Feature Review

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Focus: mTOR in immunity

Metabolic control of regulatory T cell development and function

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Foxp3$^+$ regulatory T cells (Tregs) maintain immune tolerance and play an important role in immunological diseases and cancers. Recent studies have revealed an intricate relationship between Treg biology and host and microbial metabolism. Various metabolites or nutrients produced by host and commensal microbes, such as vitamins and short-chain fatty acids (SCFAs), regulate Treg generation, trafficking, and function. Furthermore, cell intrinsic metabolic programs, orchestrated by mTOR and other metabolic sensors, modulate Foxp3 induction and Treg suppressive activity. Conversely, Tregs are crucial in regulating obesity-associated inflammation and host metabolic balance, and in shaping homeostasis of gut microbiota. We review here the interplay between Tregs and metabolism, with a particular focus on how host, commensal, and cellular metabolism impinge upon Treg homeostasis and function.

Introduction

Although long considered to be two separate disciplines, metabolism and immunology have been recently merged to form the rapidly advancing field of immunometabolism, which explores the interaction between metabolic activities and immune responses [1]. Upon cognate antigen stimulation, naïve CD4$^+$ T cells are activated, differentiate into T helper cell [T_H1, T_H2, T_H17, or follicular helper (Tfh)] effector lineages dependent on instructive cytokine milieu, and some of the effector T cells eventually become memory T cells. Tregs are a specialized T cell population that provides dominant suppression over effector T cells as well as other immune cells. The majority of Tregs develop in the thymus (tTregs), where they are selected by strong or intermediate T cell receptor (TCR) signals while escaping negative selection [2]. Tregs can also be converted from naïve T cells in the periphery (pTregs), generally at mucosal surfaces that interface with the environment, or in *in vitro* assays [induced Tregs (iTregs)], particularly in the presence of the anti-inflammatory cytokine transforming growth factor β (TGF-β). Expression of the transcription factor Foxp3 is essential for Treg development and function, and is regulated by genomic regulatory elements termed conserved noncoding DNA sequences (CNS) 1–3. CNS1 is dispensable for tTreg differentiation, but crucial for pTreg generation in gut-associated lymphoid tissues (GALT). CNS2 is required for Foxp3 expression in the progeny of dividing Tregs. CNS3, the pioneer element, controls *de novo* Foxp3 expression and tTreg differentiation [3]. Stimulation through the T cell receptor (TCR) induces Foxp3 expression and promotes Treg-specific CpG hypomethylation in Treg signature genes, and the combined actions of these independent events drive Treg development [4]. Thus, Treg lineage development is governed by both genetic and epigenetic programs.

Recent studies have revealed that metabolic factors derived from both extrinsic and intrinsic sources shape Treg abundance and activity. Host-derived nutrients and hormones play an important role in the generation, proliferation, and survival of Tregs. In addition, commensal microbiota-derived metabolites, such as SCFAs, control Treg homeostasis and function in the GALT. Furthermore, compared to naïve T cells, Tregs exhibit unique metabolic activities, characterized by low to modest glycolysis and elevated mechanistic target of rapamycin (mTOR) activity and nutrient metabolism, and these Treg-intrinsic metabolic pathways program Treg generation and activity [5–7].

These exciting new studies indicate that Tregs could serve as a 'liaison' between immunity and metabolism, that is, immune function is affected by metabolic fitness through the modulation of Tregs at three levels of regulation: host nutritional status, commensal microbes, and the cellular metabolism of Tregs themselves. We first discuss how host metabolism, including vitamin and hormone production, affects Treg cellularity, trafficking, and survival. Second, we summarize recent discoveries on how commensal microbial metabolites control colonic Treg generation and activity. Third, we describe how intracellular metabolic pathways program Treg homeostasis and function. Finally, it is also important to note that the immune system could reciprocally regulate host, microbial, and cellular metabolism through Tregs. Therefore, we briefly discuss the reciprocal interaction between Tregs and metabolic disease, and the implications of this interaction for Treg-based therapeutics.

Host metabolism and Tregs

Metabolism is a set of physical and chemical processes that derive energy and macromolecules from nutrients to sustain life. The interaction between malnutrition and impaired immunity was explored nearly 100 years ago [8], but it was not until late 1950s that malnutrition was firmly established as one of the causes of increased susceptibility to

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Keywords: metabolism; vitamin; commensal microbiota; SCFA; mTOR; obesity.

1471-4906/

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infection [9]. It is now recognized that both malnourishment and over-nutrition, exemplified by the ongoing epidemic of obesity, adversely impact immunity. Further, dysregulated immune system function contributes to many metabolic disorders including insulin resistance and diabetes [10]. Recent findings have revealed that host metabolic status and multiple nutrient metabolites impact Treg homeostasis, and this may in turn have a bearing upon metabolic disorders and associated inflammation.

Diverse vitamins and their metabolites control Treg trafficking, de novo generation, and survival

Vitamins are essential organic compounds that are either synthesized or obtained through dietary sources. A variety of immunological disorders can result from deficiency of various vitamins [11]. Among these, vitamins A, D, B_3, and B_9 have been linked to Treg biology. Dietary sources of vitamin A include all-*trans*-retinol, retinyl esters, and β-carotene. These are first converted to all-*trans*-retinal by alcohol dehydrogenases or short-chain dehydrogenases/reductases, which are ubiquitously expressed. All-*trans*-retinal is then oxidized to all-*trans* retinoic acid (RA) by retinal dehydrogenases (RALDHs), which are selectively expressed by dendritic cells (DCs) in GALT [12]. RA has pleiotropic effects on the host immune system. Specifically, RA promotes the effector functions of $CD4^+$ T cells [13], supports the generation of IgA-secreting B cells in GALT [14], mediates the balance between innate lymphoid cell (ILC) 3 and ILC2 [15], and controls secondary lymphoid organ development [16]. RA also imprints gut-homing specificity on T cells and B cells by inducing the expression of α4β7 integrin and CCR9, two receptors crucial for trafficking to the small intestine [12,14], and this process is dependent on the activity of the p38 signaling pathway in mucosal DCs [17].

