in intestinal T helper cell populations are often associated with dysbiosis, a poorly defined term describing alterations in the microbiota composition. Whilst these alterations in microbiota composition would once have been dismissed as an epiphenomenon, it is becoming increasingly apparent that compositional changes in the microbiota may both precede and play a causative role in the changes in immune cell function that drive these pathologies. In fact, it is now recognized that the immunomodulatory effects of dysbiosis are predominantly mediated through changes in the overall metabolic function of the microbiota resulting in an altered overall metabolite profile.[11]

While it is clear that the microbiota also interacts with other T cell subsets, such as intraepithelial lymphocytes (IEL),[12] γδ-T cells,[13,14] and MAIT cells,[15,16] as well as non T cell populations,[2] this review will focus on the intestinal CD4 T cell compartment. We will also highlight the impact of microbial CD4 T cell antigen mimicry in autoimmune disease and the role of the microbiota in cancer immune checkpoint blockade (ICB) therapy as examples of mechanistic (and immunological) links between intestinal CD4 T cells, microbes, and metabolites. Most of the current understanding has been obtained using gnotobiotic mouse models and mechanistic human data is sparse. Wherever possible, we will

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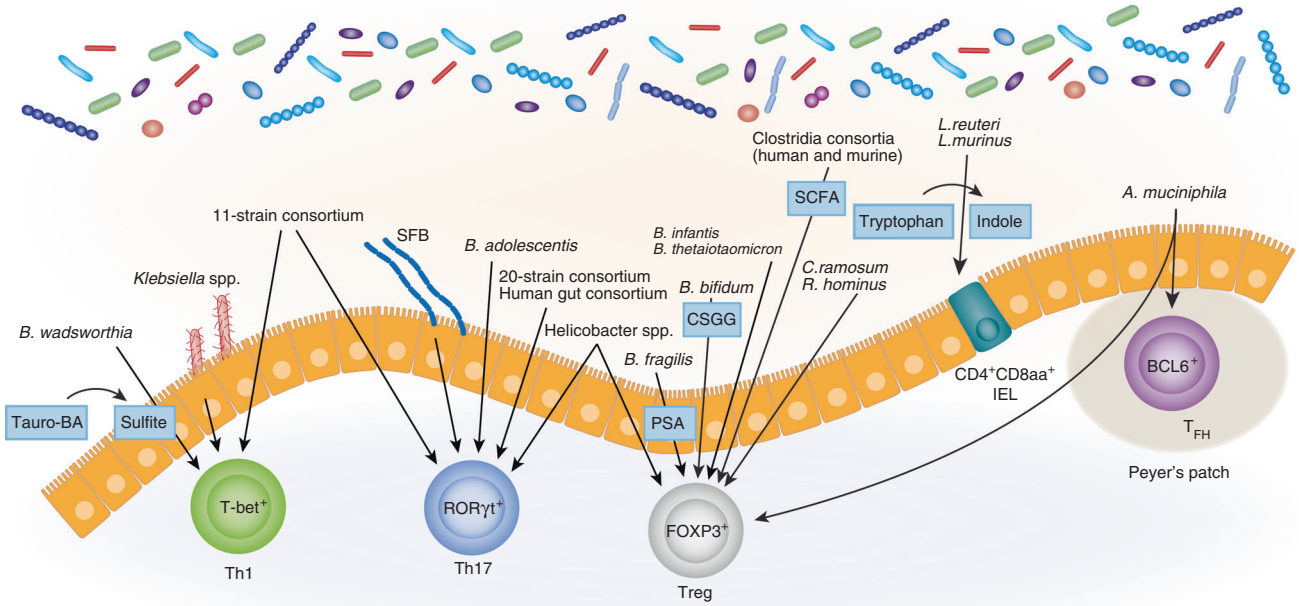

Fig. 1 Microbiota - CD4 T cell interaction. Microbial colonization has a profound impact on the intestinal $CD4^+$ T cells compartment and provides signals leading to the differentiation of naïve T cells into $Tbet^+$ Th1, $GATA3^+$ Th2, $ROR\gamma t^+$ Th17, $FOXP3^+$ Treg, and $BCL\text{-}6^+$ T follicular helper cells (T_{FH}). From left to right: *Bilophila wadsworthia* a sulfite-reducing bacterium that flourishes on the sulfur contained in taurine induces a pro-inflammatory Th1 response. Ectopic colonization of oral *Klebsiella* species in the intestine induces a strong pro-inflammatory Th1 response. A consortium of 11 human commensals elicit intestinal $Tbet^+$ Th1 and $ROR\gamma t^+$ Th17 cells. Potent Th17 induction is also observed by epithelium adhering microbes such as Segmented filamentous bacteria (SFB), and *Bifidobacterium adolescentis*. A human gut consortium and a consortium of 20 strains isolated from a patient with ulcerative colitis can induce Th17 cells. During intestinal inflammation, *Helicobacter* species induce antigen-specific Th17 cells. Conversely, under homeostatic conditions *Helicobacter* induces Treg with specificity for the same epitopes. *Bacteroides fragilis* and *Bifidobacterium bifidum* promote the induction of Treg in a polysaccharide A (PSA) and cell surface β- glucan/galactan (CSGG) polysaccharide-dependent manner, respectively. Treg are further induced by *Bifidobacterium infantis*, *Bacteroides thetaiotaomicron, Roseburia hominis*, and Clostridium strains which are able to synthesize short chain fatty acids (SCFAs). *Lactobacillus reuteri* and *Lactobacillus murinus* metabolize tryptophan to indole which signals through aryl hydrocarbon receptor (AhR) in $CD4^+$ intraepithelial lymphocytes (IEL) thereby contributing to the differentiation into $CD4^+CD8\alpha\alpha^+$ IELs. *Akkermansia muciniphila* induces an antigen-specific T_{FH} response during homeostasis.

highlight the link to human data. Finally, we will also discuss open challenges in the field.

