

REVIEW

Functional heterogeneity of gut-resident regulatory T cells

Maik Luu, Ulrich Steinhoff and Alexander Visekruna

Regulatory T cells (Treg cells) have a central role in the maintenance of intestinal homeostasis by restraining inappropriate immune responses in the healthy gut. Although distinct intestinal immune cell populations have been described to exhibit regulatory activity, several genetic and functional studies provided a strong evidence for a pivotal role of forkhead box P3 (Foxp3)⁺CD4⁺ Treg cells in prevention of dysregulated mucosal immune reactions and development of chronic immunological disorders such as celiac disease, food allergies and inflammatory bowel disease. Treg cells provide an important layer of intestinal defense by suppressing immune responses against innocuous food and commensal-derived antigens. Recent functional studies suggest that Treg cells are also involved in several other processes such as controlling microbial diversity in the gut, immunoglobulin A selection and supporting tissue repair in response to intestinal tissue damage. A better understanding of the functional heterogeneity as well as of the molecular signals, which regulate distinct intestinal Treg cell subsets, will encourage strategies aimed at transplanting the optimal Treg cell subset for cellular therapy in humans.

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Mucosal surfaces are not only continuously exposed to the gut commensal microbiota and dietary compounds but also to potentially invasive pathogens.¹ Multiple defense layers such as acquisition of commensal microbiota, a compact mucus layer, an intact epithelium and a robust mucosal immunity have been developed to protect the host from pathogen invaders.^{2,3} At the same time, the intestinal tissues are equipped with unique regulatory mechanisms and immune cell subpopulations that help maintain sustained intestinal tolerance to harmless dietary antigens and immunogenic structures derived from commensal bacteria.^{4,5,6} The role of commensal microbiota in the maintenance of intestinal homeostasis is generally accepted, and alterations in the composition of the gut community can result in the disruption of the mucosal tolerance and onset of immunological disorders that originate in dysregulated host–microbiota interactions.⁷ In the healthy intestine, the microbiota–host cross talk is essential for development, maturation and function of mucosal immune system.⁸ Furthermore, our diet intake has a substantial influence on the gut microbiota, their metabolic activity and their communication with the host immune system.^{9,10} Despite the recent advances in the field of mucosal immunology, the underlying mechanisms providing the mutualistic relationship between the gut microbiota and mucosal immune system remain incompletely understood.

Forkhead box P3 (Foxp3)⁺CD4⁺ regulatory T cells (Treg cells) comprise two distinct populations fulfilling separate tasks in the organisms.^{11,12,13} The majority of Foxp3⁺CD4⁺ Treg cells are generated in the thymus due to interaction of high-affinity T-cell receptors with major histocompatibility complex class II molecules presenting self-antigens.¹⁴ After leaving the thymic Treg cell niche, thymus-

derived (t) Treg cells, which are now enabled to recognize self-antigens and thus to suppress autoimmune responses, populate the secondary lymphoid organs such as spleen and lymph nodes as naive Treg cells (Figure 1). The importance of tTreg cells for the host was elegantly demonstrated in seminal studies performed by Sakaguchi and colleagues who adoptively transferred CD25-depleted CD4⁺ T cells into athymic nude mice lacking T lymphocytes and observed the development of systemic autoimmune diseases. Importantly, the co-transfer of CD25⁺CD4⁺ tTreg cells into same animals prevented autoimmune pathologies caused by CD25⁻ T cells in multiple organs indicating that tTreg cells have an indispensable role in controlling autoreactive T-cell responses.¹⁵

Development of tTreg cell precursors requires not only the strong T-cell receptor stimulation but also co-stimulation through CD28 and presence of common- γ -chain cytokines such as interleukin (IL)-2 and IL-15.^{16,17} A careful examination of proximal, so-called conserved non-coding sequences (CNS) in the *Foxp3* locus revealed three regulatory elements (CNS1–3) essential for controlling Foxp3 protein expression and establishing a stable Treg cell lineage.¹⁸ Distinct transcription factors bind to the promotor and CNS1–3 regulatory elements within the *Foxp3* gene. Although several transcriptional networks contribute to induction of Foxp3 in Treg cells, the nuclear factor- κ B member c-Rel was suggested to act as 'pioneer' transcription factor by binding to *Foxp3* promoter as well as CNS3 region and inducing changes in the chromatin structure at the *Foxp3* locus.¹⁹ It was demonstrated that the binding of c-Rel to the CNS3 element allows other transcription factors such as NFAT, CREB, STAT5 and Smad to access the *Foxp3* locus, which leads to the formation of c-Rel-