RA has an important role in shaping Treg development and function in the gut. Of note, different T cell lineages do not represent irreversibly differentiated endpoints. In particular, iTregs and T_H17 subsets exhibit considerable plasticity in that they can be reciprocally regulated by cytokines and cellular metabolism [7,18,19]. Mucida *et al.* showed that treatment of naïve $CD4^+$ T cells *in vitro* with RA and TGF-β induced the expression of Foxp3 and gut-homing receptors, while suppressing T_H17 differentiation [20]. Further studies attributed this Treg promoting effect in part to RA-mediated inhibition of proinflammatory cytokine production from $CD4^+CD44^{hi}$ effector T cells [21]. RA synthesis from vitamin A in the intestine is dependent upon $CD103^+$ DCs in the mesenteric lymph nodes (MLN) and the small-intestine lamina propria (LP). $CD103^+$ DC-derived RA, in combination with TGF-β, induces Foxp3 expression in naïve T cells, and these pTregs preferentially home to MLN and the small intestine [22–24]. This mechanism has significant physiological implications because it underlies tolerance induced by oral or food-derived antigens, known as oral tolerance, which is crucially dependent on pTreg generation [25]. Further, TGF-β/RA-converted Tregs ameliorate inflammatory responses in animal models of colitis [20]. Thus, RA promotes TGF-β-mediated pTreg generation and homing in GALT, and maintains mucosa immune tolerance.

Vitamin D_3 is synthesized in the skin from 7-dehydrocholesterol under sunlight, or is acquired from the diet. Vitamin D_3 is further metabolized into 25-dihydroxyvitamin D_3 [$25(OH)VD_3$], and into the most biologically active form, 1,25 $(OH)_2VD_3$, in the liver, kidney and many immune cells. The cellular action of 1,25 $(OH)_2VD_3$ is mediated by the vitamin D receptor (VDR), a ligand-dependent transcription factor. 1,25 $(OH)_2VD_3$ inhibits T cell proliferation and cytokine production, as well as inducing Foxp3 expression and enhancing the suppressive activity of Tregs [26–29]. Moreover, it can induce tolerogenic DCs that enhance Treg generation and mediate transplantation tolerance [30]. Vitamin D response elements have been identified in the conserved noncoding sequence region of the human *FOXP3* gene, and these could underlie how vitamin D_3 induces Foxp3 expression [31]. However, vitamin D_3 was shown to be required for human T cell activation [32]. In addition, mice lacking VDR contain normal numbers of functional Tregs in spleen and thymus [33] and do not develop overt systemic autoimmune disorders [34]. Nonetheless, they do have low-grade inflammation in the colon manifested by increased expression of interleukin (IL)-1β and tumor necrosis factor (TNF)-α [34]. Therefore, the physiological relevance of vitamin D_3 metabolites on Treg induction remains to be ascertained.

Tetrahydrofolate, which is derived from vitamin B_9 [also known as folic acid (FA)], is required for DNA synthesis, repair, and methylation, and is particularly involved in cell proliferation and survival [35]. tTregs constitutively express high level of folate receptor 4 (FR4), which binds FA and delivers FA derivatives into cells [36]. The importance of dietary FA to Tregs is revealed by the selective reduction of intestinal Tregs in mice fed with a FA-deficient diet or treated with an inhibitor that disrupts FA metabolism [37]. FA promotes Treg cellularity by inhibiting apoptosis [37,38]. Further, FA deficiency leads to increased colonic inflammation, which can be ameliorated by transfer of $FR4^+$ tTregs [37]. Tregs purified from human peripheral blood mononuclear cells (PBMC) also preferentially express FR4 relative to other lymphocytes [36], but the impact of FA on human Tregs remains to be examined. Thus, vitamin B_9 metabolism maintains gut Treg survival and restricts intestinal inflammation.

Vitamin B_3, also known as niacin and nicotinic acid, is an essential nutrient. Deficiency of vitamin B_3 in human leads to pellagra, a disease characterized by intestinal inflammation, diarrhea, dermatitis, and dementia [39]. Vitamin B_3 signals through the G protein-coupled receptor (GPR) 109a. This interaction induces anti-inflammatory properties, including the expression of RALDHs in colon macrophages and DCs, which in turn induce Treg differentiation. GPR109a-deficient mice contain reduced colonic Tregs and show increased susceptibility to colonic inflammation [40]. Thus, vitamin B_3 promotes colonic Treg generation and maintains colon homeostasis.

Leptin limits Treg proliferation

One of the characteristics of Tregs is their lack of proliferation upon TCR engagement alone *in vitro*, known as *in vitro* anergy. In an effort to explain this phenomenon, Matarese and colleagues found that human PBMC Tregs produce leptin (also known as OB) and constitutively

express leptin receptor (LEPR, also known as OBR), which can be further increased upon TCR engagement [41,42]. Leptin, a 16 kDa adipokine produced primarily by adipocytes, exerts its canonical function of restraining food intake and promoting energy expenditure by interacting with LEPR in the central nervous system [43]. In tTregs, leptin acts to inhibit TCR-induced proliferation; administration of an anti-leptin antibody breaks *in vitro* anergy, and tTregs from leptin or LEPR-deficient mice exhibit increased proliferative potential in response to TCR stimulation [41,42]. These data indicate that leptin is partly responsible for the *in vitro* anergic phenotype of Tregs. *In vivo*, leptin- or LEPR-deficient mice have reduced susceptibility to autoimmunity [44–47], which is associated with increased tTreg numbers as well as reduced naïve T cell proliferative potential [41]. In addition, the interplay between leptin signaling and Tregs could underlie the immunosuppressive state induced by fasting or starvation [42]. Furthermore, these observations could potentially explain the significant decrease of visceral adipose tissue (VAT)-associated Tregs in obese mice because these cells express increased level of leptin in the fat tissue [48,49]. Whole-body energy metabolism also regulates Tregs independently of leptin. For instance, deletion of *Sirt1* in hypothalamic agouti-related peptide-expressing (AgRP) neurons promotes appetite and decreases body metabolism, and this is associated with the reduced abundance of tTregs and impaired immunosuppressive activity in a leptin-independent manner [50].