DIFFERENTIAL INDUCTION OF INTESTINAL T HELPER CELL RESPONSES BY COLONIZATION WITH INDIVIDUAL COMMENSAL BACTERIA OR CONSORTIA

Colonization of germ-free mice with a diverse specific pathogen-free (SPF) microbiota results in the accumulation of CD4 T cells within the intestinal lamina propria.[17,18] Under homeostatic conditions this occurs in the absence of overt inflammation indicating that this reflects a necessary immune adaption in response to intestinal colonization. The literature discussed here demonstrates that the nature of CD4 T cell subsets induced depends on what bacterial species are present and what metabolites are produced. These studies would not have been possible without the renaissance of experimental mouse gnotobiology, as precisely controlled in vivo experiments are essential to mechanistically study host-microbial interactions.[19]

T helper type 17 (Th17) cell induction by segmented filamentous bacteria (SFB)

The notion that different consortia of commensal bacteria or different individual bacterial species induce distinct intestinal T helper cell effector subtypes was initially sparked by the observation that mice obtained from various vendors displayed different intestinal T helper effector profiles, which correlated with differences in microbiota composition.[20] Specifically, mice lacking segmented filamentous bacteria (SFB) displayed reduced Th17 levels in their intestines compared to genetically identical mice that were colonized with a microbiota that contained SFB.[21,22] Using SFB monocolonized mice demonstrated that SFB was sufficient to induce Th17 cells, particularly in the small intestine (Fig. 1, Table 1). Induction of Th17 by SFB is dependent on MHCII expression by dendritic cells (DCs)[23] and monocyte-derived macrophages,[24] and these Th17 cells expressed a T cell receptor (TCR) specific for SFB antigens.[23,25] Epithelial adhesion of SFB stimulates the production of serum amyloid A (SAA) and reactive oxygen species (ROS) by epithelial cells, important factors for Th17 cell differentiation.[26,27] Th17 cells have pleiotropic functions and can act in a homeostatic or pro-inflammatory fashion and this balance was recently shown to be modulated by SAA proteins.[28] In summary, epithelial adhesion of SFB triggers the transfer of T cell antigens into intestinal epithelial cells (IEC) which induces the activation of lamina propria SFB-specific Th17 cells.[29] However, this mechanism does not seem to be a general feature of epithelium adherent bacteria. Hence, although epithelial adherence has been identified as an important characteristic for Th17-inducing bacteria, the downstream molecular mechanisms are likely to be microbe-specific.

As SFB has not yet been identified in humans, a large set of individual commensal species derived from the human gut were screened for their ability to induce intestinal Th17 cells in mice.[30] *Bifidobacterium adolescentis* was identified as one of

Table 1. Commensals with modulatory effect on CD4 T cell compartment.

Bacteria	Effect on CD4 T cells	References
Bacteroides fragilis	Induction of IL-10 producing FOXP3$^+$ Treg in colon	45,47,86
Bacteroides thetaiotaomicron	Increase of RORγ^+Helios$^-$FOXP3$^+$ Treg in colon	42
Bifidobacterium adolescentis	Induction of Th17 cells in the small intestine	30
Bifidobacterium breve	Induction of IL-10 producing Tr1 cells in colon	146
Bifidobacterium bifidum	Induction of Nrp1$^-$Helios^{lo}FOXP3$^+$ pTreg in colon	88
Bifidobacterium infantis 35624	Induction of FOXP3$^+$ Treg in lamina propria	147
Clostridia cluster VI, XIVa and XVIII (46 murine Clostridia, 17 human Clostridia)	Increase of Helios$^-$RORyt$^+$FOXP3$^+$ Treg in colon	34–36
Clostridium butyricum	- Induction of IL-10$^+$FOXP3$^+$ Treg in colon, - Expansion of IL-17A producing γδT cells and CD4$^+$ T cells in the colon	13,148,149
Clostridium leptum	Increase of CD25$^+$FOXP3$^+$ Treg in spleen and MLN	150
Faecalibacterium prausnitzii	Accumulation of CD4$^+$CD8αα$^+$FOXP3$^-$ T_R1 cells in colon (human)	82,151
Clostridium ramosum	Increase of colonic Helios$^-$RORy$^+$FOXP3$^+$ Treg	42
Segmented filamentous bacteria (SFB)	Induction of Th17 cells in small intestine	21,22
Lactobacillus murinus	Increase of IL-10 producing FOXP3$^+$ Treg in colon	152
Lactobacillus reuteri	- Increase of FOXP3$^+$ Treg in spleen, - Induction of CD4$^+$CD8αα$^+$ IEL	12,153
Lactobacillus rhamnosus	Increase of CD25$^+$FOXP3$^+$ Treg in spleen	154
Helicobacter hepaticus	- Accumulation of RORγt$^+$FOXP3$^+$ pTreg in colon and T_{FH} in caecal patch, - Expansion of colitogenic Th17 cells in IL-10$^{-/-}$ dependent colitis	43
Klebsiella spp.	Induction of pro-inflammatory IFNγ$^+$ Th1 cells in the colon	55
Klebsiella pneumoniae	Induction of Th17 cells in colon and liver	155
Akkermansia muciniphila	- Induction of T_{FH} cells in Peyer's Patches; - Induction of colonic Treg	44,59
Commensal A4	Inhibition of Th2 development in lamina propria	156
Roseburia hominis	Induction of CD25$^+$FOXP3$^+$ T cells in lamina propria	48,49
3 strain mix (*Lactobacillus paracasei, Lactobacillus plantarium* DSM15312 and DSM15313)	Induction of FOXP3$^+$ Treg in MLN	157
Altered Schaedler flora (*Lactobacillus acidophilus (ASF360), Lactobacillus murinus (ASF361), Bacteroides distasonis (ASF519), Mucispirillum schaedleri (ASF457), Eubacterium plexicaudatum (ASF492)*, and three Clostridium species (ASF356, ASF502, ASF500)	Expansion of colonic CD103$^+$Helios$^-$FOXP3$^+$ Treg	38
Stable Defined Moderately Diverse Microbiota from mice (sDMDMm2)/ Oligo-MM12 (*Clostridium innocuum* I46, *Bacteroides caecimuris* I48, *Lactobacillus reuteri* I49, *Enterococcus faecalis* KB1, *Acutalibacter muris* KB18, *Bifidobacterium animalis* supbsp. *animalis* YL2, *Muribaculum intestinale* YL27, *Closdridium clostridioforme* YL32, *Akkermansia muciniphila YL44, Turcimonas muris* YL45, *Blautia coccoides* YL58.	Induction of RORγt$^+$Helios$^-$ FOXP3$^+$ pTreg	39
VSL#3 *(Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus bulgaricus and Streptococcus thermophiles)*	Increase of IL-10 and TGFβ- expressing T cells	158,159
11 strain consortium (*Parabacteroides distasonis, Parabacteroides gordonii, Alistipes senegalensis, Parabacteroides johnsonii, Paraprevotella xylaniphila, Bacteroides dorei, Bacteroides uniformis, Eubacterium limosum, Ruminococcaceae bacterium cv2, Phascolarctobacterium faecium, Fusobacterium ulcerans)*	- Induction of IFNγ$^+$CD8$^+$ T cells in intestine and systemic organs, - To a lesser extend, induction of colonic IFNγ$^+$ Th1 and IL-17$^+$ Th17 cells	56
Library of various intestinal microbial communities (human)	Various effects	51
Library of 53 intestinal bacterial species (human)	Various effects	50

