# Regulatory T Cell Adaptation in the Intestine and Skin

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## Abstract

The intestine and skin are distinct microenvironments with unique physiological functions that are continually exposed to diverse environmental challenges. Host adaptation at these sites is an active process involving the interaction between immune and tissue cells. Regulatory T cells (Tregs) play a pivotal role in enforcing homeostasis at barrier surfaces, illustrated by the development of intestinal and skin inflammation in diseases caused by primary Treg deficiency. Tregs at barrier sites are phenotypically distinct from their lymphoid organ counterparts and these "tissue" signatures often reflect their tissue-adapted function. We discuss current understanding of Treg adaptation in the intestine and skin, including unique phenotypes, functions and metabolic demands, and how increased knowledge of barrier site Tregs might guide precision medicine therapies.

## Introduction

Regulatory T cells play a non-redundant role in the maintenance of immune homeostasis and were first discovered based on their ability to actively suppress self-reactive inflammatory T cell responses and resulting autoimmunity [1-3]. Subsequent studies showed Treg activity is enriched within $CD4^+CD25^+$ cells [4]. Forkhead Box Protein 3 (Foxp3) was identified as the canonical marker of Tregs and is essential in their development, maintenance and function [5-8]. The central role of Tregs in peripheral tolerance is highlighted by the development of fatal autoimmunity in *scurfy* mice, which lack the *Foxp3* gene, and mice with *Foxp3* deficiency specifically in T cells [9, 10]. Moreover, individuals with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, who have mutations in the *FOXP3* gene, are susceptible to many autoimmune diseases [11-13]. Importantly, Treg-deficient mice and IPEX patients often present with gastrointestinal and skin manifestations [12, 14], highlighting the critical role of Tregs in maintenance of homeostasis at barrier sites.

Barrier sites, such as the intestine and skin, perform distinct physiological functions and are environments with a unique composition of nutrients and physiological factors. The intestine

and skin are in constant contact with the outside world and face the challenge of upholding vital physiological processes and maintaining homeostasis to the resident microbiota and innocuous environmental factors while concomitantly being poised to defend against infectious or noxious environmental insult. To achieve homeostasis, each barrier site harbours a diverse, tissue-specialised cellular network. This network includes epithelial cell subsets in direct contact with the outside world, stromal cell populations that provide support to the tissue, neuronal networks that relay chemical messages across the body and a diverse population of tissue-adapted immune cells. Immune cells, including Tregs, are central to the tissue cellular network and an iterative dialogue shapes both immune cell and tissue responses. Overall, the dynamic, diverse environments of barrier sites represent a unique challenge to Tregs, that need to be adaptable to prevailing environmental conditions while mediating key tissue-specific effector functions.

It is now appreciated that immunity at tissue sites differs vastly to lymphoid sites. Studies on Tregs within tissues, such as adipose tissue and muscle, have identified phenotypic differences from their counterparts in secondary lymphoid organs (SLOs) and uncovered novel tissue-specific Treg functions [15-17]. For example, peroxisome proliferator-activated receptor gamma (PPAR$\gamma$), the “master regulator” of adipocyte differentiation, defines visceral adipose tissue (VAT) Tregs and is required for Treg-mediated control of insulin sensitivity [15]. Important advances in our understanding of Treg adaptation to the intestine and skin have been made, including an emerging theme that Treg phenotype and function at these sites are closely linked to local environmental and physiological cues [17]. However, much knowledge on the mechanisms that drive Treg phenotype and function in the gut and skin is still to be unravelled [18, 19]. Importantly, defective Treg immunity is associated with intestinal and skin inflammatory disease, thus, a comprehensive understanding of Treg adaptation at these sites may guide development of new therapies. In this Review, we discuss our current understanding on Treg phenotypic and functional adaptation in the intestine and skin, and highlight how metabolic adaptation might contribute to Treg biology at these sites. While other $CD4^+$ T cells with regulatory function are recognised, this Review focusses on the $Foxp3^+$ Treg subset.

## Treg development: from thymus to tissue

Like all $\alpha\beta$ T cell receptor $(TCR)^+$ T cells, Treg development begins in the thymus, where two distinct pathways begin to diverge [8] (Figure 1A). Thymic-derived Tregs (tTregs) differentiate into $Foxp3^+$ Tregs in the thymus following TCR recognition of self antigen. In contrast,

peripherally-derived Tregs (pTregs) exit the thymus as naïve $CD4^+$ T cells and differentiate into $Foxp3^+$ Tregs in the periphery following recognition of their cognate antigen, which is regarded as being non-self. Although not perfect markers, expression of Helios and Nrp1 by tTregs but not pTregs can differentiate these Treg subsets [20]. A major challenge in the Treg field has been understanding the antigen specificity of the Treg response, partly owing to the difficulty in studying cell populations of such low frequency [21]. There is evidence that colonic pTregs display a TCR repertoire with reactivity to intestinal bacteria [22] and recent studies have shown that members of the bacterial genus, *Helicobacter*, can drive antigen-specific pTreg responses [23, 24] (Figure 2). These results suggest that resident gut bacteria may be dominant drivers of antigen-specific pTregs in the intestine. Intriguingly, tTregs from the colon also express TCR repertoires that are reactive to the intestinal microbiota [25]. This appears contradictory to the dogma that tTregs express self-reactive TCRs. One possibility is that microbial antigens are presented in the thymus, although this has not been documented. Perhaps a more likely scenario is the expression of self-reactive TCRs by tTregs that display cross-reactivity with microbial antigens. Indeed, Treg TCRs specific for a particular bacterial species can be cross-reactive with other bacteria and dietary antigens [23, 26, 27]. Thus, it would appear that Treg specificity covers both self and foreign antigens, in keeping with the crucial role of Tregs in maintaining homeostasis of both peripheral and barrier sites. However, our understanding of Treg specificity is currently limited to a few examples and further information on tTreg and pTreg specificities will be crucial for antigen-specific modulation of Treg function.

It is believed that following TCR stimulation in SLOs, Tregs migrate to tissues in response to tissue homing signals [28]. However, despite extensive study, little is known about Treg cell priming and migration to gut and skin sites. Available evidence supports the idea that the mesenteric lymph nodes (mLNs), which drain the intestine, are a major site for pTreg differentiation. Here, pTregs upregulate gut homing receptors, such as CCR9 and $\alpha4\beta7$, for trafficking to the intestinal lamina propria and expansion in response to local signals [20, 29-32] (Figure 1A). It is unclear whether Treg priming occurs at other sites, such as solitary isolated lymphoid tissues (SILTs) or in the lamina propria itself. A similar developmental trajectory for skin Tregs has not been described, although skin homing receptor expression is upregulated in skin draining LN (dLN) Tregs and Tregs can traffic between dLNs and skin [32, 33]. Tregs displaying an activated phenotype, such as expression of CD44, ICOS, GITR and IL-10, are present in SLOs and are termed effector Tregs [34] (Figure 1A). Effector Tregs are associated with phenotypic specialisation and an increased ability to migrate to tissue sites and may represent an intermediate Treg developmental stage in the trajectory from thymus to tissue [35, 36]. In this regard, partial tissue signatures have been observed in SLO Tregs,

such as a partial VAT Treg signature in splenic tTregs [35, 36]. Tregs can also upregulate expression of effector T cell (Teff)-associated transcription factors, such as T box transcription factor (Tbet) (T helper 1 (Th1)), GATA binding protein 3 (GATA3) (Th2), signal transducer and activator of transcription 3 (STAT3)/ retinoic acid receptor-related orphan receptor γt (RORγt) (Th17) and B cell leukemia/lymphoma 6 (Bcl6) (T follicular helper cell (Tfh)), which is associated with regulation of the corresponding Teff response [34, 37-42]. The cues and timings that drive these diverse Treg phenotypes are poorly defined. However, a recent study using a TCR-transgenic system demonstrated that a combination of TCR activation, Foxp3 expression and direct effects of local tissue cytokines were required for accumulation of tTregs in VAT and full acquisition of the VAT Treg signature [36]. Thus, Treg priming and migration to gut and skin sites are likely to occur in multiple sites in response to cognate antigen and local tissue recruiting cues. However, additional studies using antigen-specific Tregs are needed to further understand the developmental trajectory of pTregs and tTregs from thymus to tissue.