Taken together, host nutritional status, including the uptake and generation of various vitamin metabolites, as well as the production of leptin in response to the accumulation of body fat mass, modulates Treg abundance and function. This interaction in turn affects immune homeostasis, particularly in GALT and adipose tissue (Figure 1). Moreover, GALT Tregs are further shaped by commensal microbiota metabolism, which will be described below.

Commensal microbial metabolism and Tregs

It is estimated that the human body harbors 100 trillion commensal microbes, exceeding the number of host cells by more than 10-fold. Multiple studies into how the microbiota and the host immune system interact have revealed the fundamental importance of the microbiome in shaping host immune responses, and how the immune system in turn affects the composition of the microbiota; these concepts have been reviewed elsewhere [51]. Here we focus on the impact of microbial metabolism and the associated metabolites on the differentiation and function of Tregs.

Specific microbial species induce Treg generation in the colon

In mice, both tTreg and gut-induced pTregs are necessary to maintain intestinal immune tolerance [52–54]. The importance of commensal microbiota for mucosa Treg abundance is underscored by the severe reduction of Tregs in the colon LP in germ-free (GF) mice or vancomycin-treated mice [55]. In addition, the beneficial effects of probiotics

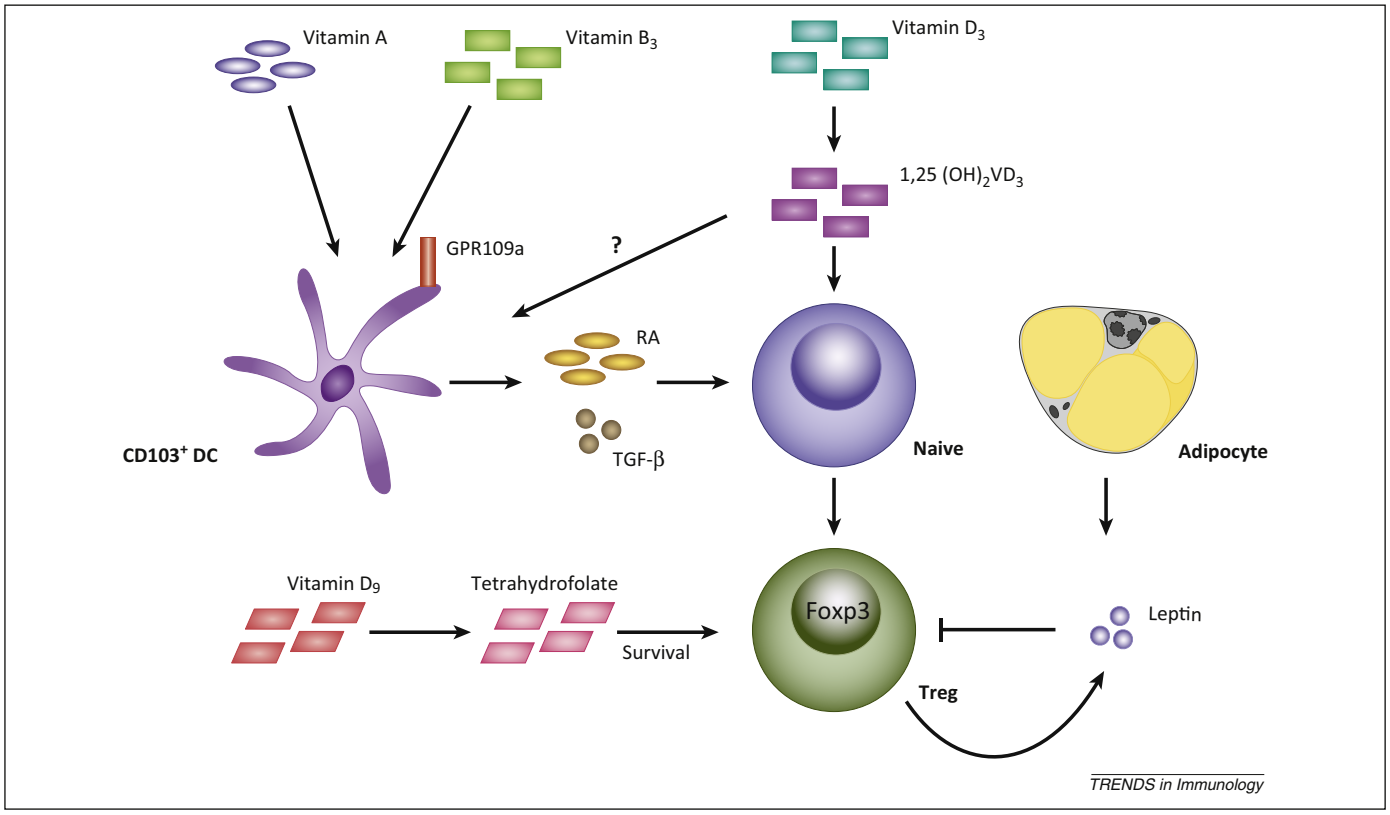

Figure 1. Host metabolism modulates regulatory T cell (Treg) homeostasis. Retinoic acid (RA), a vitamin A metabolite produced by gut-associated lymphoid tissues (GALT) $CD103^+$ dendritic cells (DCs), induces the conversion of naïve $CD4^+$ T cells to Tregs. Vitamin B_3 binds to G protein-coupled receptor GPR109a on GALT DCs and other innate immune cells, allowing these cells to induce Foxp3 expression in naïve T cells. Vitamin D_3, through its metabolite 1,25 $(OH)_2VD_3$ and the vitamin D receptor (VDR), enhances Foxp3 gene transcription by binding to the Foxp3 promoter locus. The vitamin B_9 metabolite tetrahydrofolate maintains Treg survival. Leptin, produced by adipocyte and Tregs, restrains Treg proliferation and abundance.