FOXP3 forkhead box P3, *IELs* intraepithelial lymphocytes, *IFN* interferon, *IL* interleukin, *MLN* mesenteric lymph nodes, *Th* T helper, *Treg* regulatory T cells, *pTreg* peripherally generated Treg, T_{FH} T follicular helper cell, *Tr1* type 1 regulatory T cells, *RORγt* retinoic acid receptor-related orphan receptor γt.

several human symbiotic bacteria that elicited a robust intestinal Th17 population without provoking mucosal or systemic inflammation in monocolonized mice[30] (Fig. 1, Table 1). Like SFB,[26] *B. adolescentis* is also closely associated with the intestinal epithelium.

Regulatory T cell induction

Treg are crucial to maintain intestinal homeostasis by restraining inappropriate immune responses. It is generally accepted that there are two main subsets of Treg that express the FOXP3 transcription factor. These include thymus-derived natural Treg (nTreg), expressing Helios and Neuropilin-1, and peripherally induced Treg (pTreg), which are thought to be negative for Helios and Neuropilin-1.[31] Microbially-induced pTreg can also express the transcription factor RORγt and these Treg have been shown to be particularly suppressive in intestinal inflammation.[31] Importantly, the use of these markers is still controversial and is only useful in mouse models and not in humans.[32,33]

In addition to inducing Th17 cell differentiation, SFB colonization also induces Treg gene signatures in mice.[21,34] This likely explains why SFB induction of Th17 cells does not result in overt inflammation. Due to their therapeutic potential, induction of Treg through modification of the microbiota composition and its metabolic function is a promising approach. A number of individual commensal species or consortia able to induce intestinal Treg response have been identified (Fig. 1, Table 1).

Treg induction by consortia

Initial studies identified several consortia of mouse or human-derived Clostridia species as potent inducers of intestinal Treg cells (Fig. 1, Table 1).[35–37] Clostridia from clusters IV and XIVa collectively induced secretion of transforming growth factor (TGF)-β by intestinal epithelial cells, which drove the differentiation and expansion of pTreg cells in the colon.

Colonization with the murine altered Schaedler flora (ASF) also induced intestinal Treg responses (Table 1).[38] In response to ASF colonization, control and regulation of Th1 and Th17 cells was mainly mediated by Treg production of IL-10, as Treg-deficiency or blocking the IL-10 receptor resulted in induction of Th1 and Th17 cells. Potent Treg induction in the colon was also observed following DSS treatment of ASF colonized mice, indicating an interplay between homeostatic and damage/inflammation-associated Treg induction.[38] More recently, intestinal pTreg induction was also described following colonization with a slightly more complex stable defined moderately diverse murine microbiota (Table 1) (sDMDMm2/Oligo-MM12).[39]

Treg induction by individual species

Induction of intestinal Treg following colonization with microbial consortia raised the question whether individual commensal species alone can also promote this. It is important to realize that in vivo screening of individual commensal species that have the capacity to induce Treg (or any other T helper subtype) is limited by the inherent biology of commensal bacteria and bacterial consortia that often rely on cooperation to successfully colonize. For example, some obligate anaerobic species are not able to colonize a germ-free intestine, likely due to the increased oxygen levels, and require prior colonization of facultative anaerobes to reduce oxygen levels first.[40] Alternatively, stable maintenance of a community structure likely relies on metabolic cooperation.[41]

Despite these limitations, an elegant study by Sefik et al. identified a number of individual human-derived commensal species with the potential to induce RORγ$^+$ Treg following monocolonization.[42] *Clostridium ramosum* and *Bacteroides thetaiotaomicron* were among the strongest inducers, consistent with the notion that certain *Clostridia* and *Bacteroides* species promote induction and maintenance of intestinal Treg (Fig. 1, Table 1). Another study found that pathobionts such as *Helicobacter* spp. induce antigen-specific Treg whose function is dependent on the transcription factor cMAF. Conversely, in disease-susceptible IL-10 deficient mice, *Helicobacter* spp. induced colitogenic Th17 cells with specificity for the same epitopes[43] (Fig. 1, Table 1). In addition, *Akkermansia muciniphila* has also been shown to induce antigen-specific colonic Treg[44] (Fig. 1, Table 1). Another prominent example for intestinal Treg induction, although less of a typical human commensal, is *Bacteroides fragilis*, which promotes Treg induction and IL-10 mediated anti-inflammatory responses from T cells and DCs in a toll-like receptor (TLR2)-dependent manner[45–47] (Fig. 1, Table 1).

Monocolonization of germ-free mice with the human commensal *Roseburia hominis* also increased Treg in the lamina propria[48] and the abundance of *R. hominis* is decreased in the fecal microbiota of patients with ulcerative colitis.[49]

A number of studies have performed large scale screens to mine the human gut microbiota for strains with immunomodulatory capacity (Table 1).[50,51] However, the effect on the intestinal CD4 T compartment was not always precisely analyzed.

Importantly, the diet has a significant impact on the metabolism of individual commensal species and on the composition of consortia. Indeed, using antigen-free mice (germ-free being fed an elemental diet), it was shown that the vast majority of pTreg in the small intestine are induced by dietary antigens.[52] Furthermore, a recent report demonstrated that the ketogenic diet causes a reduction in intestinal Th17 cells.[53] The specific diet used in studies is therefore an important experimental factor to be considered. This is particularly important as mouse diets used by different research institutions are not always well defined or standardized. Therefore, careful experimental controls for the diet used should be included in any future studies.