## Tregs in the intestine

The intestine is a major component of the gastrointestinal tract and involves the small intestine and large intestine, which is composed of the caecum and colon. The primary physiological function of the intestine is food digestion, nutrient uptake and waste excretion (Figure 1B). The small intestine is responsible for the majority of nutrient absorption, although the abundant microbial community of the large intestine is essential in the fermentation of indigestible material such as fibres and starch. The large intestine also plays a key role in water and mineral absorption as well as excretion of waste. The intestine is additionally armed with the task of protecting the host against harmful pathogens and other noxious agents while maintaining tolerance to the resident microbiota and innocuous dietary factors. Multiple defence mechanisms in the gut cooperate to maintain homeostasis. These include a single epithelial cell layer that functions as a physical barrier, alongside its key involvement in nutrient absorption. The epithelial barrier is supported by and communicates with a rich and robust immune network residing predominantly in the lamina propria and in specialised lymphoid structures, such as SILTs found throughout the intestine and Peyer's patches (PPs) in the small intestine. Tregs are central players in intestinal homeostasis and together with specialised antigen presenting cells, Th17 cells and the production of IgA and antimicrobial proteins that are secreted into the lumen, maintain gut health.

### Treg subsets and function in the intestine

In keeping with an abundant microbial community and regular uptake of food in the intestine, a cardinal function of Tregs at this site is control of pro-inflammatory responses towards the microbiota and dietary factors [2, 12, 14, 43] (Figures 2, 3). Indeed, IPEX patients often present with severe gastrointestinal disorders and food allergies [12, 43]. Tregs can suppress pro-inflammatory responses by a variety of mechanisms, although the precise mechanisms used in vivo are poorly understood (Box 1). Both tTregs and pTregs are present in the intestine, although available evidence suggests that homeostasis towards the microbiota and dietary factors is mediated primarily by pTregs (Figure 2). Mice deficient in the Foxp3 intronic enhancer, conserved nucleotide sequence 1 (CNS1), lack pTregs and display a dysbiotic microbiota and elevated Th2 immunity in the colon [44, 45]. Moreover, pTregs were reported to function by preventing aberrant Th1 responses to food antigens in the gut [46]. A role for Tregs in promoting a stable and diverse microbiota through regulation of Tfh responses in germinal centres and IgA production has been documented, although the Treg subsets involved are unknown [47, 48]. The Helios$^-$Nrp1$^-$ pTreg population in the intestine is substantial, albeit variable, with different pTreg subsets dominating the colon and small intestine. The colonic Treg pool is enriched in a pTreg population expressing the Th17 master transcription factor, RORγt, whose maintenance is dependent on the microbiota [40, 41, 46, 49] (Figure 1B). Conversely, a RORγt$^-$ pTreg subset responsive to food antigens is particularly important in small intestine homeostasis [46]. A dominant effect of the microbiota and dietary factors on colonic and small intestinal Tregs, respectively, highlights the intricate relationship between local environmental signals and Treg adaptation in the intestine.

A significant Helios$^+$ tTreg population that expresses GATA3 and a phenotypic signature linked to tissue repair, such as the IL-33 receptor, ST2, and amphiregulin (Areg), resides in the gut [37, 39, 40] (Figure 2). Indeed, tissue repair is a central physiological function at tissue sites and Areg-expressing tTregs mediate repair in muscle and lungs [50]. GATA3$^+$ Tregs have been identified in human blood, but this subset has not been reported in the human intestine to date [37]. GATA3$^+$ Tregs are associated with protection against aberrant chronic inflammation and GATA3 is important for Foxp3 expression and Treg accumulation in the intestine [37-39]. Along these lines, the gut homing receptors, CCR9 and α4β7, are highly expressed by intestinal ST2$^+$ Tregs [51]. Therefore, GATA3$^+$ tTregs appear to be adapted to home to sites of damage in the intestine and mediate repair, although this is yet to be conclusively proven.

Box 1.

Tregs possess a variety of suppressive mechanisms, including expression of the inhibitory receptor, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), production of anti-inflammatory cytokines, such as IL-10 and transforming growth factor beta 1 (TGF-β1), and regulation of growth factor availability, such as IL-2 and adenosine triphosphate (ATP) [8]. CTLA-4 expression by Tregs has been shown to control inflammatory responses, including Th2 responses, in the intestine [41, 52]. CTLA-4 is a central mediator of intestinal homeostasis, as evidenced by the development of colitis in patients receiving anti-CTLA-4 antibody therapy [53]. Treg suppressive function in the gut has also been shown to involve IL-10 and TGF-β1 and genetic mutations in *IL10RA*, *IL10RB, TGFBR1* or *TGFBR2* are associated with severe early onset inflammatory bowel disease (IBD) [54, 55]. Latent TGF-β1 complexed with GARP on the Treg cell surface is activated by integrin αvβ8 binding [56]. Interestingly, GARP-deficient Tregs and integrin αvβ8-deficient Tregs are defective in controlling T cell-mediated colitis [57, 58], suggesting that GARP/αvβ8-dependent TGF-β1 activation may be an important mechanism of Treg suppression in the intestine. Overall, it is unclear which mechanisms are favoured by different Treg subsets to maintain intestinal homeostasis in vivo, although Tregs likely use an array of suppressive mechanisms in a context dependent manner. Of note, Treg expression of the Th1/Th17 markers, *Tbx21*, *Cxcr3* and STAT3, and the Th2-associated transcription factors GATA3 and IRF4 is associated with suppression of Th1/Th17 and Th2 inflammatory responses in the gut [40, 41, 59]. Thus, intestinal Treg phenotypes appear to mirror those of the responses which they regulate [60].

Intestinal Tregs are also important in host defence against invading pathogens [61] (Figure 3). Interestingly, mice depleted of Tregs in vivo were more susceptible to infection by the intestinal pathogen *Citrobacter rodentium*, which was associated with impaired protective Th17 immunity [62]. Tregs can sequester IL-2 to promote Th17 responses [63]. However, in vivo IL-2 blockade was unable to fully restore Th17 immunity and protection against *C. rodentium* in Treg-depleted mice, implying that an alternative mechanism is involved [62]. More recently, a role for Tregs in epithelial stem cell renewal in the small intestine has been documented [64]. Treg addition or IL-10 stimulation of organoid cultures promoted intestinal stem cell renewal, in contrast to promotion of stem cell differentiation by Teffs and their cytokines [64]. This finding was supported in vivo following Treg depletion, whereby intestinal stem cell proportions were decreased and differentiated epithelial cells were increased [64]. The promotion of intestinal stem cell renewal by Tregs adds to a growing number of vital tissue-adapted functions that Tregs perform at tissue sites [17-19]. However, the role of different Treg subsets in these processes is unknown and further studies are required to determine the

developmental relationships and antigen specificities of intestinal Treg populations in order to better understand their shared and unique functions.

## Host pathways shaping intestinal Tregs

### *IL-2/ TGF-β1*

IL-2 and TGF-β1 are regarded as essential cytokines in the development and maintenance of Tregs [8]. Indeed, Tregs constitutively express the high affinity IL-2 receptor, CD25. Tregs are significantly decreased in the intestine of IL-2-deficient mice and can be expanded following treatment with agonistic IL-2/anti-IL-2 antibody complexes, positioning IL-2 as a key regulator of the total intestinal Treg pool [37, 65]. IL-2 appears central to the maintenance of intestinal GATA3$^+$ tTregs, as this subset was severely diminished in the small intestine of IL-2-deficient mice [37]. Similarly, in vivo blockade of IL-2 in a mouse model of oral tolerance impaired the conversion of adoptively transferred naïve OT-II T cells to pTregs in mLNs and PPs [66]. However, the relative contributions of IL-2 to RORγt$^+$ and RORγt$^-$ pTreg induction is unknown.

TGF-β1 is regarded as being an essential pTreg differentiation cytokine in the intestine [67]. TGF-β1 is required for the differentiation of Tregs in vitro (termed induced Tregs (iTregs)) and is found at high concentrations in the intestine [67]. In a mouse model of oral tolerance, TGF-β1 and GARP expression by recipient Tregs were involved in the differentiation of adoptively transferred naïve OT-II T cells to Tregs in mLNs and PPs [66]. Along these lines, dendritic cells (DCs) deficient in integrin αvβ8, which is required for activation of latent TGF-β1, are impaired in the induction of Tregs in mLNs [68]. Intestinal pTreg generation is dependent on CNS1, which contains binding sites for several transcription factors including TGFβ1-activated Smad3 [44, 69, 70]. However, mice with a specific deletion of the Smad3 binding site in CNS1 display minimal reduction in intestinal Treg frequencies [71]. Moreover, colonic Treg frequencies were reported to be normal in mice with a deletion of TGFβR1 specifically in Tregs [72]. Treg subsets were not investigated in that study and it cannot be ruled out that pTreg generation was impaired with a concomitant expansion of tTregs in the intestine. Overall, IL-2 and TGF-β1 appear to be central in Treg homeostasis in the intestine, although further in vivo studies are warranted to fully understand their involvement in the development and maintenance of intestinal tTreg and pTreg subsets.