have been partly attributed to the induction of Tregs [56–59]. Given the crucial role of Tregs in maintaining intestinal homeostasis, it is probably not surprising that many microbial species have the potential to induce Tregs [60,61]. Consistent with preferential accumulation of Tregs in the colon during ontogeny [55], Treg expansion induced by microbial colonization occurs specifically in the colon LP [60,61]. Atarashi *et al.* screened both mouse and human microbiota for specific microbe species that are capable of inducing Tregs in the colon. They discovered that colonization of mice with murine feces-derived *Clostridia* clusters IV and XIVa, or clusters IV, XIVa, and XVIII isolated from human feces, expands the population of Tregs and enhances their activity in colonic LP, as indicated by the increased expression of IL-10 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). These Gram-positive, spore-forming bacteria preferentially colonize the cecum and proximal colon, consistent with their preferential impact on colonic Tregs. Mechanistically, they are attached to colon intestinal epithelium cells (IECs) and activate IECs to produce TGF-β, a major cytokine that promotes Treg differentiation in the intestine [55,62]. In addition, colonization of GF mice with *Clostridia* stimulates colonic conventional T cells to produce IL-2, an essential cytokine for Treg proliferation. IL-2 induces expression of the epigenetic regulator Uhrf1 in colonic Tregs, which silences the expression of the cell cycle inhibitor p21 (encoded by *Cdkn1a*) by maintaining DNA methylation at the promoter region of *Cdkn1a*. Deletion of Uhrf1 in $CD4^+$ T cells leads to reduced Tregs specifically in the colon LP and the development of spontaneous colitis [63]. How commensal microbiota stimulate IL-2 production in conventional T cells remains to be defined. Hence, *Clostridia* represents a major commensal microbial species that promotes colonic Treg differentiation through TGF-β and IL-2 production from IECs and conventional T cells, respectively.

Polysaccharide A (PSA) produced by *Bacteroides fragilis*, a prominent symbiotic anaerobe colonizing mammalian lower gastrointestinal tract, has important immunoregulatory functions [64]. *B. fragilis*-derived PSA engages Toll-like receptor 2 (TLR2) expressed by T cells, which promotes pTreg generation from naïve $CD4^+$ T cells in GALT and enhances Treg production of regulatory cytokines including IL-10 and TGF-β2. Moreover, PSA promotes IL-10-producing Treg generation in peripheral lymphoid organs. Of note, PSA administration or *B. fragilis* colonization protects mice from intestinal inflammatory diseases induced by chemical agents or by *Helicobacter hepaticus* colonization [65–67], and ameliorates experimental autoimmune encephalomyelitis (EAE), a murine model for human multiple sclerosis (MS) [68–70]. These findings suggest that PSA production by *B. fragilis* induces the generation of Tregs with enhanced immunoregulatory activity and protects the host from inflammatory diseases. However, the ability of *B. fragilis* to induce colonic Tregs is significantly less potent compared to that of *Clostridia* [55], suggesting that different bacteria may elicit distinct responses, in terms of colonic Treg induction, via potentially different mechanisms.

Specific microbes have also been linked to the generation of effector T cell lineages in the intestine. Colonization of mice with a single commensal microbe, segmented filamentous bacterium (SFB), induces T_H17 cells but not other effector lineages or Tregs in the small intestine LP, and this in turn promotes antibacterial immunity as well as autoimmune arthritis in pathological settings [71–73]. Notably, commensal microbe-derived ATP drives intestinal T_H17 generation, suggesting that microbiota metabolism controls T_H17 lineage differentiation in the GALT [74]. The discovery of microbiota-dependent induction of specific T cell subsets suggests that different T cell subsets may recognize distinct commensal microbe antigens. Indeed, intestinal DCs present SFB antigens to drive mucosal T_H17 cell differentiation at different lymphoid tissues in an MHC-II-dependent mechanism [75,76]. Importantly, the TCR repertoire of gut T_H17 cells is mostly restricted to SFB antigens, which implies that microbiota antigens could dictate the fate of antigen-specific T cells [77]. This raises the interesting possibility that the TCR repertoire of gut Tregs is also shaped by specific microbial antigens; future work will be necessary to test this model.

Microbial metabolites promote Treg generation in the colon

Mammals depend upon bacteria to break down particular dietary components, including resistant starch and dietary fibers [78]. SCFAs are major metabolites from the bacterial fermentation of dietary fiber, and are highly enriched in the colon [79]. To explore the immunoregulatory functions of SCFAs, several groups performed a series of quantitative analysis of SCFA composition in cecal content between specific-pathogen free (SPF) mice and GF mice [80,81], or between mice fed with high-fiber diet or low-fiber diet [82]. They reported that butyrate, acetate, and propionate are the major bacteria-derived SCFAs that control the mucosa Treg differentiation and function.

SCFAs promote Treg differentiation through several mechanisms. First, signaling mediated by GPRs for SCFAs stimulates Treg differentiation. Expression of GPR43, which binds multiple SCFAs, on neutrophils and eosinophils has been implicated in dampening gut inflammatory responses [83]. Smith *et al.* found that colonic Tregs also express GPR43, and that this expression required the presence of gut microbiota. Furthermore, propionate-induced Treg differentiation and potentiation of suppressive activity is abolished in GPR43-deficient mice [81]. GPR109a, which specifically binds butyrate, is expressed on colon IECs and innate immune cells, but not lymphocytes [84]. The interaction between butyrate and GPR109a promotes production of IL-10 and RALDHs from macrophages and DCs, thereby inducing Treg generation [40]. Thus, SCFAs signal via distinct receptors expressed on Tregs and innate immune cells to induce Treg differentiation.