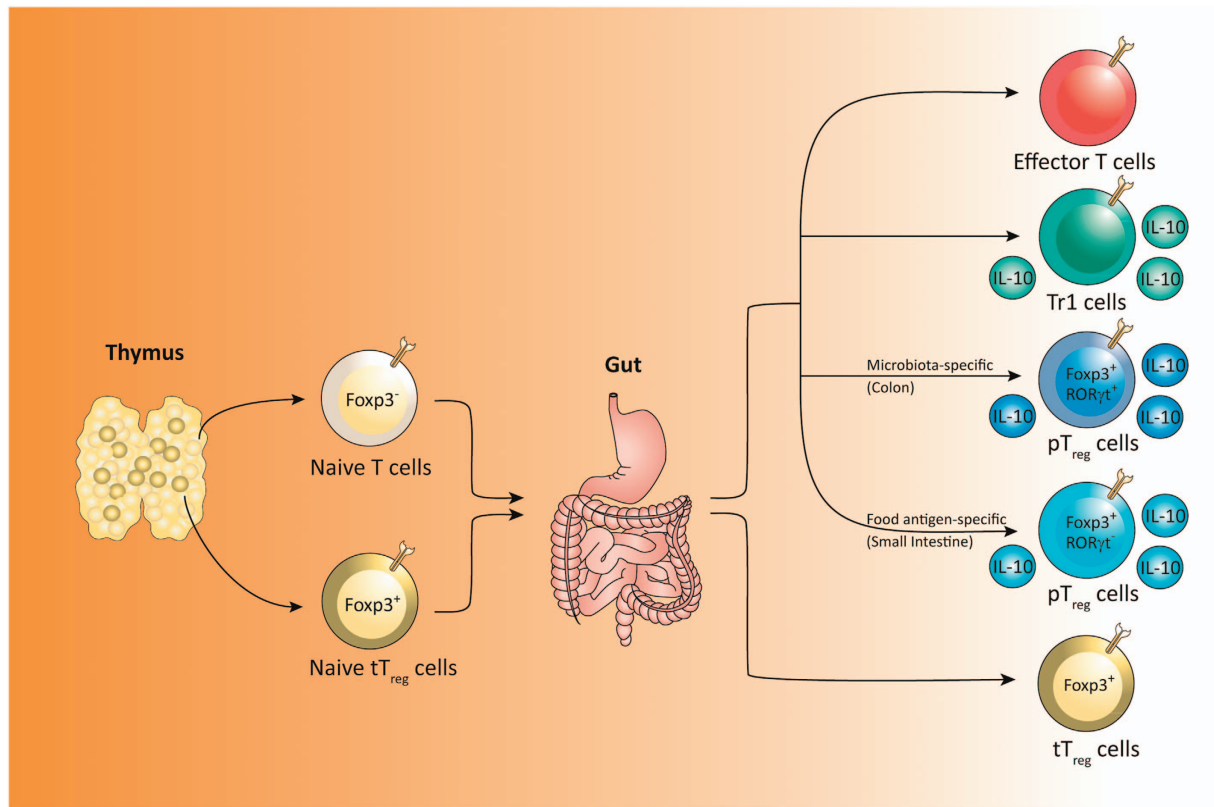


Figure 1 The heterogeneity of CD4⁺ Treg cell population in the gut. During the thymic selection process, the strength of T-cell receptor (TCR) signaling determines the thymocyte fate. Whereas high TCR self-reactivity induces the generation of the Foxp3⁺CD4⁺ tTreg cell population, low TCR self-reactivity leads to the survival of naive Foxp3⁻CD4⁺ thymocytes. After leaving the thymus, naive CD4⁺ T cells encounter harmless antigens in the gut and develop into either specialized Foxp3⁺CD4⁺ pTreg cell populations (colonic Foxp3⁺RORγt⁺ Treg cells specific for microbiota and small intestinal Foxp3⁺RORγt⁻ Treg cells reactive to food antigens) or Foxp3⁻CD4⁺ Tr1 cell subset. All these Treg cell subpopulations, together with intestinal tTreg cells, promote mucosal tolerance by producing IL-10 and other immunomodulatory factors.

containing enhanceosome and potentiation of *Foxp3* induction.^{20,21,22} In addition to its crucial role for induction of *Foxp3* expression, c-Rel also controls several other crucial steps leading to the generation of mature tTreg cells from CD25⁺Foxp3⁻ Treg cell precursors.^{23,24,25} Therefore, it is not surprising that mice lacking the molecules of upstream nuclear factor-κB signaling, for example, CARMA1, Bcl10 and MALT1 have been described to exhibit a significant loss of Treg cells.^{19,26}

