

REVIEW

Transforming growth factor β : a master regulator of the gut microbiota and immune cell interactions

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The relationship between host organisms and their microbiota has co-evolved towards an inter-dependent network of mutualistic interactions. This interplay is particularly well studied in the gastrointestinal tract, where microbiota and host immune cells can modulate each other directly, as well as indirectly, through the production and release of chemical molecules and signals. In this review, we define the functional impact of transforming growth factor-beta (TGF- β) on this complex interplay, especially through its modulation of the activity of local regulatory T cells (Tregs), type 17 helper (Th17) cells, innate lymphoid cells (ILCs) and B cells.

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INTRODUCTION

The host colonization by microbiota is a gradual and complex process initiated by the exposure of newborns to the maternal vagina or skin microbiota, according to the mode of delivery, which fluctuates throughout life due to external and internal factors, including dietary habits, antibiotic treatments and host diseases.¹ The adult human intestine contains approximately 4×10^{13} microorganisms including viruses, fungi and 500 to 1000 bacterial species. The bacterial density increases along the gastrointestinal tract to reach 3.8×10^{13} bacteria per gram of fecal matter in the colon, and the composition of this microbiota also varies according to spatial localization.^{2,3} The small intestine is colonized by a complex microbiota including *Lactobacillus sp.*, *Streptococcus sp.*, *Clostridium sp.*, *Escherichia coli*, whereas the colon is enriched in dietary fiber-consuming bacteria, such as *Clostridium sp.* and *Bacteroides* species.⁴ Depending on the origin of their vendor, the small intestine of mice can also be colonized by segmented filamentous bacteria (SFB).⁵ The gut microbiota and microbiota-derived metabolites are essential for healthy host functions, influencing the immune system, the nervous system (the gut brain axis), the endocrine system and the metabolism.⁶

Despite its key role within the organism, the microbiota encompasses millions of proteins and putative antigens to which the host must be tolerant. Physical and chemical protective barriers, such as the mucus layer, production of antimicrobial peptides, the secretion of immunoglobulin A (IgA) and the epithelium *per se*, allow microbiota sequestration within the lumen and prevent damage associated with bacterial dissemination. When the gut barrier integrity is impaired, the immune system can respond to antigens of the microbiota, inducing chronic and noxious inflammation, as well as a disequilibrium in

bacterial species composition with direct consequences on the different key physiological systems influenced by the microbiota.⁶

The role of the intestinal microbiota in shaping the immune system was clearly demonstrated by the use of axenic mice. These animals, born and raised under germ-free conditions, display a strong decrease in the expression of most cytokines (except type 2 cytokines), antimicrobial peptides, IgA, CD4⁺ T cells and B cells.⁷ The immune system can also influence the equilibrium of the microbiota in favor of certain species. One well-described example of this reciprocal interaction is the IL-23/IL-22 network. In response to microbiota-triggered Toll-like receptor (TLR) activation, CX3CR1⁺ myeloid cells overexpress the pro-inflammatory cytokines IL-23 and IL-1 β ,⁸ which in turn induces IL-22 expression by CD4⁺ T cells⁹ and innate lymphoid cell 3 (ILC3).¹⁰ IL-22 contributes to the gut barrier integrity and can influence the composition of the microbiota by inducing the expression of antimicrobial peptides within intestinal epithelial cells (IECs),¹¹ as well as the restoration of mucus production after inflammation¹² or the maintenance of IEC fucosylation.¹³ Although the role of pro-inflammatory cytokines, including IL-23, IL-1 β , IL-17A and IL-6 has largely been described in shaping both the immune system and the intestinal microbiota,¹⁴ renewed investigations suggested that certain anti-inflammatory cytokines, in particular transforming growth factor-beta (TGF- β), a highly conserved cytokine, are also key modulators of the microbiota and the host immune cell cross talk. In lower life forms, including in the soil-inhabiting, bacterial-feeding nematode, *Caenorhabditis elegans*, and in insects such as *Drosophila melanogaster*, two TGF- β homologs, namely DBL-1 and Dpp, found in these organisms, respectively, increase in response to bacterial entry in the gut and largely contribute to the gut immune response by enhancing antimicrobial peptides production, thus