### *The Th17 signalling axis*

RORγt$^+$ Tregs share expression of RORγt with conventional Th17 cells. Accordingly, the generation of RORγt$^+$ Tregs in the colon has been shown to depend on IL-6 and intrinsic STAT3 signalling, which are required for induction of RORγt in Th17 cells [41, 73] (Figure 2). IL-1β and IL-23 are additional cytokines involved in Th17 differentiation and maintenance, although a concrete role for these cytokines in intestinal RORγt$^+$ Treg responses has not been shown [40, 41, 73]. Indeed, IL-23 is associated with inflammatory Th17 responses and is a negative regulator of IL-33-dependent Treg responses in vitro and Treg responses in the intestine [39, 73, 74].

The transcription factor c-Maf is highly expressed in RORγt$^+$ Tregs and is required for their development in the colon [24, 40, 49]. Although the mechanism of c-Maf-mediated RORγt$^+$ Treg development in the intestine is unknown, a recent study has identified c-Maf as a positive regulator of Th17 responses through suppression of IL-2, which is known to inhibit Th17 development [75]. Given the dichotomy between the requirement for IL-2 in Treg and Th17 development, it will be interesting to determine whether c-Maf mediated inhibition of IL-2 is involved in RORγt$^+$ Treg development. Interestingly, deletion of c-Maf in Tregs led to spontaneous inflammation in mice, which was not observed in mice with a specific deletion of RORγt in Tregs [24]. Therefore, c-Maf may be central to RORγt$^+$ Treg function in the gut. IL-10 production is positively regulated by c-Maf in several cell types and RORγt$^+$ Tregs are associated with high IL-10 expression, potentially linking IL-10 to c-Maf-mediated RORγt$^+$ Treg function [24, 76].

*IL-33*

IL-33 is an alarmin that is released by stromal and epithelial cells at tissue sites in response to damage [77]. The IL-33 receptor, ST2, is highly expressed by GATA3$^+$ tTregs in the intestine and IL-33 treatment in vivo increases both splenic Treg and colonic RORγt$^-$ Treg numbers, while expansion of *St2*$^{-/-}$ Tregs is impaired [39, 40]. Although IL-33 in combination with TGF-β1 can induce iTreg differentiation in vitro, RORγt$^+$ pTregs remain unchanged following IL-33 treatment in vivo or in *Il33r*$^{-/-}$ mice. Therefore, IL-33 appears to positively regulate tTregs but not pTregs in vivo [39, 40].

*The tumour necrosis factor receptor superfamily (TNFRSF)/nuclear factor kappa-light–chain--enhancer of activated b cells (NF-κB) axis*

To survive and function at barrier sites, Tregs must adapt to prevailing environmental conditions. Tregs across tissue sites display an activated phenotype, including expression of CD44 and KLRG1, highlighting continual exposure to challenge [78]. Interestingly, activated Tregs in both SLOs and tissue sites such as the intestine and skin express a core TNFRSF signature [32, 79]. This includes expression of GITR, OX40 and TNFR2, and is in contrast to conventional $CD4^+$ T cells and $CD44^{lo}$ Tregs [32, 79]. Stimulation of TNFRSF members activates the NF-κB signalling pathway and provides crucial survival signals for Tregs [32, 79, 80]. Along these lines, Treg survival and accumulation in the colon depends on OX40 expression, which is also required for Treg-mediated protection against T cell transfer colitis [81]. Activation of the NF-κB transcription factor, RelA, is associated with Treg survival in SLOs and adaptation of both tTregs and pTregs to the colon [79]. Therefore, the TNFRSF/NF-κB axis might represent a central signalling module in Treg maintenance in the gut [82, 83].

**Environmental pathways shaping intestinal Tregs**

*The microbiota*

The intestine is home to a dense microbiota, which increases in abundance from the small intestine to the colon. Accordingly, pTreg maintenance in the colon is heavily influenced by the resident microbiota. In germ free mice or mice treated with broad-spectrum antibiotics colonic pTregs are dramatically reduced [20, 22, 40, 46, 84]. Moreover, colonisation of germ-free mice with a complex microbiota or a mix of 17 human *Clostridia* strains robustly induces pTregs in the colon [40, 41, 85, 86]. In contrast, the colonic Treg pool in germ-free mice is predominantly $Helios^+$ tTregs. Although the maintenance of both $ROR\gamma t^+$ and $ROR\gamma t^-$ pTregs is affected by the microbiota, $ROR\gamma t^+$ Tregs appear especially sensitive to microbial shifts [40, 41, 46, 84].

A wide range of bacterial genera can promote pTregs in the colon. These include members of *Clostridium*, *Bacteroides*, *Bifidobacterium*, *Lactobacillus* and *Helicobacter* [23, 24, 40, 85-88]. Of these bacteria, *Helicobacter* species have been shown to drive antigen-specific Tregs in the colon [23, 24]. Specifically, adoptively transferred *Helicobacter hepaticus*-reactive naïve $CD4^+$ T cells from TCR transgenic mice differentiated into $ROR\gamma t^+$ Tregs in the colon of recipient mice colonised with *H. hepaticus* [24]. While a bias towards $ROR\gamma t^+$ Treg induction by *H. hepaticus* is in line with a dominant role for the microbiota in the maintenance of this subset, larger numbers of antigen-reactive Tregs will need to be investigated to conclusively prove this. It will also be important to determine whether other known Treg-inducing bacteria,

such as *Clostridium* and *Bacteroides* species, function in a TCR-dependent manner [21, 23, 89]. Of note, *H. hepaticus* associates with the epithelium in the large intestine, likely providing a constant antigen source to the immune system. This is reminiscent of segmented filamentous bacteria (SFB), which attach to the small intestinal epithelium and are robust inducers of antigen-specific intestinal Th17 cells [90, 91]. Continual exposure to cognate antigen is crucial in VAT tTreg maintenance [36, 92] Along these lines, ROR$\gamma$t$^+$ Tregs were severely diminished in the colons of major histocompatibility complex (MHC) class II-deficient mice [41]. Therefore, it is possible that key bacterial drivers of antigen-specific Treg responses will be mucosa-associated, thus constantly exposing the immune system to antigen.

In addition to providing antigens, bacteria also act as adjuvants to shape the Treg response. Broad-spectrum antibiotics are much more potent in depletion of ROR$\gamma$t$^+$ Tregs compared to individual antibiotics [40]. In this regard, the shared functions of microbes rather than individual microbial members are likely to have the largest impact on Tregs. For example, toll-like receptor 2 (TLR2) activation by bacterial components appears to be a common Treg promoting mechanism by intestinal bacteria, and has been shown for *B. fragilis* Polysaccharide A (PSA) and cell surface $\beta$-glucan/galactan (CSGG) polysaccharides from *Bifidobacterium bifidum* [27, 93]. A large polysaccharide produced by *H. hepaticus* also signals through TLR2 and induces an anti-inflammatory signature in macrophages, including IL-10 production, that may impact intestinal Treg responses [94]. Along these lines, IL-10 production by Tregs appears to be especially dependent on the intestinal microbiota, as IL-10 producing Tregs are strongly reduced in the colons of germ free or antibiotic-treated mice [87, 93]. Moreover, colonisation of germ-free mice with *Clostridium* strains or *B. fragilis* increases IL-10$^+$ Tregs [87, 93]. Treg expression of CTLA-4 is also strongly enhanced by the microbiota [87, 93]. Overall, the microbiota is central in promoting Treg maintenance and function in the intestine, although much remains to be learned mechanistically about how the microbiota shapes Treg responses.