After exiting thymus, some tTreg cells and naive CD4⁺ T cells migrate to the intestinal tissues where additional microbial and dietary signal molecules, as well as an encounter with an antigen could lead to the conversion of naive Foxp3⁻CD4⁺ T cells to the peripheral (p) Foxp3⁺ Treg cells²⁷ (Figure 1). On the basis of molecular markers such as Helios and neuropilin-1 (Nrp-1) that are used for discrimination of tTreg from pTreg cells in mice,^{28,29,30} a significantly higher proportion of pTreg cells in the murine gut tissues has been found as compared to secondary lymphoid organs.^{6,31} Interestingly, the Treg cell-specific ablation of *Il10* allele revealed that IL-10 production by Treg cells was essential for limiting inflammation at environmental interfaces such as the colon and lung.³² In addition to frequently occurring tTreg and pTreg cells, several other regulatory cell populations such as IL-10-producing macrophages, tolerogenic dendritic cells (DCs) as well as IL-10- and transforming growth factor transforming growth factor-β1-secreting Foxp3⁻ T cells have been described in the gut.^{33,34,35} Among them, Foxp3⁻CD4⁺ regulatory type 1 (Tr1) cells that secrete high amounts of IL-10 seem to exhibit the strongest immunosuppressive

capacity and have a non-redundant role in the regulation of intestinal chronic inflammation^{36,37} (Figure 1). The binding of IL-27 to its receptor triggers the expression of transcription factors c-Maf and Blimp-1 that act together with aryl hydrocarbon receptor to induce *Il10* and *Il21* expression in Tr1 cells.^{38,39,40} IL-21 secreted by Tr1 cells acts in an autocrine manner to stabilize the Tr1 cell phenotype. Recently, the transcription factors IRF1 and BATF have also been shown to be required for Tr1 cell differentiation.⁴¹

In this review, we discuss the contribution of bacterial and dietary components such as short-chain fatty acids (SCFAs), vitamin A-derived retinoic acid (RA) and dietary antigens to the development, functional maturation and maintenance of intestinal Foxp3⁺ Treg cells that are crucial for providing tolerogenic microenvironment in the gut.

DIETARY ANTIGENS INDUCE TREG CELLS IN THE SMALL INTESTINE

One of the major tasks of the intestinal mucosa is to provide protective responses against harmful antigens while tolerating harmless antigens from commensal microbiota and food. This task is very sophisticated and requires a rigorous differentiation between potentially dangerous and innocuous antigens. Furthermore, oral antigen uptake may result in local as well as systemic non-responsiveness, a process termed intestinal tolerance or oral tolerance, respectively. Oral tolerance refers to a process in which the repeated exposure to the same food antigen does not elicit an immune response, even after systemic challenge with the same antigen together with a strong adjuvant.^{42,43} In contrast,