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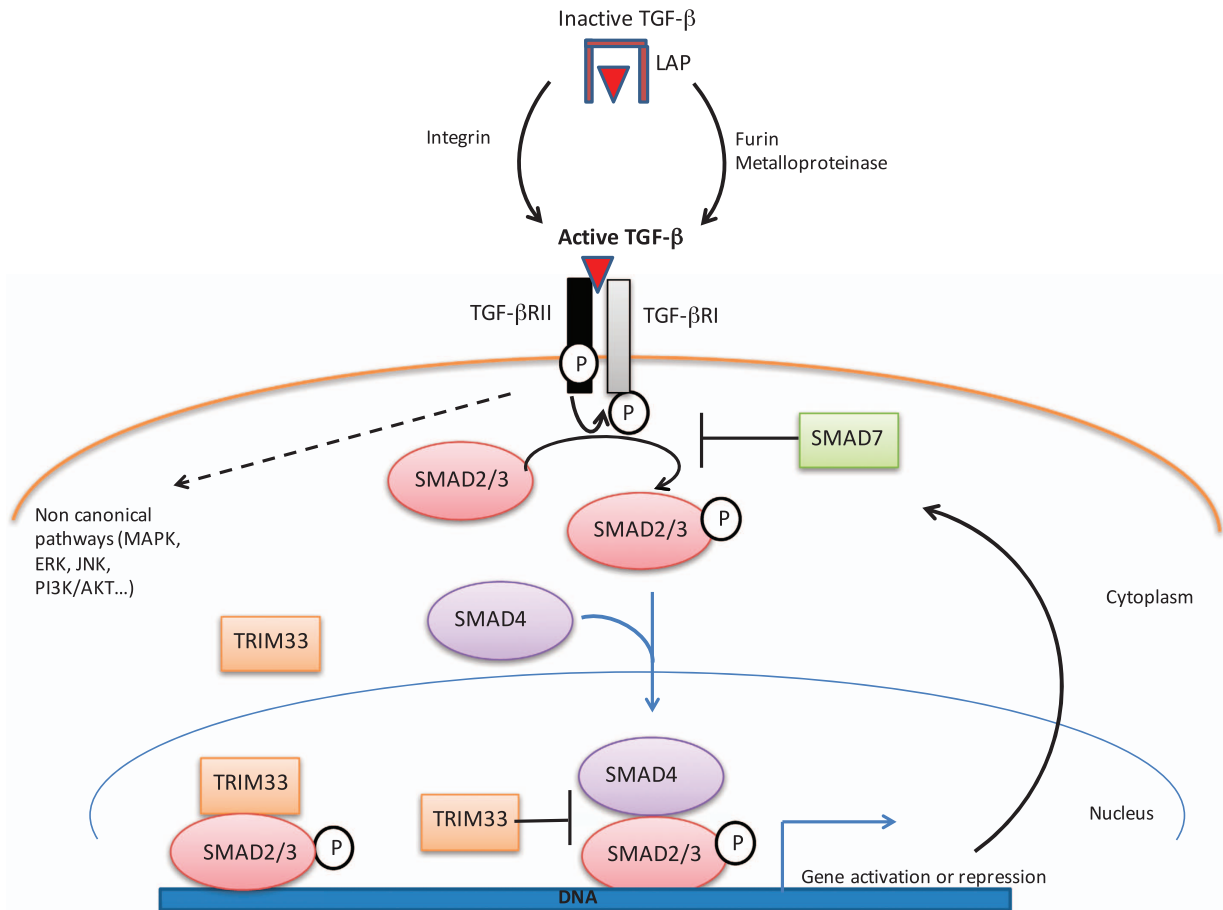