SCFAs also regulate Treg differentiation through epigenetic modifications. Butyrate is a known histone deacetylase (HDAC) inhibitor. When naïve $CD4^+$ T cells are cultured in iTreg differentiation conditions, butyrate treatment enhances acetylation at histone H3 lysine 27 (H3K27) at the Foxp3 promoter and CNS1 and CNS3 enhancers. These epigenetic modifications lead to increased Foxp3 induction [80,82]. Moreover, butyrate, through its HDAC inhibition activity, potentiates the

capability of DCs to induce Treg differentiation by repressing the expression of lipopolysaccharide (LPS) response genes [80], and also inhibits macrophage activation by rendering them hyporesponsive to TLR stimulation [85]. However, the developmental origin of SCFAs-induced colonic Tregs remains controversial. Although some studies found that SCFAs only promote the conversion of naïve $CD4^+$ T cells to Tregs [80,82], one group reported that SCFAs expand existing tTregs in the colon [81]. While further investigation is necessary to resolve the discrepancy, these studies collectively demonstrate that SCFAs, generated by gut microbial metabolism, promote colonic Treg differentiation and contribute to immune homeostasis in the colon (Figure 2).

Cellular metabolism and Tregs

In addition to aforementioned extrinsic metabolic factors that control Treg differentiation and function, cell intrinsic metabolic programming also plays a crucial role in Tregs. The progression of T cell activation, effector cell differentiation, and memory cell formation is accompanied by a series of metabolic transitions [86,87]. Furthermore, different T cell lineages display distinct metabolic features; in particular, Tregs exhibit high levels of fatty acid oxidation, nutrient metabolism, and mTOR activity versus naïve T cells [6,87]. The evolutionarily conserved mTOR pathway senses diverse environmental cues, including nutrient availability and metabolic activities, to regulate cell growth and differentiation. The mTOR pathway consists of two complexes, mTORC1 and mTORC2, with scaffold proteins Raptor and Rictor as their defining components, respectively. Both mTORC1 and mTORC2 contain the catalytic subunit, the serine/threonine kinase mTOR. A major upstream activator of mTORC1 is Rheb, a small GTPase, which mediates early, but not late, mTORC1 activation following T cell activation [88]. The immunosuppressive drug rapamycin preferentially inhibits mTORC1, but also interferes with mTORC2 activity. mTORC1 promotes anabolic metabolism, especially protein and lipid biosynthesis, and inhibits autophagy, whereas mTORC2 regulates cytoskeleton dynamics. The detailed composition and function of mTOR pathway have been reviewed elsewhere [89,90]. We focus here mainly on mTOR-dependent regulation of Treg generation and function (Figure 3A).

In cell culture experiments, inhibition of mTOR with rapamycin or by restricting essential amino acids (EAAs) induces Foxp3 expression and promotes Treg generation from naïve $CD4^+$ T cells. Conversely, mTOR activation