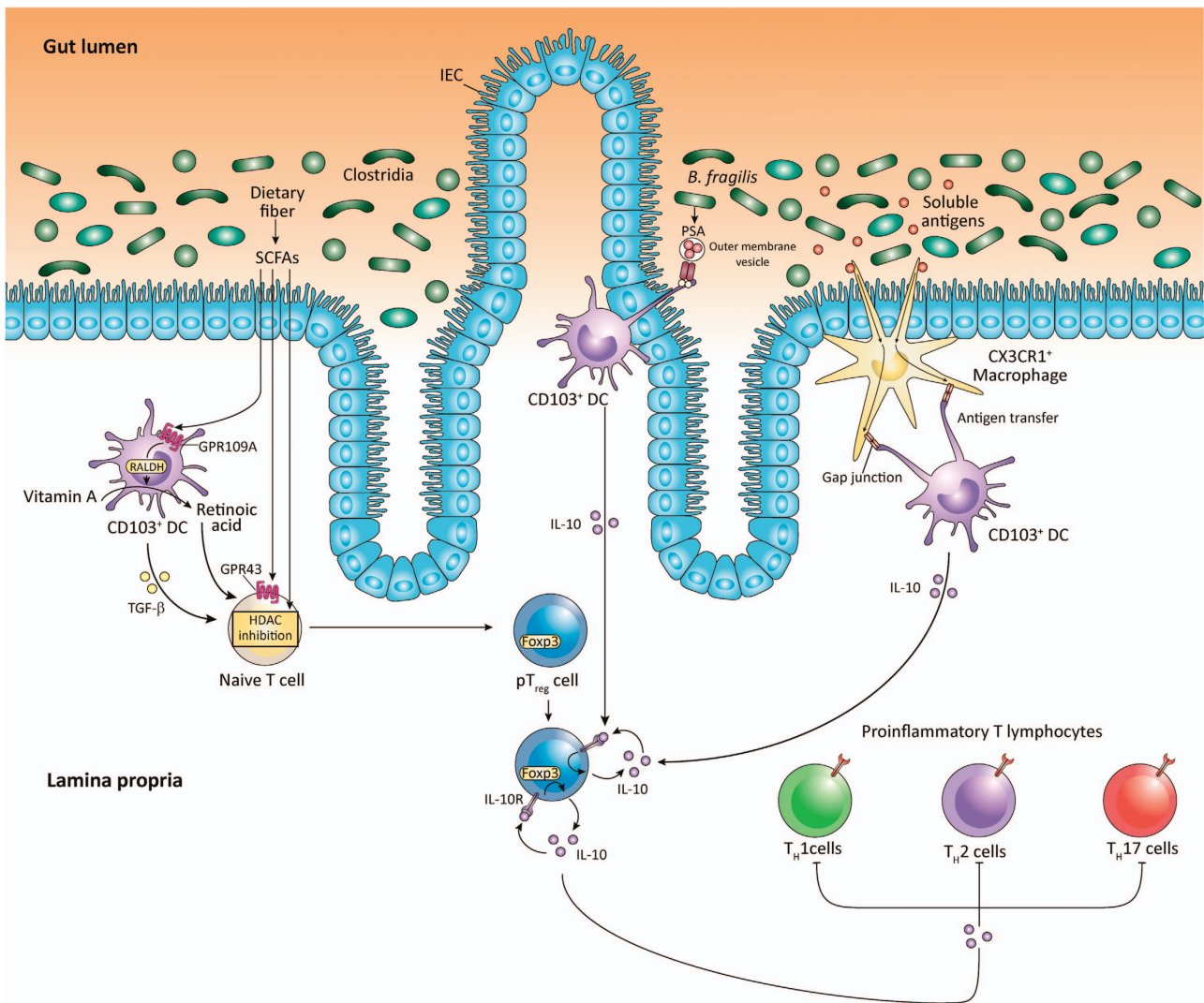


Figure 2 Molecular and cellular mechanisms of pTreg cell induction in the gut LP. The induction of pTreg cells in the gut is dependent on signals derived from diet and commensal bacteria. Anaerobic species like *Clostridia* produce SCFAs by fermentation of dietary fiber. SCFAs diffuse across the epithelium and bind to the GPR109A on CD103⁺ DCs and increase the retinaldehyde dehydrogenase-mediated conversion of vitamin A into RA. In combination with DC-derived transforming growth factor- β (TGF- β), RA is able to promote pTreg cell differentiation. Further, SCFAs passing intestinal epithelial cells (IECs) act directly on naive CD4⁺ T cells by binding to GPR43 and increasing histone acetylation through histone deacetylase (HDAC) inhibition at the *Foxp3* locus, which results in the expansion of pTreg cells. Upon binding of *B. fragilis*-released PSA in outer membrane vesicles, CD103⁺ DCs secrete IL-10 inducing autocrine IL-10 production by pTreg cells to suppress pro-inflammatory Th1, Th2 and Th17 immune responses. In addition, the gap junction-dependent antigen transfer from CX3CR1⁺ macrophages to CD103⁺ DCs drives differentiation of pTreg cells reactive to soluble food antigens.

intestinal tolerance acts locally in the gut to prevent inflammatory reactions, ranging from mild allergy to anaphylaxis.

Most of the current knowledge is derived from transgenic animals expressing a T-cell receptor-specific for ovalbumin (OT-II mice) allowing to specifically trace the T-cell reactivity against a defined oral antigen. This model revealed that tolerance toward orally applied ovalbumin requires the uptake of intestinal antigen and subsequent migration of chemokine receptor CCR7⁺ antigen-presenting cells (APCs) from the intestinal lamina propria (LP) to the draining mesenteric lymph nodes (mLNs) where the generation of antigen-specific Foxp3⁺ Tregs takes place.^{44,45} Detailed analysis revealed that several cell types are involved in this process: first, CX3CR1⁺CD11c⁺F4/80⁺ macrophages are able to efficiently capture antigens by sending protrusions into the gut lumen and transfer the antigen to CD103⁺ DCs via a gap junction-mediated process (Figure 2).^{46,47} Gap

junction-mediated interactions between neighboring cells have been shown to trigger several immunological activities, including activation of DCs,⁴⁸ the function of Treg cells⁴⁹ and the communication between DCs and Treg cells.⁵⁰

However, it remained unclear whether intestinal pTreg cells are also induced in response to a physiological diet. This question was elegantly approached by investigating animals fed on a chemically defined, elemental diet, which is devoid of dietary antigens.⁵¹ To exclude antigenic stimulation by microbial antigens, germ-free (GF) mice were raised directly after weaning on such antigen-free (AF) diet. AF mice exhibited normal numbers of Foxp3⁺CD4⁺ T cells in the periphery but reduced Treg cell numbers in the LP of the small intestine (siLP). In contrast, both, specific pathogen-free and GF mice raised on conventional diet revealed ~4–5 times higher numbers of this cells, indicating that the reduction was due to food and not microbial

antigens. Recently, Nrp-1, which is not a specific marker of human Treg cells,⁵² was shown to be highly expressed on murine tTreg but not on pTreg cells.^{29,30} Using this marker, Kim *et al.*⁵¹ could show that AF mice were lacking Nrp-1^{low} pTreg cells in siLP, demonstrating that dietary antigens are essential for the induction of most Nrp-1^{low}Foxp3⁺ pTreg cells in the small intestine. Further experiments revealed that pTreg cells of the siLP exhibit a rapid turnover with a short lifespan when deprived of food antigens, indicating that pTreg cells are continuously generated and replaced in the presence of dietary antigens.