Another shared function of the microbiota is the production of metabolites that influence intestinal Treg responses [95]. The best characterised are the short-chain fatty acids (SCFAs), such as butyrate, propionate and acetate, which are fermented from dietary fibre by the gut microbiota. Certain bacteria are particularly adept at fermenting fibre, and include members of *Clostridium* and *Bacteroides*, which are abundant in the large intestine and are associated with induction of Treg responses [95]. Accordingly, SCFAs are highly enriched in the large intestine [95]. There is substantial evidence that SCFAs promote pTreg responses in the gut, particularly the colon [96-98]. For example, administration of butyrate, propionate and acetate

either individually or in combination increased colonic Treg numbers in specific pathogen free (SPF) mice [98]. SCFA treatment in mice and humans reduces intestinal inflammation and pathology [99, 100], although whether this effect is Treg-mediated has not been shown. SCFAs are thought to promote intestinal Tregs via two main mechanisms: through recognition by certain G-protein coupled receptors (GPCRs), such as GPR43 and GPR109A, which are expressed by colonic Tregs and colonic epithelial cells and innate immune cells, respectively, as well as through SCFA histone deacetylase (HDAC) inhibitor activity [96-98, 101]. Although SCFAs have been reported to induce ROR$\gamma$t$^+$ Tregs in one study, this remains to be demonstrated conclusively [40, 41]. Interestingly, intestinal tTreg expansion by SCFAs has also been reported [98], indicating that microbial metabolites may shape tTregs in the gut.

*Metabolites*

The aryl hydrocarbon receptor (AhR) is a nuclear sensor of many environmental compounds derived from the diet, microbiota and non-physiological xenobiotic compounds such as pollutants [102]. These include indole-3-carbinol and 3, 3'-diindolylmethane, derived from cruciferous vegetables, and tryptophan, which can be metabolised by the host enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2, 3- dioxygenase (TDO) expressed in innate cells [102]. Members of the gut microbiota, such as *Lactobacillus*, can also metabolise tryptophan to generate AhR ligands [102]. Metabolism of tryptophan produces a wide array of AhR ligands, including kynurenine, which selectively enhances Foxp3$^+$ Treg generation in vitro in the presence of TGF-$\beta$1 [103]. Colonic and small intestinal Tregs preferentially express AhR compared to Tregs from other tissue sites and SLOs [40, 84], suggesting that AhR ligands may be particularly important in shaping intestinal Tregs responses. The role of the AhR in Treg biology is controversial [104], however, a recent study has provided evidence that AhR activation regulates Treg responses in the gut. Mice with deletion of AhR in Tregs displayed a specific decrease in pTreg numbers but not tTregs in the intestine [84]. Moreover, intestinal Tregs from mice with a conditional knockout of AhR in Tregs produced increased amounts of IFN$\gamma$ and IL-17A and were defective in protection against T cell transfer colitis [84], arguing for a functional role for AhR in intestinal Tregs. However, more studies are needed to conclusively prove a role for the AhR and its ligands in intestinal Treg biology.

Another dietary factor that shapes Tregs in the intestine is vitamin A. Vitamin A is a fat soluble vitamin that is metabolised to its bioactive form, RA, through a series of metabolic steps that include oxidation by retinal dehydrogenases (RALDHs) [105]. Like TGF-$\beta$1, vitamin A is found at high concentrations in the intestine and is central in pTreg homeostasis in the gut [18]. In

the presence of TGF-β1, RA induces pTreg differentiation and the expression of CCR9 and α4β7 [29, 31, 106]. Moreover, CNS1, which is required for pTreg generation, contains binding sites for the retinoic acid receptor (RAR) and retinoid X receptor (RXR) heterodimer, which is activated by RA [70]. $CD103^+$ DCs are particularly equipped to promote Treg responses in the gut by RALDH-mediated RA generation and activation of latent TGF-β1 via integrin αvβ8 [29, 31, 107, 108]. RALDHs are also expressed by intestinal epithelial cells, although their contribution to Treg induction is unknown [109]. $ROR\gamma t^+$ pTreg development may be particularly dependent on vitamin A. Mice fed a vitamin A-deficient diet or treated with a RA receptor inhibitor display reduced frequencies of $ROR\gamma t^+$ Tregs, although whether their absolute numbers were decreased was not reported [41]. A human Treg subset expressing CD161 that is $RORC^+$ and reactive to RA, reminiscent of murine $ROR\gamma t^+$ Tregs, has been described [110]. In contrast to $ROR\gamma t^+$ Tregs, RA feeding was unable to rescue $ROR\gamma t^-$ Treg defective generation in the setting of an antigen-free diet [46]. Moreover, $Helios^+$ tTregs in the gut were unaffected by a vitamin A-deficient diet [41]. Therefore, vitamin A appears to specifically drive $ROR\gamma t^+$ Tregs in the intestine. Additional essential nutrients, such as folate, niacin and branched chain amino acids, also positively regulate Treg responses in the intestine [18, 101, 111]. Overall, the microbiota and dietary factors have a strong impact on shaping intestinal Treg responses and much is to be discovered in this area.

## Tregs in the skin

The skin is the largest organ of the body and is essential to many physiological processes, including thermoregulation, hydration, insulation and sensation (Figure 1B). These physiological functions involve the actions of skin appendages, such as hair follicles, sweat ducts and sebaceous glands. A cardinal function of the skin is to create a physical barrier for protection against external physical injury. To achieve this, the skin is composed of a complex, multi-layered epithelium called the epidermis, which is composed of an outer layer of dead keratinocytes called the stratum corneum that is underlayed by proliferating keratinocytes interspersed with select immune populations, such as Langerhans cells. Below the epidermis lies the dermis, which contains a dense network of immune cells, including a sizeable Treg population [19].

### Treg subsets and function in the skin

Given the hallmark feature of barrier sites being constantly exposed to environmental challenges, it might be predicted that pTreg differentiation would be favoured in the skin due

to diverse antigenic exposure. Intriguingly, pTregs comprise only a minor fraction of skin Tregs, in stark contrast to the intestine. Instead, the skin Treg pool is dominated by tTregs expressing GATA3 and Nrp1/Helios, which can comprise up to 80% of all skin Tregs [37, 112-114] (Figure 1B). A Helios$^+$ tTreg population expressing retinoid-related orphan receptor $\alpha$ (ROR$\alpha$) but not ROR$\gamma$t has also been described in the skin, although whether these Tregs co-expressed GATA3 was not reported [114]. Thymic Tregs appear to be central in preventing aberrant inflammatory responses in the skin, as mice with GATA3 or ROR$\alpha$ deletion in Tregs develop heightened type 2 skin inflammation spontaneously or upon challenge, respectively [114, 115]. Along these lines, IPEX patients frequently present with inflammatory skin disorders, such as atopic dermatitis and eczema [116]. IPEX patients also display susceptibility to skin infections, such as *Staphylococcus aureus*, which is a common skin commensal microbe. Indeed, type 2 inflammation elicited in the skin of *Foxp3$^{Cre}$xGata3$^{fl/fl}$* mice was associated with a deranged response to the microbiota [115]. Treg-mediated tolerance to the resident skin microbiota appears to be initiated shortly after birth and is associated with a sharp influx of Tregs to the skin during neonatal development [117]. Tregs are also required for skin host defence against the pathogenic parasite *Leishmania major* [118]. Therefore, Tregs in both the skin and gut are central to maintain homeostasis with the microbiota and protection against pathogens (Figure 3). However, this shared function appears to be performed by different Treg developmental lineages at each site.

A cardinal function of the skin is tissue repair and Tregs have been shown to facilitate the healing process following skin wounding [119]. Expression of epidermal growth factor receptor (EGFR) by Tregs was required for their accumulation into wounds and control of Ly6C$^{hi}$ inflammatory cell accumulation in the skin [119]. Similarly, Tregs inhibited Ly6C$^{hi}$ inflammatory responses in the skin during a mouse model of psoriasis, although tissue repair in this setting was not investigated [120]. Although tTregs are associated with tissue repair at multiple tissue sites, this has yet to be documented in the skin [50, 113]. Another important physiological process in the skin is hair follicle morphogenesis. Interestingly, Tregs have been shown to stimulate hair follicle regeneration by promoting stem cell proliferation and differentiation in the skin [121]. Indeed, hair follicles are proposed as a preferential accumulation site for Tregs in mouse and human skin [19]. Thus, it appears that Tregs in the skin and gut are both involved in regulation of stem cell development but promote opposing outcomes, namely stem cell differentiation in the skin and stem cell renewal in the gut.

**Host pathways shaping skin Tregs**

Unlike the intestine, the host pathways that drive Tregs in the skin are less well defined. The skin Treg pool is dominated by GATA3$^+$ tTregs, which have been identified at multiple tissue sites. Given the potential universal role of GATA3$^+$ tTregs in tissue repair across tissue sites [50], it might be predicted that the host factors known to promote this subset, such as IL-2 and IL-33 are common across sites. In support of this, GATA3 expression in splenic Tregs is enhanced following systemic administration of IL-2 in vivo [37]. Moreover, skin Langerhans cells and dermal fibroblasts promote Treg proliferation in an IL-2-dependent manner in vitro [122, 123]. However, IL-7 and not IL-2 was required for Treg maintenance in the skin in vivo [124]. IL-33 treatment was shown to increase skin dLN Tregs in a model of allogeneic skin graft [125], however a conclusive role for this cytokine in tTreg maintenance in the skin has not been demonstrated. The Th2-associated cytokine, thymic stromal lymphopoietin (TSLP), has been linked to local Treg expansion in the skin following vitamin D receptor (VDR) stimulation [126]. Overall, the host pathways that shape skin Tregs remain unclear (Figure 4).