Figure 1 TGF- β , secreted under its inactive form due to its association with the Latent Associated Peptide (LAP), is activated by integrin- or metalloproteinase. Activated TGF- β can then bind to TGF β RII, and induce the auto-phosphorylation of TGF β RII, which in turn phosphorylates TGF β RI, leading to the activation of the canonical (SMAD2/3) and non-canonical (ERK, JNK, MAPK, etc.) pathways. Phosphorylated SMAD2/3 form complexes with SMAD4 and translocate into the nucleus to control gene expression, including *Smad7*, a negative regulator of the canonical pathway. Within the nucleus, SMAD2/3 complexes can be dissociated of SMAD4 by TRIM33, to bind this later and control the expression of similar or different genes.

controlling bacterial homeostasis.^{15–17} The role of TGF- β in the interplay between gut bacteria and the immune system is even more evident in mammals with a more complex gut flora and immune system, implying a primacy of this cytokine in the evolution of the relationship between the host organism and the microbiota.

In this review, we highlight recent findings and provide an insight into the cross talk between the TGF- β , the intestinal microbiota and the lymphoid cells of the lamina propria of the mammalian gut.

TGF- β SIGNALING PATHWAYS AND GUT IMMUNITY

TGF- β is a potent immunosuppressive cytokine involved in the development and functions of numerous immune cells, including T and B cells, but also dendritic cells (DCs) and natural killer (NK) cells.¹⁵ There are three isoforms of TGF- β in mammals: TGF- β 1, 2 and 3. TGF- β 2 and TGF- β 3 mainly have a role in muscle and bone development, whereas TGF- β 1 (TGF- β) expression predominates in immune cells. All three isoforms signal through a common serine–threonine kinase receptor complex composed of TGF- β RI and TGF- β RII subunits.¹⁶ Signaling is initiated by binding to TGF- β RII, which leads to its auto-phosphorylation, and in turn phosphorylates the TGF- β RI subunit (Figure 1). The latter then phosphorylates the receptor-associated R-SMADs, SMAD2 and/or SMAD3, which bind to SMAD4 (CoSMAD) and translocate into the

nucleus to regulate gene transcription. Among the TGF- β -activated genes, *smad7* encodes a protein that binds to the cytoplasmic domain of TGF- β RI and negatively regulates TGF- β signaling.¹⁷ Interestingly, SMAD2/3-dependent and SMAD4-independent signaling pathways have been described, including the SMAD2/3 interaction with TRIM33 (TIF-1 γ) and IKKs, which can act either in combination or in competition with SMAD4 during the differentiation steps of a given cell.^{18–20} Moreover, TGF- β RI can also activate other pathways, such as the MAPK/MEK, JNK/p38 and AKT/PI3K,²¹ which could phosphorylate the variable linker regions of SMAD2–3.²²

In the gut, IECs as well as selective immune cells, including DCs are an important source of bioactive TGF- β .²³ TGF- β is a polypeptide secreted as an inactive form bound to the latency-associated peptide, which masks its binding site to TGF- β RII (Figure 1). To be activated, the latent complex needs to undergo conformational changes or protein cleavage. Several molecules can mediate this activation, including furin, metalloproteinases and the integrins (Itg) α v β 6 and α v β 8.^{24,25} The TGF- β R complex is widely expressed by immune cells, as well as other cells. The importance of TGF- β signaling in gut immune cell homeostasis has been highlighted by studies that used either genetically altered TGF- β signaling pathways, the deletion of factors important for TGF- β activation or administration of anti-TGF- β . In mice, germ-line ablation of SMAD3 is associated with gut inflammation and abscesses, which are totally absent when animals