As mentioned above, APCs, including CX3CR1⁺ macrophages and CD103⁺ DCs, have been claimed to be essential for induction of pTreg cell differentiation. Analysis of the DC populations in AF mice revealed a significantly reduced number of CD103⁺CD11b⁺ DCs within the siLP but a normal composition of DC subsets in the mLNs, which is the tissue site of pTreg education. It has to be mentioned that CD103⁺CD11b⁺ DCs isolated from the siLP of AF mice were still able to stimulate T cells *in vitro* and to induce the expression of Foxp3 in OT-II T cells.⁵¹ A recent study addressed the type of APCs required for induction of pTreg cells and oral tolerance. Through myeloid lineage-specific depletion of APCs, it was elegantly shown that pre-DC-derived classical DCs (cDCs) but not monocyte-derived APCs were required for efficient generation of pTreg cells.⁵³ Various cDC subsets from mLNs co-cultured with OT-II T cells revealed a hierarchical role of the different DC subpopulations to induce a pTreg cell pattern in ovalbumin-specific CD4⁺ T cells. *In vitro*, a Treg cell-specific gene cluster was most efficiently induced by IRF8-dependent migratory CD103⁺CD11b⁻ DCs followed by CD8 α ⁺ DCs and CD103⁺CD11b⁺ DCs, which induced an intermediate phenotype, while CD103⁻CD11b⁺ DCs were less efficient to induce Treg cell-specific gene signature.⁵³ Surprisingly, oral tolerance was intact in both, mice lacking migratory CD103⁺CD11b⁻ DCs⁵³ and neonatal mice deficient of siLP pTreg cells upon weaning onto a normal chow.⁵¹ Whether this simply reflects a functional redundancy of cDCs or a rapid generation of pTreg cells upon antigen encounter remains still to be answered. Obviously, the mechanisms that are responsible for the maintenance of oral tolerance appear to be more intricate and robust as depicted by our current immunological view. We thus cannot currently exclude that additional, yet undiscovered mechanisms of oral/intestinal tolerance contribute to maintain mucosal and peripheral homeostasis upon the encounter of dietary antigens.

MICROBIOTA-MEDIATED ACCUMULATION OF TREG CELLS IN THE COLON

Despite the growing evidence of essential contribution of commensal microbiota to the induction and accumulation of colonic Treg cell population, the particular role for the individual microbial factors and molecular mechanisms underlying induction of pTreg cells by commensals are only partially understood. Although the whole gut commensal community includes only five different phyla (two major phyla Firmicutes and Bacteroidetes as well as significantly less abundant Proteobacteria, Actinobacteria and Verrucomicrobia), there is a broad diversity at the level of microbial species and their abundance.¹⁰ The stable composition of commensal bacteria has a pivotal role in the shaping of the intestinal immune system, and the abnormal alteration in the gut microbial community is considered to be one of the important pathogenic factors leading to chronic inflammation and development of inflammation-associated carcinogenesis in the colon.⁵⁴ Among all these individual species, *Bacteroides fragilis* and several commensal *Clostridium* strains have been shown to induce colonic Treg cell differentiation and functional

maturation.^{31,55,56} Similar effects on the accumulation of pTreg cells in the colonic LP have been reported for other *Bacteroides* strains such as *Bacteroides thetaiotamicron* and *Bacteroides caccae*, although the underlying mechanisms are less characterized.⁶ The mono-colonization of GF mice with *B. fragilis* increases the secretion of IL-10 by Treg cells and DCs. Interestingly, this effect is dependent on only one bacterial component, polysaccharide A (PSA), which is released by this bacterium in outer membrane vesicles.^{6,55} Outer membrane vesicles containing PSA can then be taken up by colonic DCs. The induction of IL-10 production by DCs is again crucial for function of Treg cells, as DC-derived IL-10 acts directly on Treg cells to induce their IL-10 secretion (Figure 2). Moreover, a single bacterial molecule such as purified PSA has been shown to protect against experimental colitis in mice by inducing production of IL-10 to suppress pro-inflammatory Th17 responses.^{57,58} There seem to be overlapping mechanistic effects on colonic Treg cells between *Bacteroides* and *Clostridium* species, as the murine mixture of 46 *Clostridium* strains have a strong ability to induce accumulation of colonic IL-10-secreting Treg cells.³¹ Similarly, 17 *Clostridium* strains isolated from healthy humans are able to trigger expansion of IL-10-producing pTreg cells in the colon of mice and rats.⁵⁶ On the other hand, some differences also exist between *Clostridium*- and *Bacteroides*-mediated Treg cell induction. The colonization of mice with *Clostridium* but not with *Bacteroides* strains increases the expression of transforming growth factor- β 1 by intestinal epithelial cells, a factor that is needed for *de novo* induction of pTreg cells in the gut.³¹ Further, the mono-colonization of GF mice with a single *Clostridium* strain *Clostridium butyricum* was found to prevent the acute colitis in mice by strongly inducing IL-10 production in intestinal macrophages without affecting IL-10 production by Treg cells in the gut.⁵⁹