T helper type 1 (Th1) cell induction

In conventionally housed mice with a complex undefined microbiota, it has been demonstrated that the microbiota limits Th1 and promotes Treg responses against intestinal antigens. This microbiota-induced immune tolerance was mediated by CX_3CR1^+ mononuclear phagocytes.[54] Indeed, while a plethora of individual bacterial species with the capacity to induce intestinal Treg or Th17 cells have been identified, very few commensal species that promote a non-inflammatory homeostatic Th1 response have been described. The first study to identify Th1 inducers demonstrated that *Klebsiella* species, which normally colonize the oral cavity, can ectopically colonize the gut under dysbiotic conditions where they induce a strong Th1 response[55] (Fig. 1, Table 1). This response was pro-inflammatory and appeared to be dependent on $CD11b^-CD103^+$ DCs and TLR signaling. More recently, a defined consortia of 11 bacterial strains from healthy humans that elicits interferon-γ secreting $CD8^+$ T cells and anti-cancer immunity was identified.[56] This study also reported induction of colonic Th1 and Th17 cells (Fig. 1, Table 1).

T follicular helper (T_{FH}) cell induction

In Peyer's Patches, T_{FH} cells support affinity maturation of B cells in germinal centers and differentiation of B cells to IgA-secreting plasma cells.[57] It has been demonstrated that microbially-derived extracellular ATP regulates T_{FH} cells, which in turn regulates secretory IgA (SIgA) and microbial composition.[58] *A. muciniphila* has been reported to induce antigen-specific T_{FH} cells to promote systemic antigen-specific T-dependent IgA and IgG1 production. This finding indicates that commensal bacteria do not only modulate intestinal but also systemic adaptive immune responses during homeostasis[59] (Fig. 1, Table 1).

The studies illustrate important milestones in the discovery of individual intestinal commensal species or consortia with the capacity to induce different T helper cell subsets. The literature covered here is by no means complete (Table 1) and we apologize to authors of studies that are not mentioned due to space restraints.

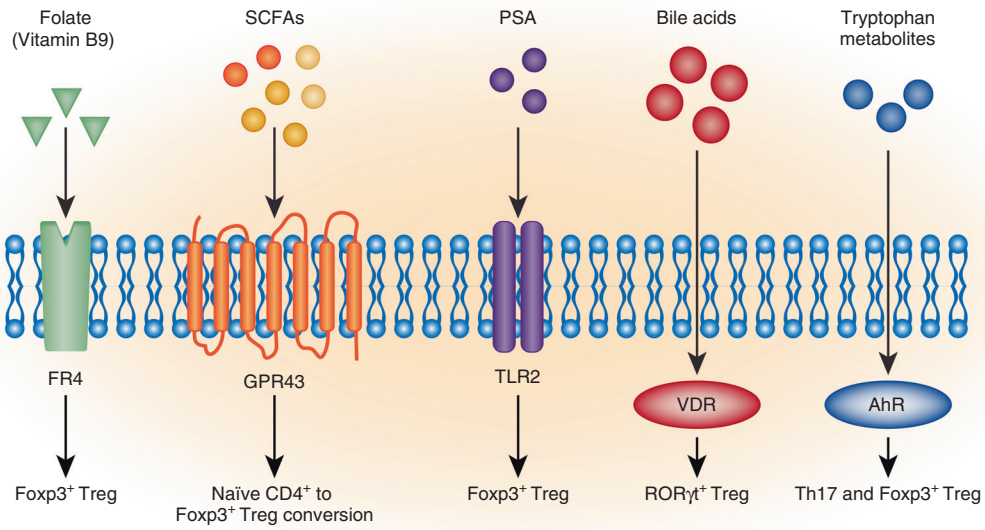

Fig. 2 Microbial metabolites and receptors expressed on CD4 T cells. Binding of metabolites that are produced or modulated by intestinal microbes to known receptors expressed on CD4 T cells.

METABOLITES INVOLVED IN THE INDUCTION OF DIFFERENT INTESTINAL T HELPER CELL SUBSETS

Although the immunological pathways and transcription factors required to induce and maintain the various T helper cell subsets are well described,[60] the observation that different intestinal commensal species promote distinct intestinal T helper subsets raises the question of how microbial metabolites are involved in this process. While TGF-β is essential for the in vivo conversion of naïve CD4 T cells into pTreg,[61] a number of microbial metabolites have been identified that further promote or enhance this process. So far, most studies have focused on the role of metabolites in Treg induction. However, recent studies are beginning to unravel the impact of metabolites on the induction of Th17 and Th1 cells.

Diet-derived metabolites

Although we will not focus specifically on the role of diet in this review, it is important to realize that the diet has a dramatic impact on the intestinal microbiota and hence on the production of microbial metabolites.[62] Furthermore, it is difficult to categorize metabolites into purely dietary, microbial, or host derived as the original metabolite can be further modified the microbes or the host. Nevertheless, we will attempt to categorize the metabolites discussed here into certain categories.

Vitamins

Retinoic acid (RA) is a host metabolite derived from dietary vitamin A and produced by intestinal CD103$^+$ DCs, IEC, and intestinal mesenchymal cells.[63] RA enhances the differentiation of pTreg in the presence of TGF-β.[64–66] Although RA promotes Treg induction and suppresses the development of Th17 cells,[65] it is also required to elicit proinflammatory T helper cells in response to infection or mucosal vaccination.[67] These findings suggest that RA functions in a context-dependent manner and is capable of mediating both anti- and pro-inflammatory effects. The vitamin B9 (folic acid), which is also derived from the diet or generated by members of the microbiota,[68] was shown to be a survival factor for Treg that express high levels of folate receptor 4 (Fig. 2, Table 2).[69,70] Furthermore, vitamin B3 (Niacin), a bacterial-derived product and GPR109a ligand, has been shown to act on DCs and favor the induction of Treg (Table 2).[71] Importantly, as vitamins are by definition essential for many biochemical processes, studies using vitamin-deficient diets to study immune function are often difficult to interpret.

Adenosine triphosphate (ATP)

Luminal adenosine-5′-tri-phosphate (ATP) can be both host- as well as microbiota-derived and has been shown to promote the differentiation of intestinal Th17 cells[72,73] (Table 2). Germ-free mice display greatly reduced numbers of lamina propria Th17 cells, which can be rescued by the administration of ATP.[72] ATP induced the production of cytokines that favor Th17 differentiation in CD70^{high}CD11c^{low} DCs through P2X receptor signaling. Of note, SFB do not produce high levels of ATP, therefore, SFB-mediated Th17 cell induction likely occurs through a mechanism independent of ATP signaling.[22]

It has also been demonstrated that ATP released by bacteria limits T_{FH} cell abundance in Peyer's Patches via the ATP-gated ionotropic P2X7 receptor, which results in decreased production of T-dependent SIgA in the small intestine.[58] Increased SIgA can lead to changes in composition of the commensal bacteria, which has been shown to affect glucose metabolism and fat deposition in mice.[58] Conversely, blocking microbial production of ATP can also increase specific IgA responses to live and inactivated oral vaccines.[74] These studies illustrate that modulation of ATP in the small intestine can affect high-affinity IgA responses against gut colonizing bacteria.