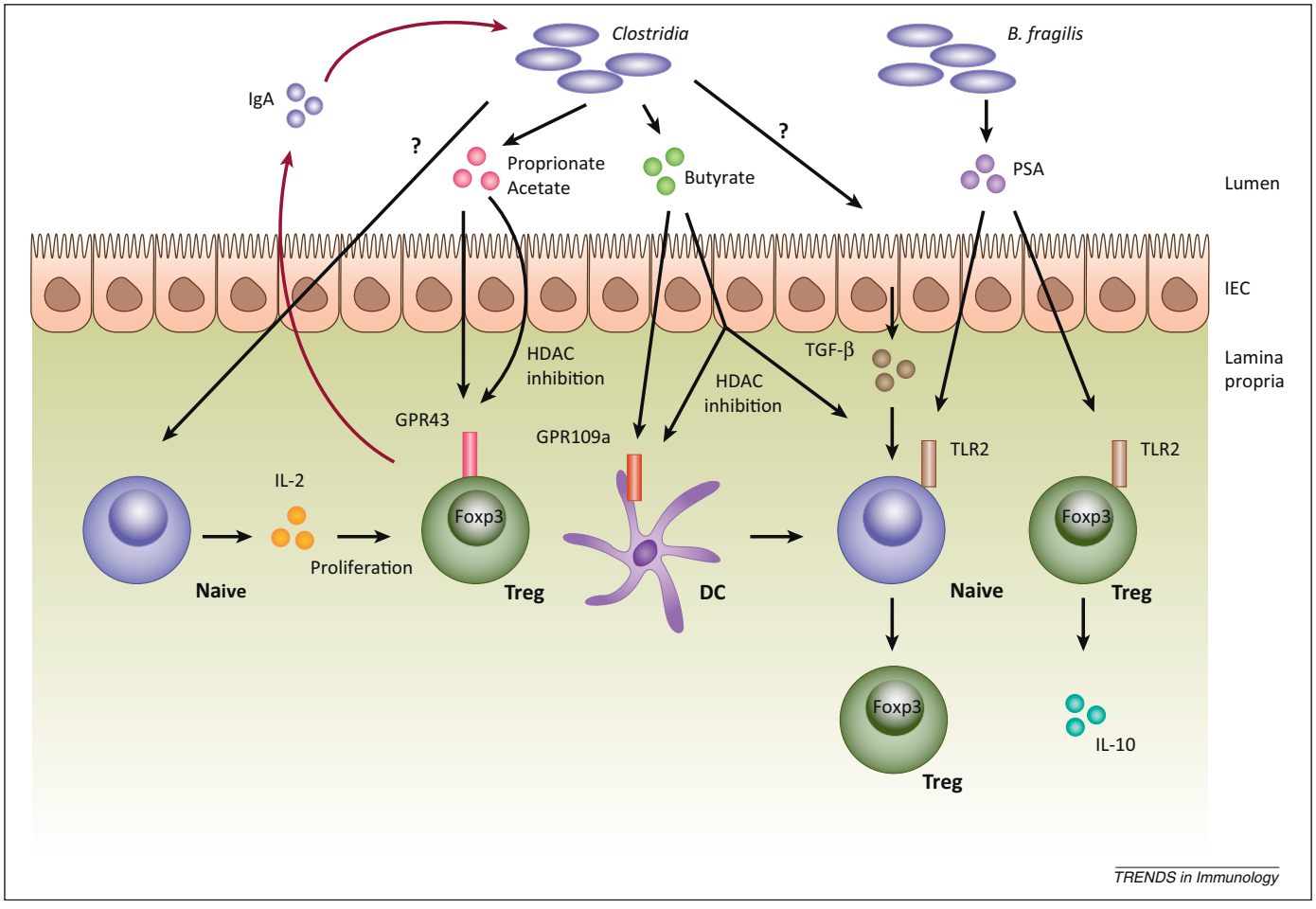

Figure 2. Commensal microbial metabolism controls regulatory T cell (Treg) homeostasis in the colon. Colonization of mice with *Clostridia* species from both mouse and human feces leads to increased Treg abundance in the colon. Short-chain fatty acids (SCFAs), metabolites produced through bacteria fermentation of dietary fiber, promote Treg expansion and *de novo* generation. *Clostridia* species also stimulate conventional T cells to produce interleukin (IL)-2, which promotes colonic Treg proliferation. *B. fragilis* produces polysaccharide A (PSA) that can induce Treg induction and potentiate Tregs to produce regulatory cytokines such as IL-10. In a feedback loop, Tregs contribute to diversification of *Clostridia* species by promoting the generation and selection of IgA. Abbreviations: DC, dendritic cell; GPR, G protein-coupled receptor; HDAC, histone deacetylase; IEC, intestinal epithelial cells; TGF, transforming growth factor; TLR, Toll-like receptor.

through the phosphoinositide 3-kinase (PI3K)–AKT (protein kinase B) axis inhibits Foxp3 expression [91–95]. Genetic analyses have confirmed these findings. Deletion of mTOR, thus eliminating both mTORC1 and mTORC2 activity, leads to failure of T_H1, T_H2, or T_H17 effector lineage differentiation, but to spontaneous generation of Foxp3$^+$ iTregs, even in the absence of Treg-polarizing cytokines [96] (see Box 1 and Figure 3B for mTOR signaling in conventional T cell differentiation). Importantly, mTOR-dependent induction of the transcription factor hypoxia-inducible factor 1α (HIF1α) suppresses iTreg generation while promoting T_H17 differentiation through modulating the glycolytic pathway and transcriptional control [7,97]. Mice with T cell specific deletion of Rheb or Rictor remain healthy, and their naïve CD4$^+$ T cells do not spontaneously differentiate into Foxp3$^+$ iTregs *in vitro*, suggesting that both mTORC1 and mTORC2 are required to inhibit iTreg generation [96,98]. Therefore, mTOR signaling is a negative regulator of *de novo* Foxp3 expression in naïve T cells.

Although initial study using T cell specific deletion of mTOR indicated that mTOR deficiency does not significantly impact upon Treg suppressive function *in vitro* [96], genetic deletion of *Rptor* (which encodes Raptor) specifically in Tregs revealed that mTORC1 has a surprisingly positive role in Treg function. Treg-specific deletion of *Rptor* leads to severe systemic autoimmune diseases and early death, whereas deletion of *Rictor* preserves immune homeostasis [5]. Raptor-deficient Tregs have intrinsic defects because they express reduced levels of the immune-suppressive molecules CTLA-4 and ICOS ('inducible T cell costimulator'), and have severely impaired suppressive activity *in vitro* and *in vivo*. These functional defects are linked to impaired lipid biosynthesis, in particular the mevalonate pathway. Pharmacological inhibition of the mevalonate pathway in Tregs using statins, inhibitors of the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), impairs their suppressive activity, whereas addition of mevalonate, the product of HMGCR, restores Treg-mediated suppression. TCR and IL-2 stimulation potently activates mTORC1, suggesting that mTORC1 couples immune signals and lipid biosynthesis to establish Treg functional competency. The role of mTORC1 in additional metabolic pathways and other relevant processes remains to be explored. Interestingly, deletion of *Rictor* partially restores the defective suppressive activity of Raptor-deficient Tregs, and Treg-specific deletion of both *Rptor* and *Rictor* slightly improves autoimmune symptoms observed in mice lacking *Rptor* in Tregs. Therefore, mTORC1 promotes Treg function partly through inhibition of mTORC2 activity, but mTORC1-dependent lipid metabolism plays a more dominant role [5]. Lipid metabolism, via peroxisome proliferator-activated receptor (PPAR)-γ-dependent program, has been associated with the homeostasis of VAT-associated Tregs [48], which will be further discussed below. Taken together, these data indicate that T cell intrinsic metabolic activity controls Foxp3 expression and Treg function, in that mTOR-mediated anabolism inhibits Foxp3 induction in naïve T cells, while mTOR-dependent lipid biosynthesis potentiates Treg function (Figure 3A).

Box 1. mTOR in effector and memory T cell differentiation

mTOR kinase activity is required for T_H1, T_H2, and T_H17 differentiation [96], but different mTOR complexes have differential functions in effector T cell differentiation (see Figure 3B), as discussed below.

T_H1: T cell specific deletion of Rheb or Rictor leads to impaired T_H1 differentiation, suggesting a positive role of mTORC1 and mTORC2 in T_H1 differentiation [98,109]. However, Raptor-deficient T cells exhibit normal T_H1 differentiation *in vitro* [110]. Further research is required to solve this discrepancy.