While the general influence of microbiota on the intestinal homeostasis is well appreciated, it remains unclear whether the most effects are mediated by microorganisms themselves or by their metabolic products. Recently, soluble microbial metabolites such as SCFAs have been shown to have a crucial role in controlling differentiation and functional activity of colonic pTreg cells.^{60,61,62} SCFAs are microbial fermentation products of indigestible food components such as dietary fiber that are mainly produced by colonic anaerobic bacteria, especially by *Clostridium* species.¹⁰ Novel results indicate that SCFAs exhibit profound effects on the cells of mucosal immune system, thereby acting as key microbial metabolites by bridging the physiological barrier between luminal commensal strains and the cells of the mucosal immune system.⁶³ Distinct cellular processes are affected by SCFAs through activation of G protein-coupled receptors (for example, GPR41, GPR43 and GPR109A) and downstream signaling cascades such as nuclear factor- κ B and mitogen-activated protein kinase pathways. In addition, SCFAs can be transported into the cytoplasm by SCFA transporters (for example, Slc5a8 and Slc16a1) or diffuse passively into the cells. In the nucleus they act as inhibitors of histone deacetylases, enzymes involved in the epigenetic regulation of gene expression⁶⁴ (Figure 2). Acetate, propionate and butyrate (the most abundant SCFAs in the gut lumen) are able to restore the normal numbers of colonic Treg cells in GF mice, which are devoid of gut microbiota and microbial metabolites.⁶³ With regard to the underlying mechanisms of SCFA-mediated Treg cell expansion, butyrate has been investigated most extensively.^{60,61} The proposed mechanism by which butyrate acts directly on Treg cells is based on the inhibition of the histone deacetylase activity that results in enhanced histone H3 and H4 acetylation. Particularly, the treatment of Treg cells with butyrate induces the histone H3 acetylation in the promoter and CNS regions of the *Foxp3* locus.⁶¹ As a consequence, the increased expression of

this transcription factor potentiates the differentiation of Treg cells. Moreover, it was shown that butyrate treatment of Treg cells results in increased acetylation of Foxp3 protein leading to its enhanced stability and protection from degradation.⁶⁰ Finally, a study by Smith *et al.*⁶² has proposed the Treg cell-intrinsic involvement of GPR43 in promoting colonic Treg cell expansion by SCFAs, as this receptor is exclusively expressed in colonic Treg cells but not in those derived from other tissues. Apart from direct effects of SCFAs on T cells, butyrate has been also shown to induce tolerogenic properties in DCs in a GPR109A-dependent manner by triggering the expression of *Il10* and *Aldh1a1* (the gene encoding for retinaldehyde dehydrogenase 1).⁶⁵ Interestingly, during the intestinal homeostasis, only the number and function of colonic Treg cells is altered by SCFA treatment or intake of high-fiber diet, while the pro-inflammatory T-cell subsets Th1 and Th17 cells are not affected. These findings gain new mechanistic insights into the way of how the host–microbe interaction via microbial metabolites provides immunological homeostasis in the gut. Surprisingly, during the inflammation, SCFAs are capable of augmenting pathogenic Th1- and Th17-mediated immune responses.^{66,67} These results indicate that while at steady state SCFAs promote the immunosuppressive function of colonic Treg cells, their effect might be less beneficial under inflammatory conditions.

FUNCTIONAL HETEROGENEITY AMONG GUT-RESIDENT TREG CELLS

Apart from their well-recognized role as regulators of immunologic responses, the tissue-resident Treg cells can respond to specific environmental cues by fulfilling organ-specific regulatory tasks. For example, specialized Treg cell fractions regulate diverse metabolic parameters in adipose tissue or support regenerative processes in injured muscles.^{68,69,70} Recently, a subset of Foxp3⁺ Treg cells co-expressing the IL-33 receptor ST2 and transcription factor GATA3 has been shown to be enriched in colonic LP.⁷¹ As IL-33 functions as danger signal following tissue injury, the ST2⁺ Treg cell subpopulation might have a crucial role in adaptation of colon to the inflammatory environment by reacting rapidly to inflammation-driven tissue damage. Several studies have demonstrated a considerable functional heterogeneity among intestinal Treg cell population as compared to Treg cells in spleen and lymph nodes.^{72,73} It is thought that the existence of distinct Treg cell subsets in the intestinal LP might be one of the key factors balancing immune responses at mucosal surfaces. Previously, it was suggested that some Treg cells seem to be capable of co-opting special aspects of the T-cell differentiation pathways required for the regulation of Th2- or Th17-mediated immune responses. IRF4 was described to be co-expressed in a specialized Treg cell subset being able to efficiently suppress Th2-mediated immune responses.⁷⁴ Similarly, Stat3-deficient Treg cells were not able to control Th17 responses and to suppress colitis.⁷⁵ Recently, it was shown that commensal microbiota-induced Treg cells in the colon paradoxically express the nuclear hormone receptor and transcription factor ROR γ t, a molecule that normally antagonizes Foxp3 induction and promotes generation of Th17 cells (Figure 1). Indeed, the majority of colonic Treg cells induced by the mixture of 17 *Clostridium* species strongly express ROR γ t, and these Treg cells are profoundly reduced in the colon of GF mice.⁷² In contrast to GATA3⁺ST2⁺Helios⁺ Treg cells, which are able to expand during the intestinal tissue damage, colonic ROR γ t⁺Helios⁻ Treg cells cannot respond to IL-33.⁷³ As ROR γ t⁺ Treg cells have enhanced suppressive capacity during the T-cell-mediated gut inflammation as compared to the total Foxp3⁺ Treg cell population,⁷⁶ one can speculate that a certain division of labor between distinct intestinal Treg cell subsets such as GATA3⁺