**Environmental pathways shaping skin Tregs**

*The microbiota*

The skin harbours a rich microbiota that is arranged into distinct communities based on the diverse topography of the skin, such as hair follicles and sweat glands [127, 128]. The microbiota may be particularly important in promoting Treg expansion during neonatal development in the skin, as germ free neonates harbour reduced numbers of Tregs compared to SPF neonates [129]. Interestingly, another study reported increased Treg numbers in the skin of adult germ free mice compared to SPF mice [130]. These studies suggest that the skin microbiota may dynamically regulate the size of the Treg pool during ontogeny [129, 130]. Although Treg subsets were not characterised in the two aforementioned studies, the majority of skin Tregs belong to the tTreg subset. Therefore, it is intriguing that this Treg subset might be strongly impacted by microbiota in the skin but not the intestine. Of note, the two studies examined different skin sites, namely trunk skin and ears, and it is well established that microbial communities vary widely depending on skin site [127, 128]. Therefore, to fully elucidate the role of the resident microbiota in skin Treg homeostasis, detailed analysis of different skin tissue will be required.

*Metabolites*

There is evidence that vitamin A may be an important driver of Tregs in the skin. Skin DCs express RALDHs and can induce Tregs in vitro, a process that can be blocked by a RA receptor inhibitor [131]. Indeed, vitamin A is essential in skin health and vitamin A deficiency in humans often presents with skin manifestations [132]. Interestingly, members of the skin microbiota, such as *Propionibacterium acnes* and those from the fungal genus *Malassezia*, can ferment SCFAs from skin lipids and generate indoles with potent AhR activity, respectively [133, 134]. Therefore, the microbiota may play an important role in generating metabolites that could shape Tregs in the skin. Overall however, very little is understood about the impact of metabolites on Treg adaptation in the skin.

*Ultraviolet radiation*

The skin is unique in its exposure to ultraviolet (UV) radiation. UVB radiation in particular has long been known to generate immunosuppression in the skin [135]. Accordingly, several skin diseases, such as psoriasis and atopic dermatitis, are treated with UV therapy [135]. UVB irradiation of the skin leads to a significant expansion of $Nrp1^+$ tTregs, which is a key mechanism of UV radiation-induced skin immunotolerance [112] (Figure 4). A population of dermal $CD11b^+Langerin^-$ DCs has been shown to be involved in UVB-mediated promotion of Tregs in vivo [136]. The effects of UV on Treg expansion are linked to vitamin D, which is metabolised under sunlight or derived from the diet [105]. UVB radiation metabolises 7-dehydrolcholesterol to the most biologically active form of vitamin D, 1,25 $(OH)_2D_3$ (vitamin D3), which signals through the VDR that is expressed by numerous cells, including T cells and APCs [105]. Accordingly, 1,25 $(OH)_2D_3$ stimulation of human $CD4^+$ T cells in vitro can induce Tregs directly or through stimulating Langerhans cells to produce TGF-β1 [137, 138]. However, a requirement for vitamin D in UVB-mediated tTreg expansion in the skin has not been investigated.

## Treg metabolic adaptation at barrier sites: an emerging theme

Metabolism is an essential process in every cell and is involved in T cell growth, differentiation and function [139]. Glucose, lipids and amino acids, such as glutamine, are key nutrients involved in T cell metabolism. Importantly, the concentration of these nutrients, and indeed others, is very different between SLOs and barrier sites, as well as between barrier sites themselves. SLOs are rich in glucose and glutamine, whereas the intestine is rich in dietary and microbial-derived nutrients, such as vitamins and fatty acids, and the skin is abundant in lipids and salt [128] (Figure 5). Which particular metabolic processes cells utilise at any given

time depends on local metabolic substrate availability. Given the unique and dynamic nutrient composition of the intestine and skin, Tregs at these sites would be predicted to display distinct metabolic profiles.

In vitro studies have demonstrated that the metabolic state of a Treg cell is flexible and dynamic, with Tregs able to use different metabolic substrates to fulfil their metabolic needs [139]. Moreover, Treg activation triggers metabolic pathways that are intricately linked to Treg function [139, 140]. However, little is understood about Treg metabolic adaptation in vivo. Single-cell RNA-sequencing analysis of colonic and skin Tregs has revealed a metabolic adaptation trajectory, whereby dLN Tregs are associated with expression of glycolysis-associated genes, in contrast to tissue-adapted colon and skin Tregs that preferentially express genes associated with fatty acid metabolism [32, 141] (Figure 5). T cells utilise glycolysis upon activation and dLNs are believed to be sites of Treg activation and priming before recruitment to tissues [140, 142, 143]. As well as activation status, a bias towards glycolytic metabolism by dLN Tregs may be due to the rich glucose environments of the dLNs compared to barrier sites. In this regard, intestinal Tregs display increased in vivo lipid uptake and higher expression of the fatty acid binding receptor CD36 compared to splenic Tregs, suggesting adaptation to the lipid rich environment of the gut [141]. Interestingly, tissue resident memory $CD8^+$ T cells in the skin depend on exogenous fatty acid uptake via fatty acid binding protein 4 (FABP4) and FABP5 for survival and maintenance [144]. Therefore, fatty acid metabolism may be central in T cell adaptation to barrier sites, which display rich lipid compositions (Figure 5). Indeed, a defining feature of the skin is an abundance of diverse and unconventional lipids unique to the skin [128]. Moreover, the cholesterol metabolites, oxysterols, are abundant in the intestine and have been identified as ligands for RORγt, suggesting involvement in intestinal $ROR\gamma t^+$ Treg development and function [145]. Understanding how lipids, as well as other metabolic substrates and physiological factors such as oxygen, pH and temperature shape Treg metabolic adaptation will be an important area of future research (Figure 5).

Several recent studies have identified a requirement for Foxp3 in regulating the expression of metabolic genes in Tregs [143, 146, 147]. Interestingly, Foxp3-dependent regulation of metabolism is associated with cellular protection against various metabolic stressors. Foxp3 expression by Tregs protects against apoptosis following exposure to saturated long-chain fatty acids, such as palmitate and stearate [146]. Similarly, Foxp3 protected Tregs from lactate-mediated inhibition of proliferation in vitro [147]. In this regard, Tregs are more resistant to the tumour environment, which is high in lactate and low in glucose and glutamine,

compared to Teffs [148, 149]. Therefore, Tregs may possess a selective advantage over Teffs in harsh environments, such as barrier sites. Autophagy may be a central mechanism enabling Tregs to thrive in nutrient poor environments, as Treg defects in autophagy are associated with dysregulated Treg fitness and function in the gut and other tissue sites [141, 150]. Moreover, defects in autophagic processes were associated with altered metabolic adaptation of Tregs in vivo [141, 150]. Thus, Tregs at barrier sites may display unique metabolic profiles that are intrinsically linked to Treg adaptation and function at these sites.

## Future perspectives

Tregs are essential in maintaining homeostasis at barrier sites, such as the intestine and skin. Defective Treg immunity at these sites is associated with chronic inflammatory diseases, such as IBD, food allergy and atopic dermatitis, which are increasing in prevalence and remain a major challenge to treat. A shift towards precision medicine is emerging and a definitive understanding of Treg adaptation at barrier sites will be essential to the success of targeted therapies. The identification of diverse Treg phenotypes in the intestine and skin and differences in their relative frequencies has uncovered greater heterogeneity in the Treg population than was previously recognised. However, much remains to be understood about Treg antigen reactivity and metabolic adaptation at tissue sites. A key advance in Treg biology has been the discovery that Tregs at tissue sites are crucial in upholding physiological functions of the tissue, aside from their classical role in suppression of pro-inflammatory responses. Investigating the cross-talk between Tregs and tissue cells, such as stromal and neuronal populations, should further advance our understanding of the role of Tregs in tissue homeostasis. Technological advances in single cell TCR sequencing, cell phenotyping, metabolic profiling and imaging are increasing the level of detail at which immune responses within tissues can be investigated. Harnessing these techniques to study Treg adaptation in the intestine and skin, as well as other barrier sites which remain largely understudied, will likely reveal further novel phenotypes and functions of tissue Tregs and better guide targeted therapies.