Short chain fatty acids (SCFA)

The SCFA acetate, butyrate and propionate are generated by microbial fermentation of dietary fibers. SCFA are cardinal examples of metabolites with an immuno-modulatory effect on the mucosal CD4 T cell compartment (Fig. 2, Table 2). Multiple bacterial groups are able to produce acetate,[11,39] and Clostridia species belonging to cluster IV and XIVa are prominent sources of butyrate in the gut.[11] Propionate is produced by species of the phylum Bacteroides and Firmicutes.[11] Acetate and butyrate, in particular, have been shown to promote intestinal Treg induction and function through multiple mechanisms.[75–77] These include the upregulation of *Foxp3* expression in CD4 T cells by enhanced Histone 3 acetylation of promoter and conserved non-coding sequence regions by histone deacetylase (HDAC) inhibition.[76,77] Moreover, the effects of SCFA are mediated by binding to free fatty acid receptors such as G-protein coupled receptors (GPCRs)

Table 2. Effects of microbiota-derived metabolites on CD4 T.

Metabolites	Species derived from	Mechanism	Effects on $CD4^+$ T cells	Reference
Short-chain fatty acids (SCFA)	Clostridia clusters VI, XIVa and XVIII	Enhance TGFβ expression by epithelial cells	Promote differentiation of IL-10 - producing Treg cells	34–36,75–77
Butyrate		Inhibition of histone deacetylation at the *Foxp3* promoter and at CNS1 region	Stabilization of *Foxp3* expression and enhanced Treg effector function	34,77
Propionate		Signals through GPR43 expressed on $CD4^+$ T cells	Induction of IL-10 producing Treg	75
Polysaccharide A (PSA)	*B. fragilis*	Signaling through TLR2 expressed on Treg and pDCs	Induction of Treg and IL-10 production	45,46,86
Cell surface β-glucan/galactan (CSGG) polysaccharides	*B. bifidum*	Signaling through TLR2 expressed on DCs	Induction of intestinal Treg	88
Tryptophan metabolite (Indole-3-lactic acid)	*L. reuteri*	Signaling through AhR expressed on $CD4^+$ IELs leading to downregulation of ThPOK	Conversion of $CD4^+$ IELs into $CD4^+CD8aa^+$ IELs	12
Folate (Vitamin B9)	*Bifidobacterium, Lactobacillus*	Signaling trough FR4	Maintenance of Treg	70
Niacin (Vitamin B3)	Commensals	Signaling through GPR109a	Regulates Treg and IL-10-producing $CD4^+$ T cell frequency in the colon	71
Adenosine triphosphate (ATP)	Various bacteria	Activation of P2X and P2Y purinergic receptors	- Induction of lamina propria Th17 cells - Limits the number of T_{FH} cells in Peyer's Patches	58,72,73
Bile acids	commensals	Signaling through VDR expressed on $CD4^+$ T cells	Induction of colonic $ROR\gamma t^+$ Treg	102
Isoallo lithocholic acid	commensals	Production of mitoROS leading to enhanced Foxp3 expression	Induction of Treg differentiation	103
3-oxo lithocholic acid	commensals	Binds to RORγt and inhibits its transcriptional activity	Inhibition of Th17 cell differentiation	103

AhR aryl hydrocarbon receptor, *B. bifidum Bacteroides bifidum*, *B. fragilis Bacteroides fragilis*, *CNS* conserved non-coding sequence, *FR4* folate receptor 4, *FOXP3* forkhead box protein P3, *GPR* G protein-coupled receptor, *IELs* intraepithelial lymphocytes, *IL* interleukin, *L. reuteri Lactobacillus reuteri*, *mitoROS* mitochondrial reactive oxygen species, *pDC* plasmacytoid dendritic cells, *RORγt* retinoic acid receptor-related orphan receptor γt, *TGFβ* transforming growth factor β, *Th* T helper, *ThPOK* helper-inducing POZ/Kruppel like factor, *TLR* Toll-like receptor, *Treg* regulatory T cells, *VDR* Vitamin D receptor.

expressed on CD4 T cells (GPR43, also known as FAAR2) and DCs (GPR109A).[71,75] Furthermore, SCFA upregulate GPR15 expression leading to Treg accumulation in the colon.[75] More recently, it has been shown that SCFA promote T cell differentiation into both effector and regulatory T cells in a context-dependent manner. Th1 and Th17 cell differentiation was mediated by the HDAC inhibitory activity of SCFA in T cells, which subsequently resulted in enhanced mTOR-S6K activity required for differentiation and cytokine expression.[78]

In contrast to SCFA, dietary long chain-fatty acids enhance differentiation and proliferation of Th1 and Th17 cells.[79] Furthermore, dietary fats derived from a high-fat diet induced changes in the ileum microbiota composition leading to a reduction in intestinal Th17 cells.[80]

Several groups have shown a protective effect of SCFA in mouse models of colitis.[75,77] Similarly, in IBD patients microbial dysbiosis was associated with a decrease of SCFA producing bacteria and corresponding lower levels of fecal SCFA.[11,49,81,82] Furthermore, fecal microbiota transplantation (FMT) from healthy donors into pediatric ulcerative colitis patients resulted in increased diversity, abundance of Clostridia, and fecal butyrate levels.[83] These studies indicate that SCFA also play an important functional role in humans.

Microbial polysaccharides

Polysaccharide A (PSA) produced by *B. fragilis* is a good example of a bacterial compound with immuno-modulatory effects[84] (Figs. 1 and 2, Table 2). PSA was reported to protect mice from experimental colitis.[85] This protective effect was mediated by IL-10 production by intestinal Treg, in a TLR2-dependent mechanism,[46] and also via indirect effects on conventional DCs[47] or plasmacytoid DCs.[86] Furthermore, it was shown that *B. fragilis* releases PSA via outer-membrane vesicles (OMV) which are delivered to DCs.[47,87] These OMV-primed DCs induce intestinal Treg and thereby protect animals from experimental colitis. Similarly, cell surface β-glucan/galactan (CSGG) polysaccharides of *Bifidobacterium bifidum* were shown to induce intestinal Treg capable of suppressing intestinal inflammation via activation of DCs in a TLR2-dependent manner[88] (Fig. 1, Table 2).