are crossed on a Rag-KO background.^{26,27} DC-specific deletion of Itg $\beta 8$ chain causes inflammatory bowel diseases (IBD) associated with unchecked T-lymphocyte activation.²⁸ Interestingly, under inflammatory conditions, CD4⁺ Foxp3⁺ regulatory T cells (Tregs) constitute a unique $\alpha\beta 8$ -expressing T-cell subset, and are thus capable of counter-regulating gut inflammation through the provision of bioactive TGF- β .²⁹ TGF- $\beta 1$ -deficient mice spontaneously develop multi-organ autoimmune lesions by the age of 3 weeks.³⁰ T lymphocytes are key effectors of this autoimmunity, as mice specifically lacking TGF- β R in T cells develop a similar inflammation to that observed in TGF- $\beta 1$ -deficient animals.^{31–33} It is worth noting that the intestine is not severely affected in these models, probably due to the early onset of the autoimmune disease. Indeed, an incomplete deprivation of TGF- β signaling in T cells, due to the expression of a dominant negative receptor of TGF- β R, is associated with a slower disease progression with intestinal inflammation.³⁴ In patients with Crohn's disease and ulcerative colitis, the two major forms of IBD known to be associated with an excessive immune response directed against components of commensal bacteria, an impairment of TGF- β signaling has largely been reported and seems likely associated with an increase in *smad7* expression in T cells.³⁵ In addition to T cells, gut pathologies were also associated with impairment of TGF- β signaling in B cells, DCs and NK cells. The SMAD3/4 pathway enhances IgA class switching and secretion of IgA within the lumen, which contribute to maintaining gut barrier integrity against microbiota,³⁶ and the deletion of TGF- β RII in CD11c⁺ cells, affecting both DCs and NK cells, leads to spontaneous colitis.³⁷

MICROBIOTA-INDUCED TGF- β

Compared with specific pathogen-free animals, the levels of TGF- β expression are largely impaired in the gut of germ-free mice.³⁸ IECs are most likely an important source of bioactive TGF- β when considering the huge number of cells they represent in the gut.²¹ Epithelial cell injury and gut inflammation have both been demonstrated to enhance TGF- β production by IECs,³⁹ and there is growing evidence to suggest that IEC-derived TGF- β is also regulated by the microbiota. Several *Clostridium* species were shown to produce short-chain fatty acids, such as butyrate, acetate and propionate, capable of exacerbating TGF- β production by colonic IECs.⁴⁰ Though the exact mechanisms by which *Clostridium*-derived short-chain fatty acids increase TGF- β in IECs remain to be unraveled, it is noteworthy that not only do the *Clostridium* species increase TGF- β secretion by IECs in the colon, but also the expression of metalloproteinases at the surface of IECs, providing a large source of bioactive TGF- β within the colon.⁴¹ Microbiota-derived products were also reported to influence the TGF- β production by lamina propria DCs, including ATP (adenosine 5'-triphosphate), which increases the expression of TGF- β in a CD70^{high} subset of DCs of the small intestine. Similarly to the effects of short-chain fatty acids on IECs, microbiota-derived ATP can both directly increase IEC TGF- β production and indirectly increase active TGF- β levels by promoting integrin $\alpha\beta 8$ expression at the surface of DCs.⁴² After dextran sodium sulfate treatment, *Clostridium butyricum* was recently reported to enhance TGF- β production by colonic lamina propria DCs mainly in a TLR2, API-ERK pathway-dependent manner.²³ Furthermore, microbiota-mediated TLR signaling in intestinal DCs also promotes the expression of the integrin $\alpha\beta 8$ essential for TGF- β activation.⁴² The long co-evolution of commensal bacteria and the mammalian gut has resulted in multiple mechanisms to increase TGF- β levels, which include microbiota-induced TGF- β production as well as enhancement of active and hence functional TGF- β levels. Although

direct and indirect mechanisms may be used by IECs and DCs in response to different commensal bacteria and bacteria-derived products, future investigations should address the specific contributions of each mechanism during homeostasis, healing, inflammation initiation and inflammation resolution more precisely.

TGF- β SIGNALING, MICROBIOTA AND TREGS

Foxp3-expressing CD4⁺ Tregs have an essential role in intestinal homeostasis. Tregs are characterized by constitutively high levels of the forkhead family transcription factor Foxp3, which is deemed to provide them with their regulatory activity.^{38,43} Although Tregs constitute approximately 10% of the CD4⁺ T cells in most organs, they are more abundant within the lamina propria, often exceeding values of 20% and 30% in the small intestine and in the colon, respectively. In the gut, Tregs can regulate the different branches of the mucosal immune response through several mechanisms. They inhibit T-cell responses to maintain immune tolerance to dietary and microbiota-derived antigens. Tregs express high levels of CTLA-4, ICOS, IL-10, IL-35 and to some extent TGF- β . However, *Foxp3-Cre; Tgfb1^{fl/fl}* mice, with Tregs unable to produce TGF- β , remain healthy without any signs of IBD, implying that production of TGF- β by Tregs is not essential for their regulatory functions in the gut.⁴⁴ However, a cellular source of bioactive TGF- β , such as DCs, is essential to sustain the high levels of Foxp3 in Tregs and their regulatory functions.²⁸