## References

1. Sakaguchi, S., et al., *Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease.* J Exp Med, 1985. **161**(1): p. 72-87.

2. Powrie, F. and D. Mason, *OX-22high CD4+ T cells induce wasting disease with multiple organ pathology: prevention by the OX-22low subset.* The Journal of Experimental Medicine, 1990. **172**(6): p. 1701.
3. Sakaguchi, S., et al., *Regulatory T cells and immune tolerance.* Cell, 2008. **133**(5): p. 775-87.
4. Sakaguchi, S., et al., *Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases.* The Journal of Immunology, 1995. **155**(3): p. 1151.
5. Hori, S., T. Nomura, and S. Sakaguchi, *Control of Regulatory T Cell Development by the Transcription Factor Foxp3.* Science, 2003. **299**(5609): p. 1057.
6. Fontenot, J.D., M.A. Gavin, and A.Y. Rudensky, *Foxp3 programs the development and function of CD4+CD25+ regulatory T cells.* Nature Immunology, 2003. **4**: p. 330.
7. Khattri, R., et al., *An essential role for Scurfin in CD4+CD25+ T regulatory cells.* Nature Immunology, 2003. **4**: p. 337.
8. Josefowicz, S.Z., L.-F. Lu, and A.Y. Rudensky, *Regulatory T Cells: Mechanisms of Differentiation and Function.* Annual Review of Immunology, 2012. **30**(1): p. 531-564.
9. Brunkow, M.E., et al., *Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse.* Nature Genetics, 2001. **27**: p. 68.
10. Fontenot, J.D., et al., *Regulatory T cell lineage specification by the forkhead transcription factor foxp3.* Immunity, 2005. **22**(3): p. 329-41.
11. Bennett, C.L., et al., *The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3.* Nature Genetics, 2001. **27**: p. 20.
12. Gambineri, E., T.R. Torgerson, and H.D. Ochs, *Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis.* Curr Opin Rheumatol, 2003. **15**(4): p. 430-5.
13. Wildin, R.S., et al., *X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy.* Nat Genet, 2001. **27**(1): p. 18-20.
14. Powrie, F., et al., *Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice.* Int Immunol, 1993. **5**(11): p. 1461-71.
15. Cipolletta, D., et al., *PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells.* Nature, 2012. **486**(7404): p. 549-53.
16. Burzyn, D., et al., *A special population of regulatory T cells potentiates muscle repair.* Cell, 2013. **155**(6): p. 1282-95.
17. Panduro, M., C. Benoist, and D. Mathis, *Tissue Tregs.* Annual Review of Immunology, 2016. **34**(1): p. 609-633.
18. Tanoue, T., K. Atarashi, and K. Honda, *Development and maintenance of intestinal regulatory T cells.* Nature Reviews Immunology, 2016. **16**: p. 295.
19. Ali, N. and M.D. Rosenblum, *Regulatory T cells in skin.* Immunology, 2017. **152**(3): p. 372-381.
20. Nutsch, K., et al., *Rapid and Efficient Generation of Regulatory T Cells to Commensal Antigens in the Periphery.* Cell Rep, 2016. **17**(1): p. 206-220.
21. Russler-Germain, E.V., S. Rengarajan, and C.S. Hsieh, *Antigen-specific regulatory T-cell responses to intestinal microbiota.* Mucosal Immunol, 2017. **10**(6): p. 1375-1386.

22. Lathrop, S.K., et al., *Peripheral education of the immune system by colonic commensal microbiota.* Nature, 2011. **478**(7368): p. 250-4.
23. Chai, J.N., et al., *Helicobacter species are potent drivers of colonic T cell responses in homeostasis and inflammation.* Sci Immunol, 2017. **2**(13).
24. Xu, M., et al., *c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont.* Nature, 2018. **554**(7692): p. 373-377.
25. Cebula, A., et al., *Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota.* Nature, 2013. **497**(7448): p. 258-62.
26. Hegazy, A.N., et al., *Circulating and Tissue-Resident CD4(+) T Cells With Reactivity to Intestinal Microbiota Are Abundant in Healthy Individuals and Function Is Altered During Inflammation.* Gastroenterology, 2017. **153**(5): p. 1320-1337.e16.
27. Verma, R., et al., *Cell surface polysaccharides of Bifidobacterium bifidum induce the generation of Foxp3(+) regulatory T cells.* Sci Immunol, 2018. **3**(28).
28. Wei, S., I. Kryczek, and W. Zou, *Regulatory T-cell compartmentalization and trafficking.* Blood, 2006. **108**(2): p. 426-31.
29. Coombes, J.L., et al., *A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-β– and retinoic acid–dependent mechanism.* The Journal of Experimental Medicine, 2007. **204**(8): p. 1757.
30. Hadis, U., et al., *Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria.* Immunity, 2011. **34**(2): p. 237-46.
31. Sun, C.M., et al., *Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid.* J Exp Med, 2007. **204**(8): p. 1775-85.
32. Miragaia, R.J., et al., *Single-Cell Transcriptomics of Regulatory T Cells Reveals Trajectories of Tissue Adaptation.* Immunity, 2019.
33. Tomura, M., et al., *Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice.* The Journal of Clinical Investigation, 2010. **120**(3): p. 883-893.
34. Cretney, E., A. Kallies, and S.L. Nutt, *Differentiation and function of Foxp3(+) effector regulatory T cells.* Trends Immunol, 2013. **34**(2): p. 74-80.
35. Vasanthakumar, A., et al., *The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells.* Nat Immunol, 2015. **16**(3): p. 276-85.
36. Li, C., et al., *TCR Transgenic Mice Reveal Stepwise, Multi-site Acquisition of the Distinctive Fat-Treg Phenotype.* Cell, 2018. **174**(2): p. 285-299 e12.
37. Wohlfert, E.A., et al., *GATA3 controls Foxp3+ regulatory T cell fate during inflammation in mice.* The Journal of Clinical Investigation, 2011. **121**(11): p. 4503-4515.
38. Wang, Y., M.A. Su, and Y.Y. Wan, *An essential role of the transcription factor GATA-3 for the function of regulatory T cells.* Immunity, 2011. **35**(3): p. 337-48.
39. Schiering, C., et al., *The alarmin IL-33 promotes regulatory T-cell function in the intestine.* Nature, 2014. **513**(7519): p. 564-568.
40. Sefik, E., et al., *Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells.* Science, 2015. **349**(6251): p. 993-7.
41. Ohnmacht, C., et al., *The microbiota regulates type 2 immunity through RORgammat(+) T cells.* Science, 2015. **349**(6251): p. 989-93.
42. Yu, F., et al., *Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance.* Nature Immunology, 2014. **16**: p. 197.