Aryl hydrocarbon receptor (AhR) ligands

Generally, AhR is required for the development and homeostasis of various cell types, including γδIELs,[89] Th17, and Treg, with an important role in maintaining host-microbiota mutualism. Signaling through AhR has been shown to regulate Treg and Th17 cell differentiation in a ligand-specific manner.[90–92] Recent work revealed that the AhR-Foxp3-RORγt axis controls gut homing of CD4 T cells by regulating the expression of GPR15.[93]

The microbiota can modulate the immune system through activation of the AhR transcription factor. AhR ligands can be derived from many sources. Besides xenobiotic compounds, such as dioxin, AhR also recognizes natural ligands derived from cruciferous vegetables, as well as ligands produced by the microbiota through tryptophan metabolism.[94] For example, *Lactobacillus reuteri* provides indole derivates, such as indole-3 lactic acid, of dietary tryptophan that activate AhR. This induces intraepithelial $CD4^+CD8\alpha\alpha^+$ T cells via downregulation of the transcription factor ThPOK (Fig. 1, Table 2).[12]

Catabolism of tryptophan into the AhR ligand kynurenine, mediated by indoleamine-2,3-dioxygenase (IDO) activity in DCs and IEC, favors pTreg differentiation and controls effector T cell activity.[95–97] Interestingly, IEC of mice colonized with *Clostridium* express high level of IDO.[36]

Furthermore, the importance of microbial-derived AhR ligands in intestinal immune education was recently highlighted using a model of gestational colonization.[98] This study revealed that AhR ligands, produced by the maternal microbiota, were transferred to the offspring postnatally through the milk and shaped the offspring's intestinal innate lymphoid cells (ILC) 3 population.

Bile acids (BA)

BA are small molecules that are synthesized from cholesterol in the liver and are further metabolized by the intestinal microbiota, including deconjugation of glycine or taurine and biotransformation of unconjugated primary BA to secondary BA. Hence, in comparison to conventionally housed animals, the BA profile of germ-free animals is markedly different with a reduced diversity and overall abundance of secondary BA.[99] BA function as signaling molecules with pleiotropic metabolic and immune effects through dynamic interactions with host receptors and the microbiota resulting in a complex interplay between BA, the microbiota and the host immune system. For example, mice fed with a high-fat diet displayed an altered composition of conjugated BA, resulting in an expansion of *Bilophila wadsworthia*, a sulfite-reducing bacterium[100] (Fig. 1). These changes were associated with enhanced intestinal Th1 responses and the development of colitis in genetically susceptible IL-10-deficient animals. Furthermore, a study by Cao et al. showed that BA induce T cell mediated inflammation in the ileum, unless T cells are protected by the xenobiotic transporter Mdr1.[101]

Two recent studies have identified additional anti-inflammatory roles for BA metabolites that modulate intestinal CD4 T cell responses. Song et al. demonstrate the importance of microbial BA in the development of colonic $ROR\gamma t^+$ Treg in mice[102] (Table 2). Genetic ablation of BA metabolizing genes in individual symbiotic bacteria led to a reduction of Th17 cells in vivo. Conversely, restoration of the BA pool enhanced colonic $ROR\gamma t^+$ Treg and ameliorated gut inflammation through the BA/vitamin D receptor axis (Fig. 3). Moreover, Hang et al. identified two distinct derivates of lithocholic acid (LCA) as T cell regulators in mice.[103] While 3-oxoLCA inhibited the differentiation of Th17 cells by binding to RORγt and inhibiting its transcriptional activity, isoalloLCA enhanced Treg differentiation (Table 2). Collectively, these studies revealed novel mechanisms that regulate CD4 T cell responses through BA metabolites.

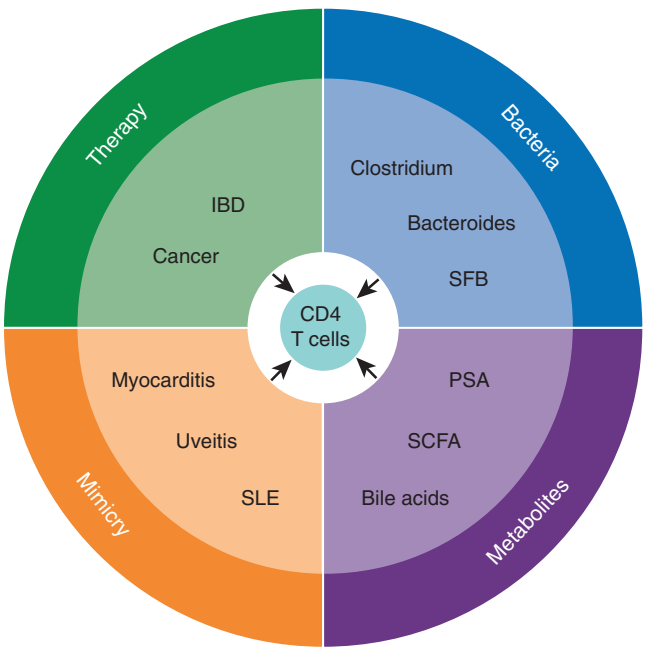

Fig. 3 Interplay between intestinal CD4 T cells, bacteria, metabolites, mimicry and therapy. This schematic summarizes the topics that were discussed in this review.

In human inflammatory bowel disease (IBD), altered fecal BA profiles characterized by an increase in conjugated primary BA and a decrease in secondary BA levels have been described.[104] Furthermore, reduced levels of stool LCA and deoxycholic acid (DCA) were reported in colectomy treated patients with ulcerative colitis compared to familial adenomatous polyposis pouch patients.[105] In this study, diminished secondary BA were linked with altered microbial composition and lower expression of genes required for the conversion of primary to secondary BA. In animal models of acute and chronic colitis, supplementation with LCA and DCA reduced intestinal inflammation. Of note, LCA has been demonstrated to impair Th1 activation, evidenced by IFNγ and TNFα production, through vitamin D receptor signaling.[106]

Collectively, these studies demonstrate that the intestinal CD4 T cell compartment can be activated by diverse microbial taxa and a variety of different mechanisms. High degrees of redundancy in these processes are crucial for the control and the maintenance of intestinal homeostasis. Of note, some of these microbes that elicit tolerogenic immune adaptations have also been implicated in disease pathogenesis in experimental models of inflammation.[107–109] This finding suggests that these beneficial effects are context-dependent and modulated by host-genetics factors.