T_H2: whereas Rheb-deficient T cells differentiate into T_H2 cells normally [98], Raptor-deficient T cells have a profound defect in T_H2 differentiation *in vitro* and *in vivo* [88]. This difference is ascribed to Rheb-independent, but Raptor-dependent mTORC1 activity [88]. Rictor-deficient T cells also have impaired T_H2 differentiation [98,109]. Thus both mTORC1 and mTORC2 promote T_H2 differentiation.

T_H17: deletion of either Rheb or Raptor impairs T_H17 differentiation [98,110], whereas Rictor deletion does not affect this process [98,109]. Thus, mTORC1 is crucial for T_H17 differentiation.

Tfh: It is currently unknown whether or how mTOR signaling regulates Tfh differentiation.

Effector and memory CD8$^+$ T cells: deletion of Raptor abolishes the generation of antigen-specific effector CD8$^+$ T cells in a *Listeria monocytogenes* infection model, whereas deletion of Rictor marginally reduces this process, indicating that mTORC1 plays a predominant role in effector CD8$^+$ T cell formation [88]. Moreover, pharmacological study using rapamycin demonstrated that mTORC1 is required for effector CD8$^+$ T cell differentiation through transcription factor HIF1 [111]. However, mTORC1 negatively controls memory CD8$^+$ T cell generation because rapamycin treatment or Raptor knockdown promotes the frequency and function of memory CD8$^+$ T cells [112–114].

Tregs influence host metabolism and microbiota diversity

The interaction between metabolism and Tregs is a two-way street: host and commensal microbiota metabolism regulate Treg homeostasis, while Tregs also play an essential role in controlling host metabolic disorders and commensal microbial diversity. For example, normal mouse VAT is enriched with Tregs compared to other lymphoid organs, but these VAT-associated Tregs are significantly reduced in obese mice. Loss- and gain-of-function studies demonstrated that VAT-associated Tregs protect against obesity-associated inflammation and insulin resistance, and improve metabolic parameters [49]. A later study showed that PPAR-γ, a master transcription factor for adipogenesis, is highly expressed in VAT-associated Tregs and crucially regulates their homeostasis. Treg-specific deletion of PPAR-γ depletes Tregs specifically in VAT, but not in other organs. Strikingly, the insulin-sensitizing effect of the commonly used drug pioglitazone, a PPAR-γ agonist, is largely dependent on PPAR-γ expression by VAT-associated Tregs. Furthermore, pioglitazone promotes lipid uptake by VAT-associated Tregs by upregulating the expression of the fatty acid transporter CD36 and potentially activating fatty acid oxidation [48]. The protective role of VAT-associated Tregs on metabolic syndromes is also evident from studies on human subjects [99–101]. These findings highlight a potential therapeutic value of modulating Tregs to improve obesity-associated metabolic disorders.

Tregs also exert an important regulatory role on the composition of gut microbiota. In mice deficient for Foxp3