tTreg and ROR γ t⁺ pTreg cells might exist. A deeper investigation of this hypothesis will require more functional studies in the future. In addition, the work done by Korn *et al.*⁷⁷ demonstrated that there seems to be a unique, major histocompatibility complex class II-independent niche for tTreg cells in the intestinal LP, suggesting that microbiota-derived signals are not only important for induction of pTreg cells but also for maintenance of gut-resident tTreg cells. Finally, the recent discovery reveals that apart from colonic microbiota-dependent ROR γ t⁺ pTreg cells, the ROR γ t⁻Nrp-1^{low} pTreg cell subpopulation can be induced by dietary antigens in the siLP (Figure 1).⁵¹ Taken together, these observations point to a great diversity and phenotypic complexity within effector Treg subsets in the intestinal mucosa.

TREG CELLS CONTRIBUTE TO THE IGA SELECTION IN PAYER'S PATCHES AND DIVERSIFICATION OF MICROBIAL COMMUNITY IN THE GUT

In the organized lymphoid tissue of small intestine such as Payer's patches (PP), highly specialized microfold (M) cells overlying this organ mediate the transport of luminal antigens and bacteria toward the mucosal immune system.⁷⁸ Although the importance of PP in the induction of tolerance to food antigens is still controversial, it is well accepted that within germinal centers of PP, B cells preferentially produce immunoglobulin A (IgA), which subsequently enters the intestinal lumen and prevents the adhesion of commensals to the mucosal surfaces.⁷⁹ There is considerable evidence that the secretion of IgA in the gut lumen suppresses inflammatory responses to commensal bacteria.¹ In PP, commensal-specific IgA selection seems to be regulated by a specific subset of Foxp3⁺ Treg cells, called follicular regulatory T cells, which are phenotypically similar to follicular T helper cells that provide essential factors to germinal center B cells undergoing somatic hypermutation. PP-derived Foxp3⁺ Treg cells have been shown to regulate the intestinal IgA secretion and to facilitate the diversification of commensal bacterial species.⁸⁰ Mice lacking only T cells (*Cd3e*^{-/-}) or both T and B lymphocytes (*Rag1*^{-/-}) exhibit reduced bacterial diversity as compared to wild-type animals. Interestingly, while the transfer of naive CD4⁺ T cells failed to re-establish the gut bacterial richness in *Cd3e*^{-/-} mice, the transfer of Foxp3⁺ Treg cells alone or co-transfer of naive CD4⁺ T cells along with Foxp3⁺ Treg cells reconstituted the bacterial diversity in these animals.⁸⁰ Thus, it appears that Foxp3⁺ follicular regulatory T cells influence the IgA selection in germinal centers of PP and contribute to the maintenance of balanced microbiota in the gut, which, in turn, promotes the expansion of intestinal Treg cells. Notably, lower concentrations of SCFAs were observed in *Cd3e*^{-/-} mice as compared to wild-type animals, suggesting that the lack of Foxp3⁺ Treg cells and defective IgA selection not only result in reduced diversity of gut microbiota but also affect the synthesis of bacterial metabolic products.⁸⁰

EFFECTIVE TREG CELL DIFFERENTIATION IN MLNS

CD103-expressing CD11c⁺ DCs seem to have a central role in the presentation of harmless foreign antigens that are taken up in the LP and transported to naive CD4⁺ T lymphocytes in mLN.⁷⁸ These DCs have a capacity to produce high levels of a vitamin A-derived RA, because of their high expression of the enzyme retinaldehyde dehydrogenase 2 (Figure 2). Because of the high level of RA and transforming growth factor- β 1 in mLN, a vast majority of naive CD4⁺ T cells in this organ acquires Foxp3 expression after encountering their cognate antigen.⁴⁵ Interestingly, RA is also known to promote induction of gut-homing molecules on pTreg cells such as integrin