The wide-spread use of targeted as well as untargeted metabolomics methodology in microbiome research will undoubtedly result in the identification of additional microbial metabolites involved in microbiota-immune crosstalk.

INTESTINAL CD4 T HELPER CELLS IN DISEASE AND THERAPY

The role of intestinal T helper cells in disease is an extremely large topic,[60] particularly when also considering the role of metabolites in disease. It is well established that intestinal T helper cells as well as metabolites are involved in intestinal disorders, which has been reviewed in detail elsewhere.[11,110,111] Some mouse models for systemic autoimmune disorders, such as experimental autoimmune encephalomyelitis (EAE), have also demonstrated a potential link to intestinal CD4 T helper cells. However, the precise immunological mechanisms as well as the role of TCR reactivity remained unclear.[109] Therefore, we will highlight recent work that elucidated the role of intestinal CD4 T helper cells in systemic (non-intestinal) diseases where the immunological mechanism involved has been identified to be microbial antigen mimicry. First, we will provide a short summary on what is known about antigen-specificity of intestinal T helper cells, which has been previously reviewed in detail.[112]

Antigen-specificity of intestinal T helper cell responses

Most of the current literature on the role of metabolites on intestinal T helper cell responses did not address whether the observed effects were antigen-specific or not. This is likely due to the fact that the role of antigen-specificity remains difficult to address experimentally. However, antigen-specificity is an important aspect, especially in the context of microbiota-autoimmune crosstalk, which includes microbial antigen mimicry as discussed later.

The precise requirements for antigen-specificity for induction and effector function of intestinal regulatory T cells remains unclear. When considering antigen-specificity of the intestinal Treg compartment both peripherally induced pTreg and thymus-derived natural nTreg populations needs to be taken into consideration.[36,38,113] nTreg have a repertoire skewed towards self-reactivity, while pTreg have a repertoire similar to that of naïve CD4 T cells.[114,115] Although elegant studies have cloned the TCR of intestinal Treg and identified potential bacterial candidates as the source of the antigen, the precise antigen itself was not identified.[116,117] As mentioned above, *Helicobacter* induce antigen-specific Treg[43] and the antigen-specificity of Th17 cells induced by SFB has been carefully characterized.[23,25,29] Furthermore, *A. muciniphila* induces antigen-specific T_{FH} and Treg cells.[44,59]

The experimental difficulties generated by the combination of a complex microbiota and the presence of a diverse TCR repertoire resulted in the establishment of several simplified experimental models that facilitate the study of antigen-specificity in host-microbiota crosstalk (Table 3). These models combine cloning of TCRs, use of transgenic TCRs with known specificity, and use of defined gnotobiotic mouse models to generate simplified experimental conditions. These tools have been instrumental in gaining valuable insight on the role of antigen-specificity of intestinal T helper cells.

Table 3. Available TCR transgenic models to study bacteria-specific $CD4^+$T responses.

TCR transgenic models	Species	recognized antigen	recognized epitope	Reference
CBir1	Commensals	CBir1 flagellin (456–475)	DMATEMVKYSNANILSQAGQ	160
Smarta	*E. coli*	OmpC_GP61	GLNGPDIYKGVYQFKSVEFD	161
7B8	SFB	P3340	FSGAVPNK	25
5A11	SFB	unknown	IRWFGSSVQ	25
1A2	SFB	unknown	QFSGAVPNK	25
CT2	Clostridia	unknown	AASAIWNTGYQNFY	116,162
CT6	Clostridia	unknown	AASGYSALGRLH	116
HH5-1	*H. hepaticus*	HH_1713	GNAYISVLAHYGKNG	43
HH7-2	*H. hepaticus*	HH_1713	QESPRIAAAYTIKGA	43
BθOM	*B. thetaiotaomicron*	BT4295 (541–554)	EEFNLPTTNGGHAT	163
Amuc124	*A. muciniphila*	Amuc_RS03735 (Am3735–1)	TLYIGSGAILS	59
	A. muciniphila	Am3740-1	LIFESSNALGLGR	59
TCR-M	Host	auto-antigen	RSLKLMATLFSTYASADR	164
	B. thetaiotaomicron	microbial antigen mimicry	TFLILMAALTATFASAQK	133
17.4-NOD and 14.6-NOD	Host	auto-antigen	VYLKTNVFL	165
	B. thetaiotaomicron	microbial antigen mimicry	KIYLKTNVYL	135

A. muciniphila Akkermansia muciniphila, *B. thetaiotaomicron* Bacteroides thetaiotaomicron, *E. coli* Escherichia coli, *GP* glycoprotein, *H. hepaticus* Helicobacter hepaticus, *OmpC* outer membrane porin C, *SFB* segmented filamentous bacteria.

Intestinal commensal-specific CD4 T cells in humans are even harder to investigate and most studies have been performed in the context of inflammatory condition using blood samples, which does not reflect true intestinal CD4 T cells.[118–121] One elegant study did demonstrate however that microbiota-specific CD4 T cells are abundant in the circulation of healthy individuals.[122]