43. Torgerson, T.R., et al., *Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene*. Gastroenterology, 2007. **132**(5): p. 1705-17.
44. Josefowicz, S.Z., et al., *Extrathymically generated regulatory T cells control mucosal TH2 inflammation.* Nature, 2012. **482**(7385): p. 395-9.
45. Campbell, C., et al., *Extrathymically Generated Regulatory T Cells Establish a Niche for Intestinal Border-Dwelling Bacteria and Affect Physiologic Metabolite Balance.* Immunity, 2018. **48**(6): p. 1245-1257 e9.
46. Kim, K.S., et al., *Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine.* Science, 2016. **351**(6275): p. 858.
47. Cong, Y., et al., *A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota.* Proc Natl Acad Sci U S A, 2009. **106**(46): p. 19256-61.
48. Kawamoto, S., et al., *Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis.* Immunity, 2014. **41**(1): p. 152-65.
49. Yang, B.H., et al., *Foxp3(+) T cells expressing RORgammat represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation.* Mucosal Immunol, 2016. **9**(2): p. 444-57.
50. Zhang, C., et al., *'Repair' Treg Cells in Tissue Injury.* Cell Physiol Biochem, 2017. **43**(6): p. 2155-2169.
51. Siede, J., et al., *IL-33 Receptor-Expressing Regulatory T Cells Are Highly Activated, Th2 Biased and Suppress CD4 T Cell Proliferation through IL-10 and TGFbeta Release.* PLoS One, 2016. **11**(8): p. e0161507.
52. Read, S., V. Malmström, and F. Powrie, *Cytotoxic T Lymphocyte–Associated Antigen 4 Plays an Essential Role in the Function of Cd25+Cd4+ Regulatory Cells That Control Intestinal Inflammation.* The Journal of Experimental Medicine, 2000. **192**(2): p. 295.
53. Gupta, A., et al., *Systematic review: colitis associated with anti-CTLA-4 therapy.* Aliment Pharmacol Ther, 2015. **42**(4): p. 406-17.
54. Barnes, M.J. and F. Powrie, *Regulatory T cells reinforce intestinal homeostasis.* Immunity, 2009. **31**(3): p. 401-11.
55. Uhlig, H.H. and F. Powrie, *Translating Immunology into Therapeutic Concepts for Inflammatory Bowel Disease.* Annu Rev Immunol, 2018. **36**: p. 755-781.
56. Edwards, J.P., A.M. Thornton, and E.M. Shevach, *Release of Active TGF-β1 from the Latent TGF-β1/GARP Complex on T Regulatory Cells Is Mediated by Integrin β8.* The Journal of Immunology, 2014. **193**(6): p. 2843-2849.
57. Salem, M., et al., *GARP Dampens Cancer Immunity by Sustaining Function and Accumulation of Regulatory T Cells in the Colon.* Cancer Res, 2019.
58. Worthington, J.J., et al., *Integrin alphavbeta8-Mediated TGF-beta Activation by Effector Regulatory T Cells Is Essential for Suppression of T-Cell-Mediated Inflammation.* Immunity, 2015. **42**(5): p. 903-15.
59. Chaudhry, A., et al., *CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner.* Science, 2009. **326**(5955): p. 986-91.
60. Schmidt, A., N. Oberle, and P. Krammer, *Molecular Mechanisms of Treg-Mediated T Cell Suppression.* Frontiers in Immunology, 2012. **3**: p. 51.
61. Belkaid, Y. and K. Tarbell, *Regulatory T cells in the control of host-microorganism interactions (*).* Annu Rev Immunol, 2009. **27**: p. 551-89.
62. Wang, Z., et al., *Regulatory T cells promote a protective Th17-associated immune response to intestinal bacterial infection with C. rodentium.* Mucosal Immunology, 2014. **7**: p. 1290.

63. Pandiyan, P., et al., *CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation–mediated apoptosis of effector CD4+ T cells.* Nature Immunology, 2007. **8**: p. 1353.
64. Biton, M., et al., *T Helper Cell Cytokines Modulate Intestinal Stem Cell Renewal and Differentiation.* Cell, 2018. **175**(5): p. 1307-1320 e22.
65. Oldenhove, G., et al., *Decrease of Foxp3+ Treg cell number and acquisition of effector cell phenotype during lethal infection.* Immunity, 2009. **31**(5): p. 772-86.
66. Edwards, J.P., et al., *The GARP/Latent TGF-beta1 complex on Treg cells modulates the induction of peripherally derived Treg cells during oral tolerance.* Eur J Immunol, 2016. **46**(6): p. 1480-9.
67. Konkel, J.E. and W. Chen, *Balancing acts: the role of TGF-beta in the mucosal immune system.* Trends Mol Med, 2011. **17**(11): p. 668-76.
68. Worthington, J.J., et al., *Intestinal dendritic cells specialize to activate transforming growth factor-beta and induce Foxp3+ regulatory T cells via integrin alphavbeta8.* Gastroenterology, 2011. **141**(5): p. 1802-12.
69. Tone, Y., et al., *Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer.* Nat Immunol, 2008. **9**(2): p. 194-202.
70. Xu, L., et al., *Positive and negative transcriptional regulation of the Foxp3 gene is mediated by access and binding of the Smad3 protein to enhancer I.* Immunity, 2010. **33**(3): p. 313-25.
71. Schlenner, S.M., et al., *Smad3 binding to the foxp3 enhancer is dispensable for the development of regulatory T cells with the exception of the gut.* J Exp Med, 2012. **209**(9): p. 1529-35.
72. Konkel, J.E., et al., *Transforming Growth Factor-beta Signaling in Regulatory T Cells Controls T Helper-17 Cells and Tissue-Specific Immune Responses.* Immunity, 2017. **46**(4): p. 660-674.
73. Zuniga, L.A., et al., *Th17 cell development: from the cradle to the grave.* Immunol Rev, 2013. **252**(1): p. 78-88.
74. Izcue, A., et al., *Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis.* Immunity, 2008. **28**(4): p. 559-70.
75. Gabrysova, L., et al., *c-Maf controls immune responses by regulating disease-specific gene networks and repressing IL-2 in CD4(+) T cells.* Nat Immunol, 2018. **19**(5): p. 497-507.
76. Saraiva, M. and A. O'Garra, *The regulation of IL-10 production by immune cells.* Nat Rev Immunol, 2010. **10**(3): p. 170-81.
77. Pichery, M., et al., *Endogenous IL-33 Is Highly Expressed in Mouse Epithelial Barrier Tissues, Lymphoid Organs, Brain, Embryos, and Inflamed Tissues: In Situ Analysis Using a Novel Il-33–LacZ Gene Trap Reporter Strain.* The Journal of Immunology, 2012. **188**(7): p. 3488.
78. Fan, X. and A.Y. Rudensky, *Hallmarks of Tissue-Resident Lymphocytes.* Cell, 2016. **164**(6): p. 1198-1211.
79. Vasanthakumar, A., et al., *The TNF Receptor Superfamily-NF-kappaB Axis Is Critical to Maintain Effector Regulatory T Cells in Lymphoid and Non-lymphoid Tissues.* Cell Rep, 2017. **20**(12): p. 2906-2920.
80. Salomon, B.L., et al., *Tumor Necrosis Factor alpha and Regulatory T Cells in Oncoimmunology.* Front Immunol, 2018. **9**: p. 444.
81. Griseri, T., et al., *OX40 is required for regulatory T cell–mediated control of colitis.* The Journal of Experimental Medicine, 2010. **207**(4): p. 699.

82. Long, M., et al., *Nuclear factor-kappaB modulates regulatory T cell development by directly regulating expression of Foxp3 transcription factor.* Immunity, 2009. **31**(6): p. 921-31.
83. Schuster, M., et al., *IkappaB(NS) protein mediates regulatory T cell development via induction of the Foxp3 transcription factor.* Immunity, 2012. **37**(6): p. 998-1008.
84. Ye, J., et al., *The Aryl Hydrocarbon Receptor Preferentially Marks and Promotes Gut Regulatory T Cells.* Cell Rep, 2017. **21**(8): p. 2277-2290.
85. Geuking, M.B., et al., *Intestinal bacterial colonization induces mutualistic regulatory T cell responses.* Immunity, 2011. **34**(5): p. 794-806.
86. Atarashi, K., et al., *Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.* Nature, 2013. **500**(7461): p. 232-6.
87. Atarashi, K., et al., *Induction of colonic regulatory T cells by indigenous Clostridium species.* Science, 2011. **331**(6015): p. 337-41.
88. Kullberg, M.C., et al., *Bacteria-triggered CD4+ T Regulatory Cells Suppress Helicobacter hepaticus–induced Colitis.* The Journal of Experimental Medicine, 2002. **196**(4): p. 505.
89. Bilate, A.M., et al., *Tissue-specific emergence of regulatory and intraepithelial T cells from a clonal T cell precursor.* Sci Immunol, 2016. **1**(2): p. eaaf7471.
90. Ivanov, I., et al., *Induction of intestinal Th17 cells by segmented filamentous bacteria.* Cell, 2009. **139**(3): p. 485-98.
91. Yang, Y., et al., *Focused specificity of intestinal TH17 cells towards commensal bacterial antigens.* Nature, 2014. **510**(7503): p. 152-6.
92. Kolodin, D., et al., *Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice.* Cell Metab, 2015. **21**(4): p. 543-57.
93. Round, J.L. and S.K. Mazmanian, *Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota.* Proc Natl Acad Sci U S A, 2010. **107**(27): p. 12204-9.
94. Danne, C., et al., *A Large Polysaccharide Produced by Helicobacter hepaticus Induces an Anti-inflammatory Gene Signature in Macrophages.* Cell Host Microbe, 2017. **22**(6): p. 733-745 e5.
95. Koh, A., et al., *From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites.* Cell, 2016. **165**(6): p. 1332-1345.
96. Arpaia, N., et al., *Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation.* Nature, 2013. **504**(7480): p. 451-5.
97. Furusawa, Y., et al., *Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells.* Nature, 2013. **504**(7480): p. 446-50.
98. Smith, P.M., et al., *The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis.* Science, 2013. **341**(6145): p. 569-73.
99. Maslowski, K.M., et al., *Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43.* Nature, 2009. **461**(7268): p. 1282-6.
100. Hamer, H.M., et al., *Review article: the role of butyrate on colonic function.* Aliment Pharmacol Ther, 2008. **27**(2): p. 104-19.
101. Singh, N., et al., *Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis.* Immunity, 2014. **40**(1): p. 128-39.
102. Shinde, R. and T.L. McGaha, *The Aryl Hydrocarbon Receptor: Connecting Immunity to the Microenvironment.* Trends Immunol, 2018. **39**(12): p. 1005-1020.
103. Mezrich, J.D., et al., *An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells.* J Immunol, 2010. **185**(6): p. 3190-8.