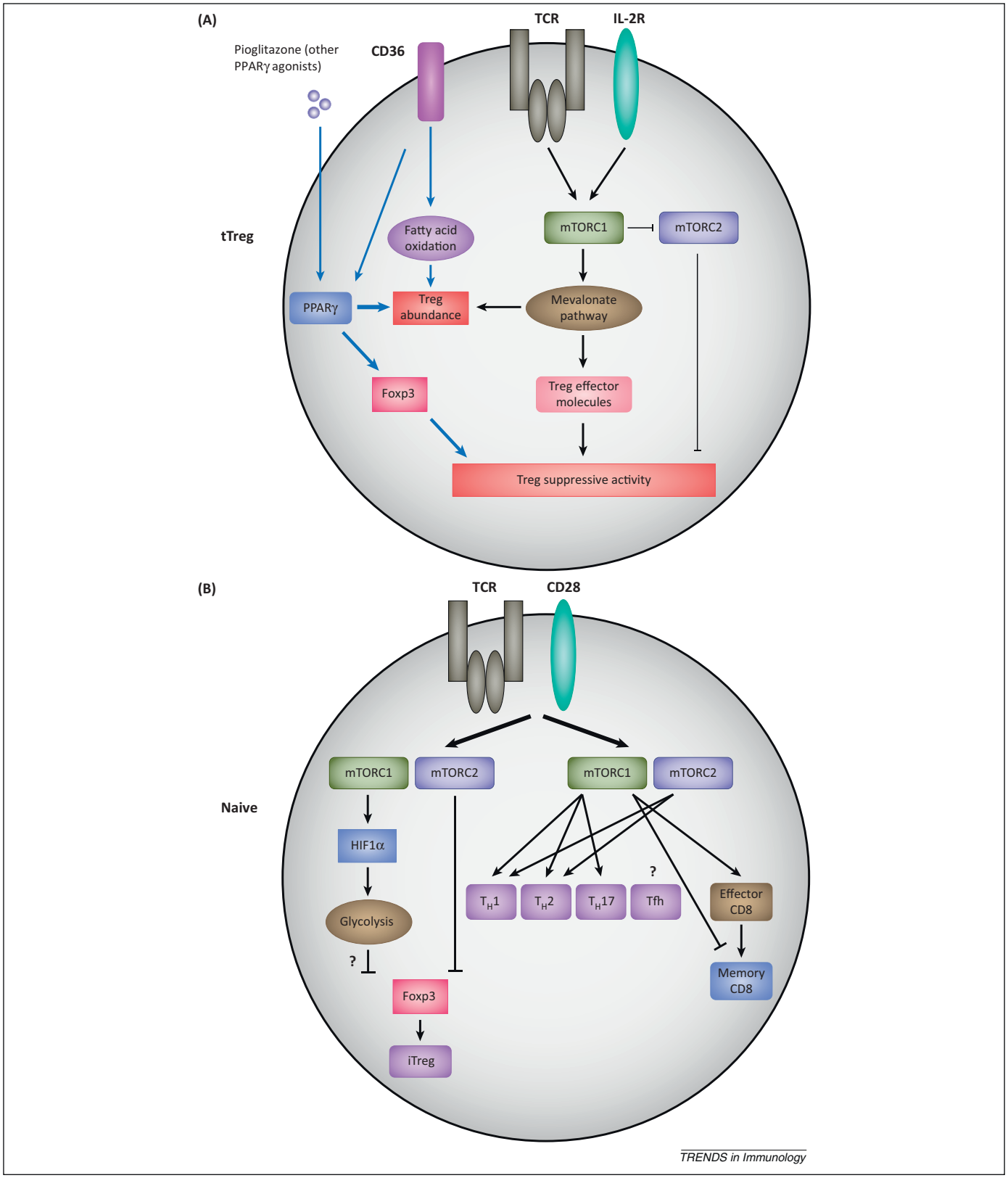

Figure 3. mTOR signaling in Tregs and conventional T cells. **(A)** In Tregs, TCR and IL-2 signaling promotes Treg proliferation and function by activating mTORC1-dependent lipid biosynthesis, particularly the mevalonate pathway. mTORC1 also promotes Treg function through inhibition of mTORC2 activity. VAT-associated Tregs exhibit increased expression of PPAR-γ, which promotes fatty acid metabolism and hence stimulates the accumulation and suppressive phenotypes of Tregs residing in adipose tissue. **(B)** In conventional T cells, mTOR signaling inhibits Foxp3 induction partly by inducing HIF1α expression and HIF1α-dependent glycolysis. mTOR signaling promotes T_H1, T_H2, T_H17, and effector $CD8^+$ T cell differentiation. However, mTORC1 signaling negatively regulates memory $CD8^+$ T cell differentiation. Whether and how mTOR signaling controls Tfh differentiation is currently unknown. Abbreviations: CD28/36, cluster of differentiation 28/36; HIF, hypoxia inducible factor; IL, interleukin; iTreg, induced regulatory T cell; PPAR, peroxisome proliferator-activated receptor; mTOR, mechanistic target of rapamycin; mTORC, mTOR complex; TCR, T cell receptor; T_H, helper T cell; tTreg, thymus-derived regulatory T cell; VAT, visceral adipose tissue.

CNS1, which is required for pTreg induction in mucosal environment, there is a reduction of relative abundance of the phylum Firmicutes, which includes *Clostridia*, in the gut [53]. A recent study demonstrates that Tregs contribute to diversification of commensal microbiota. Tregs support the production and selection of immunoglobulin A (IgA) in germinal center of Peyer's patches. Diversified IgA maintains a diverse microbial community in gut, particularly the diversification of *Clostridia* cluster IV and XIVa, which, as discussed above, induce gut Treg differentiation. Importantly, Treg-regulated microbial diversity is required for maturation of mucosal immune system [102]. Thus, Tregs and gut microbiota form a regulatory loop to mediate host–microbe symbiosis and immune homeostasis (Figure 2).

Although Tregs control metabolic disorders or dysbiosis-induced inflammatory diseases through inhibition of proinflammatory immune cells, Tregs also stimulate the generation and activity of anti-inflammatory M2 macrophages [103]. Moreover, Tregs could alter local amino acid availability by stimulating DCs to express enzymes that catabolize EAAs, and this leads to impaired T cell activation and increased Treg conversion from naïve T cells [94]. For example, the interaction between CTLA-4 on Tregs and CD80/CD86 on DCs induces DC expression of indoleamine 2,3-dioxygenase (IDO), an enzyme that consumes tryptophan [104]. Treg-mediated limitation of nutrient availability in the immune microenvironment contributes to 'infectious tolerance', which posits that Tregs enforce immune tolerance partly through converting conventional T cells into Tregs. This provides a feed-forward mechanism to amplify the immune regulatory effects of Tregs through metabolic means, adding another layer of complexity to the interplay between metabolism and Tregs.

Concluding remarks

Emerging studies highlight the complex interplay between Tregs and extrinsic and intrinsic metabolic pathways with significant impact on immune responses and diseases. Recent investigation of how specific nutrient availability and systemic metabolism affect immune cell function underlies the connection between metabolic and immune disorders. Similarly, the latest unraveling of microbial metabolite-mediated control of specific T cell lineage differentiation opens a fascinating new field of research for investigators to decipher the host–commensal interactions. Further, in light of the recent revelation that Tregs at different anatomic locations exert tissue-specific physiological functions [48,49,105,106], it is tempting to speculate that other microbiota-rich locations, such as skin and oral cavity, may harbor Tregs with tissue-specific activities that are modulated by distinct local microbes and metabolites [107]. The discovery that cell intrinsic metabolism controls T cell lineage fate and Treg function suggests the potential for metabolic modulation of immune function. Moreover, the prominence of Tregs in controlling obesity-associated inflammation indicates that immunotherapy could benefit metabolic diseases [108]. Immunometabolism is a nascent field and many questions remain open (Box 2). The exciting advances and future development may provide new opportunities to develop novel Treg-based immunological interventions for the treatment of inflammatory and metabolic diseases.

Box 2. Important areas for future research

- Determination of whether and how vitamin D affects mucosal Treg differentiation and function.
- Analysis of TCR repertoire of colon Tregs to determine their antigen specificity.
- Mechanistic study of how mTOR signaling modulates iTreg induction and function.
- Metabolic profiling of steady-state Tregs and functionally suppressive Tregs under inflammatory conditions.
- Identification of mTOR-dependent and independent metabolic factors that support Treg development and function.
- Elucidation of the mechanism underlying lipogenesis-mediated Treg functional maturation.
- Investigation of Tregs in other barrier environments, such as the skin and oral cavity, and their relationship with local microbiota.

Acknowledgements

This work was supported by grants from the National Institutes of Health (AI101407, AI105887, CA176624, and NS064599), American Cancer Society, National Multiple Sclerosis Society, Crohn's & Colitis Foundation of America.

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