Antigen mimicry and autoimmune disease

A large number of studies linking microbiota, intestinal T helper cells, and disease are correlative in nature and hence lack a detailed molecular or immunological mechanism.[123] We will focus here on recent literature implicating molecular mimicry of microbial and host CD4 T cell epitopes in autoimmune diseases. Molecular mimicry of cross-reactive antigenic epitopes between microbes and host self-peptides has been thought of as a potential mechanism to drive activation of auto-reactive T cells.[124,125] In addition to the previously mentioned EAE study,[109] the microbiota and CD4 T cell responses have also been implicated in mouse models of arthritis.[126,127] While no specific cross-reactive microbial antigen mimic has yet been identified in arthritis, it has been shown that intestinal Th17 cells expressing dual TCRs are involved in lung autoimmunity, which is a major cause of arthritis-related mortality.[128] While this is not classical molecular antigen mimicry, it was one of the first studies reporting the involvement of TCR specificity of intestinal CD4 T cells in systemic autoimmunity. Microbiota-dependent activation of an autoreactive TCR was also involved in the pathogenesis of autoimmune uveitis.[129–131] In this study, the activation of retina-specific T cells involved signaling through the autoreactive TCR induced by a microbial antigen and was independent of the endogenous retinal autoantigen.[129] However, the precise bacterial species and antigen involved have not been identified. Furthermore, skin, oral, and gut commensal orthologs of the human lupus autoantigen Ro60 were able to activate Ro60-specific CD4 memory T cell clones from lupus patients, implicating T cell cross-reactivity and microbial antigen mimicry in lupus.[132] A recent study provided proof of the existence of a specific microbial peptide mimic within β-galactosidase expressed by *B. thetaiotaomicron*. This molecular mimic induced autoimmune T helper cells that also recognize the autoantigen myosin heavy chain 6 (MYH6), thus causing cardiomyopathy.[133] This study also linked their findings to a human patient population. Microbial antigen mimicry in autoimmune disease has not only been identified for CD4 T cells but also for CD8 T cells in Type 1 diabetes, for example.[134] It is also important to note that microbial mimicry of host autoantigens does not necessarily have to be detrimental but can also be protective, as demonstrated in a mouse model of colitis.[135] Taken together, these studies clearly demonstrate microbial antigen mimicry as an important mechanism by which intestinal CD4 T helper cell responses can either promote or protect from systemic autoimmune disease. It will be interesting to study whether bacterial or host metabolites are involved in determining whether antigen mimicry is detrimental or beneficial in an autoimmune setting.

Microbiota-mediated T helper cell responses in cancer ICB immunotherapy

We are using ICB as an example for how commensal bacteria-mediated intestinal CD4 T cell responses can be used to promote disease therapy. The most widely used current ICB strategies include monoclonal antibodies targeting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and/or programmed cell death protein (PD-1) as well as its ligand PD-L1. Seminal work using murine tumor models demonstrated that the gut microbiota influences the efficacy of ICB therapy[136,137] and more recent work translated these findings to cancer patients.[56,138–140] For example, enrichment of *A. muciniphila* in the fecal microbiota of patients with epithelial cancer correlated with a favorable clinical outcome following ICB therapy.[138] Furthermore, a study involving patients with metastatic melanoma receiving ICB therapy observed an increased abundance of eight microbial species including *Bifidobacterium longum* in ICB responders.[140] Another study on ICB therapy in metastatic melanoma patients found a significant enrichment of the *Faecalibacterium* genus in responders, whereas non-responders displayed a higher abundance of the Bacteroidales order.[139]

Beyond these correlative patient data, it has been shown that germ-free or antibiotic treated tumor-bearing mice receiving fecal microbiota transplantation (FMT) from patients responding to ICB improved the response to anti-PD-1 treatment.[138–140] In contrast, mice receiving FMT from non-responders showed poor response to ICB therapy. Of note, oral gavage of *A. muciniphila* following FMT with non-responder's feces restored efficacy of ICB therapy in vivo, suggesting that primary resistance to ICB therapy may partially be due to the absence of specific bacteria.[138] The beneficial effect of *A. muciniphila* in tumor-bearing mice was dependent on increased secretion of IL-12 by DCs promoting the accumulation of $CCR9^{+}CXCR3^{+}CD4^{+}$ T cells in lymph nodes and tumor.[138] Similarly, *B. fragilis*-mediated Th1 immune responses following anti-CTLA-4 treatment were also IL-12 dependent.[137]

Collectively, these studies provide compelling evidence that the intestinal microbiota influences anti-tumor immunity. Although the specific microbes associated with enhanced clinical response vary between reports, it is likely that various members of the human microbiota can modulate the response to ICB therapy. A study by Tanoue et al. revealed that a consortium of 11 bacteria isolated from the human gut microbiota induced IFNγ-producing CD8 T cells and, to a lower extent, also CD4 T cells. These bacteria-induced anti-tumor immunity by itself and further promoted the efficacy of ICB inhibitors in multiple tumor models.[56] The authors suggested that circulating metabolites produced or induced by these microbes might modulate systemic T cells. Indeed, several immunomodulatory metabolites, such as mevalonate and dimethylglycine, were elevated in the caecal contents and serum of gnotobiotic mice colonized with the 11 strain consortium. The challenge in this field is now to move on from identifying bacteria that enhance ICB therapy towards molecular mechanisms of how these bacteria improve ICB-therapy efficacy.

CONCLUSION

The various topics discussed in this review are summarized in Fig. 3. While understanding microbial composition, diversity, and richness are already complex enough, it is now clear that a functional understanding of the microbiota requires a more in-depth understanding of the immunomodulatory capacity of microbial metabolites. This added complexity poses both experimental and analytical challenges. One important experimental consideration is the impact of differing diets on microbiota composition and metabolic activity. It is therefore important to continue to experimentally dissect the interplay between microbes, metabolites, diet, immune system, and the host to study host-microbial crosstalk and how this modulates the dynamics of intestinal T helper cell phenotypes and function. Continuing development of computational analysis tools for the analysis of metabolomic data, similar to the tools available for microbiota composition analysis, is important to overcome analytical challenges non-specialist labs currently face.[141] Another important challenge is the translation from mouse models to humans. In vitro screening methods for human-derived commensal microbes are key for translating mouse data to the human situation.[142] Furthermore, recent studies in wild mice that harbor a wild microbiota, which is considerably more diverse than standard SPF laboratory mice, have demonstrated immune phenotypes that more closely resemble the human situation.[143] Disease phenotypes and therapies in these wild mice also better reflected what is

observed in human patients.[144] Therefore, while defined experimental germ-free and gnotobiotic mouse models are essential to identify molecular disease mechanisms together with specific microbial species and metabolites involved in a given disease model, wild mice might provide an important tool to translate these mechanistic findings into an experimental in vivo model that more closely resembles the human situation.

Charles Janeway famously referred to the use of adjuvants by experimental immunologist as their "dirty little secret", which lead to the formulation of his hypothesis of the existence of evolutionary conserved pattern recognition receptors.[145] Wild mice with a very complex and undefined microbiota may hold their own "dirty little secret", which will help us to better translate disease mechanisms identified in defined mouse models to the human situation.

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AUTHOR CONTRIBUTIONS

M.B.G. and R.B. contributed equally to the writing of this manuscript. R.B. generated the drafts for the figures.

ADDITIONAL INFORMATION

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