104. Stockinger, B., et al., *The aryl hydrocarbon receptor: multitasking in the immune system.* Annu Rev Immunol, 2014. **32**: p. 403-32.
105. Mora, J.R., M. Iwata, and U.H. von Andrian, *Vitamin effects on the immune system: vitamins A and D take centre stage.* Nat Rev Immunol, 2008. **8**(9): p. 685-98.
106. Mucida, D., et al., *Reciprocal TH17 and Regulatory T Cell Differentiation Mediated by Retinoic Acid.* Science, 2007. **317**(5835): p. 256.
107. Annacker, O., et al., *Essential role for CD103 in the T cell–mediated regulation of experimental colitis.* The Journal of Experimental Medicine, 2005. **202**(8): p. 1051.
108. Travis, M.A., et al., *Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice.* Nature, 2007. **449**(7160): p. 361-5.
109. Iwata, M., et al., *Retinoic acid imprints gut-homing specificity on T cells.* Immunity, 2004. **21**(4): p. 527-38.
110. Povoleri, G.A.M., et al., *Human retinoic acid-regulated CD161(+) regulatory T cells support wound repair in intestinal mucosa.* Nat Immunol, 2018. **19**(12): p. 1403-1414.
111. Ikeda, K., et al., *Slc3a2 Mediates Branched-Chain Amino-Acid-Dependent Maintenance of Regulatory T Cells.* Cell Rep, 2017. **21**(7): p. 1824-1838.
112. Yamazaki, S., et al., *Homeostasis of Thymus-Derived Foxp3+ Regulatory T Cells Is Controlled by Ultraviolet B Exposure in the Skin.* The Journal of Immunology, 2014.
113. Delacher, M., et al., *Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues.* Nat Immunol, 2017. **18**(10): p. 1160-1172.
114. Malhotra, N., et al., *RORalpha-expressing T regulatory cells restrain allergic skin inflammation.* Sci Immunol, 2018. **3**(21).
115. Harrison, O.J., et al., *Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury.* Science, 2018: p. eaat6280.
116. Halabi-Tawil, M., et al., *Cutaneous manifestations of immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome.* Br J Dermatol, 2009. **160**(3): p. 645-51.
117. Scharschmidt, Tiffany C., et al., *A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes.* Immunity, 2015. **43**(5): p. 1011-1021.
118. Belkaid, Y., et al., *CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity.* Nature, 2002. **420**: p. 502.
119. Nosbaum, A., et al., *Cutting Edge: Regulatory T Cells Facilitate Cutaneous Wound Healing.* The Journal of Immunology, 2016. **196**(5): p. 2010.
120. Stockenhuber, K., et al., *Foxp3(+) T reg cells control psoriasiform inflammation by restraining an IFN-I-driven CD8(+) T cell response.* J Exp Med, 2018. **215**(8): p. 1987-1998.
121. Ali, N., et al., *Regulatory T Cells in Skin Facilitate Epithelial Stem Cell Differentiation.* Cell, 2017. **169**(6): p. 1119-1129.e11.
122. Seneschal, J., et al., *Human Epidermal Langerhans Cells Maintain Immune Homeostasis in Skin by Activating Skin Resident Regulatory T Cells.* Immunity, 2012. **36**(5): p. 873-884.
123. Clark, R.A. and T.S. Kupper, *IL-15 and dermal fibroblasts induce proliferation of natural regulatory T cells isolated from human skin.* Blood, 2007. **109**(1): p. 194.
124. Gratz, I.K., et al., *Cutting Edge: Memory Regulatory T Cells Require IL-7 and Not IL-2 for Their Maintenance in Peripheral Tissues.* The Journal of Immunology, 2013. **190**(9): p. 4483.
125. Gajardo, T., et al., *Exogenous interleukin-33 targets myeloid-derived suppressor cells and generates periphery-induced Foxp3+ regulatory T cells in skin-transplanted mice.* Immunology, 2015. **146**(1): p. 81-88.

126. Leichner, T.M., et al., *Skin-derived TSLP systemically expands regulatory T cells.* J Autoimmun, 2017. **79**: p. 39-52.
127. Grice, E.A., et al., *Topographical and temporal diversity of the human skin microbiome.* Science, 2009. **324**(5931): p. 1190-1192.
128. Chen, Y.E., M.A. Fischbach, and Y. Belkaid, *Skin microbiota–host interactions.* Nature, 2018. **553**: p. 427.
129. Scharschmidt, T.C., et al., *Commensal Microbes and Hair Follicle Morphogenesis Coordinately Drive Treg Migration into Neonatal Skin.* Cell Host & Microbe, 2017. **21**(4): p. 467-477.e5.
130. Naik, S., et al., *Compartmentalized Control of Skin Immunity by Resident Commensals.* Science, 2012. **337**(6098): p. 1115.
131. Guilliams, M., et al., *Skin-draining lymph nodes contain dermis-derived CD103(-) dendritic cells that constitutively produce retinoic acid and induce Foxp3(+) regulatory T cells.* Blood, 2010. **115**(10): p. 1958-68.
132. Galimberti, F. and N.A. Mesinkovska, *Skin findings associated with nutritional deficiencies.* Cleve Clin J Med, 2016. **83**(10): p. 731-739.
133. Sanford, J.A., et al., *Inhibition of HDAC8 and HDAC9 by microbial short-chain fatty acids breaks immune tolerance of the epidermis to TLR ligands.* Sci Immunol, 2016. **1**(4).
134. Magiatis, P., et al., *Malassezia yeasts produce a collection of exceptionally potent activators of the Ah (dioxin) receptor detected in diseased human skin.* J Invest Dermatol, 2013. **133**(8): p. 2023-30.
135. Schwarz, T., *25 years of UV-induced immunosuppression mediated by T cells-from disregarded T suppressor cells to highly respected regulatory T cells.* Photochem Photobiol, 2008. **84**(1): p. 10-8.
136. Yamazaki, S., et al., *Ultraviolet B–Induced Maturation of CD11b-Type Langerin-Dendritic Cells Controls the Expansion of Foxp3+ Regulatory T Cells in the Skin.* The Journal of Immunology, 2018. **200**(1): p. 119.
137. Jeffery, L.E., et al., *1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3.* J Immunol, 2009. **183**(9): p. 5458-67.
138. van der Aar, A.M., et al., *Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells.* J Allergy Clin Immunol, 2011. **127**(6): p. 1532-40 e7.
139. Newton, R., B. Priyadharshini, and L.A. Turka, *Immunometabolism of regulatory T cells.* Nat Immunol, 2016. **17**(6): p. 618-25.
140. Zeng, H., et al., *mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function.* Nature, 2013. **499**(7459): p. 485-90.
141. Kabat, A.M., et al., *The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation.* Elife, 2016. **5**: p. e12444.
142. Michalek, R.D., et al., *Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets.* J Immunol, 2011. **186**(6): p. 3299-303.
143. Gerriets, V.A., et al., *Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation.* J Clin Invest, 2015. **125**(1): p. 194-207.
144. Pan, Y., et al., *Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism.* Nature, 2017. **543**(7644): p. 252-256.
145. Santori, F.R., et al., *Identification of natural RORgamma ligands that regulate the development of lymphoid cells.* Cell Metab, 2015. **21**(2): p. 286-298.

146. Howie, D., et al., *Foxp3 drives oxidative phosphorylation and protection from lipotoxicity.* JCI Insight, 2017. **2**(3): p. e89160.
147. Angelin, A., et al., *Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments.* Cell Metab, 2017. **25**(6): p. 1282-1293 e7.
148. Klysz, D., et al., *Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation.* Sci Signal, 2015. **8**(396): p. ra97.
149. Pollizzi, K.N. and J.D. Powell, *Integrating canonical and metabolic signalling programmes in the regulation of T cell responses.* Nat Rev Immunol, 2014. **14**(7): p. 435-46.
150. Wei, J., et al., *Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis.* Nat Immunol, 2016. **17**(3): p. 277-85.