$\alpha 4\beta 7$ and chemokine receptor CCR9.³⁴ Thus, RA supports both processes in parallel, the *de novo* generation of intestinal pTreg cells and their migratory properties by imprinting intestinal homing receptors.⁴⁵ Once, the Foxp3⁺ Treg cells are generated acquiring their full functional phenotype in mLNs, they can emigrate to the LP and become a part of colonic (commensal-derived antigens) or small intestinal (food antigens) pTreg cell fraction. On the basis of this model, the establishment of Treg cell-mediated intestinal tolerance requires two steps: the generation of food-specific (or commensal antigen-specific) Foxp3⁺ Treg cells in mLNs; and the homing of antigen-specific pTreg cells in the gut LP where they further proliferate.⁷⁸

In conclusion, multiple mechanisms have been developed in the gut contributing simultaneously to the accumulation of Treg cells and establishment of robust intestinal tolerance. First, the continuous microbial synthesis of microbiota-derived metabolites such as SCFAs leads to local pTreg cell induction and the maintenance of tTreg cell niche in the colonic LP. Second, the induction of antigen-specific pTreg cells after presentation of harmless antigens on LP-derived CD103⁺ DCs takes place in mLNs and other parts of the gut-associated lymphoid tissues such as PP in the small intestine. Interestingly, a novel study has shown that high-fiber feeding protects mice against food allergy by enhancing the retinaldehyde dehydrogenase 2 expression and tolerogenic activity of CD103⁺ DCs, which results in increased conversion of naive T cells into Foxp3⁺ Treg cells in mLNs.⁸¹ Together, these data highlight synergetic activity of dietary fiber (the primary source of SCFAs) and a vitamin A-derived RA that together have a pivotal role in promoting tolerogenic microenvironment in the intestine by contributing efficiently to the generation of gut-resident Treg cells (Figure 3).

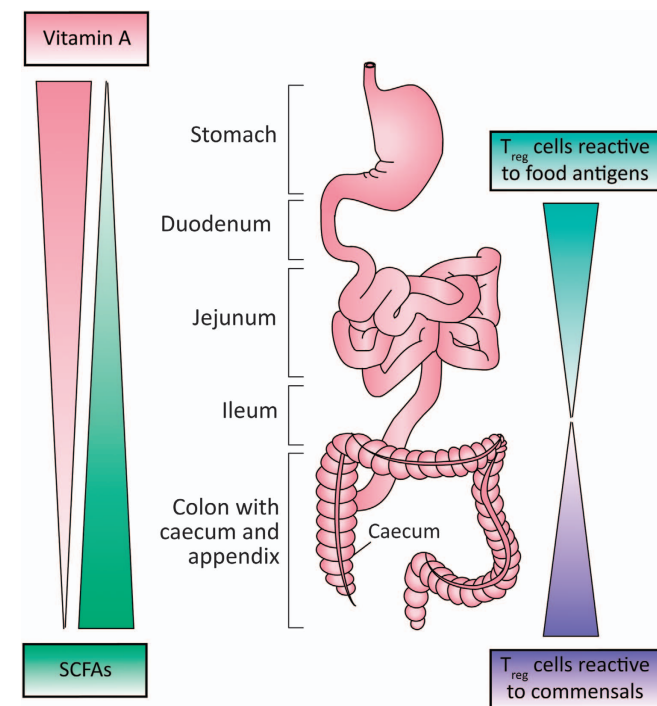


Figure 3 A proximal–distal distribution of pTreg cell-inducing factors and pTreg cells reactive to harmless environmental antigens along the intestine. Whereas the amount of food-derived vitamin A decreases from proximal to distal part of the intestine, the number of commensal microbes and their metabolic products SCFAs increases in the same direction.

CONCLUSION REMARKS

The promotion of immune tolerance in the intestinal tissues is achieved by cooperative network of several immune cell populations, including central players such as Foxp3⁺ Treg cells and Tr1 cells. Dysregulated intestinal responses to dietary antigens and commensal microbiota frequently lead to severe immunological disorders in humans such as celiac disease, food allergy and inflammatory bowel disease (IBD). Although the Treg cell-based therapy always present a certain risk by increasing susceptibility for infections, the disruption of the mucosal barrier and consequent chronic inflammation in IBD patients might be improved by generating therapeutic Treg cells. Highly efficient fecal microbiota transplantation into patients with antibiotic-resistant *Clostridium difficile* infection is a successful example of how manipulation of our microbiota might help to combat diseases in humans. The transplantation of commensal *Clostridium* species to IBD patients with restricted composition of intestinal microbiota or supplementation of purified bacterial components such as PSA from *B. fragilis* might be the next important clinical step allowing for the amplification of specific anti-inflammatory signals required for restoring the disrupted intestinal homeostasis. Future studies focusing on the better understanding of molecular mechanisms responsible for acquiring the stable Treg cell phenotype with colonic tissue-specific features could provide novel therapeutic approaches for treatment of gastrointestinal inflammatory disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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