Tregs differentiate either in the thymus (tTreg) or in the periphery of naive T cells (pTreg). The tTreg cells and pTreg cells appear to have non-redundant immunoregulatory functions in the gut.⁴⁶ TGF- β has a key role in both thymic and peripheral Treg differentiation. The deprivation of TGF- β signaling in thymocytes was associated with a delay in the tTreg cell development potentially due to their apoptosis.^{33,47} Within the Foxp3 locus, conserved noncoding DNA sequences (CNS1-3) promote stability, size and composition of the Foxp3⁺ Treg populations.⁴⁸ In the gut, the binding of SMAD2/3/4 to the CNS1 induces Foxp3 expression in peripheral naive T cells.⁴⁹ CNS1-deficient mice display unaffected tTreg cell populations, but a defective induction of Foxp3 in the gut-associated lymphoid tissue.^{48,49} Unlike Foxp3-deficient mice, CNS1-deficient mice do not develop autoimmunity spontaneously, but display Th2-mediated intestinal inflammation.⁵⁰ The relative proportion of tTreg cells and pTreg cells in the intestine remains a matter of debate.^{51,52} Although dietary antigens induce the development of pTreg cells in the small intestine, independently of microbiota, commensal bacteria and their derived products have an instrumental role in the differentiation of pTregs within the colon.⁵³ *Clostridium butyricum* enhances TGF- β production by lamina propria DCs of colon, which was associated with pTreg differentiation.²³ Among bacterial products, butyrate, a *Clostridium*-derived short-chain fatty acid, not only induces TGF- β expression by IECs and potentially helps enforce pTreg-cell differentiation, but also acts directly on pTreg-cell differentiation by inhibiting histone deacetylase, resulting in histone 3 lysine 27 acetylation (H3K27ac) on CNS1 (and CNS2 at high concentrations of butyrate), opening the chromatin and leading to the transcription of *Foxp3*.^{54,55} It is noteworthy that in the colon of patients with ulcerative colitis, the levels of butyrate and the capacity of Treg cells to suppress effector T cells are reduced,⁵⁶ whereas the levels of TGF- β are largely reported to be increased.⁵⁷ This could either be due to the increased expression of *smad7* in T cells (including Tregs) from patients with IBD⁵⁷ or to different but complementary roles of TGF- β and microbiota-products in pTreg-cell differentiation. Retinoic acid (RA), a vitamin A-derived metabolite largely produced in the gut by

CD103⁺ DCs, and IECs not only contributes to mucosal homing but also has a role in tolerance and inflammation. RA cooperates with TGF- β to enhance pTreg-cell differentiation. CD103⁺ DCs exhibit an exacerbated ability to induce pTreg-cell differentiation *in vitro*, which is dependent on their RA production and TGF- β signaling in T cells.⁵⁸ On stimulation, in the presence of TGF- β , T cells were shown to upregulate the RA receptor alpha.⁵⁹ TGF- β -induced high expression levels of IL-6R at the surface of T cells, which constrains Treg differentiation, can be reversed by the addition of RA into the culture medium.⁵⁸ Of note, vitamin A-deficient mice were reported to have a comparable proportion and number of total intestinal Tregs⁶⁰ implying that tTregs can replenish the intestinal Treg compartment in the absence of RA-induced pTreg. Interestingly, the gut microbiota was recently reported to repress RA synthesis.⁶¹ Though this repressive role was observed in the context of colitis-associated cancer and the contribution of specific bacteria or bacterial-derived products repressing RA remains unclear, these recent works suggest that microbiota could finely tune the differentiation of colonic pTreg cells by either promoting TGF- β or repressing RA synthesis.

Within the gut, a large proportion of Tregs express the transcription factor ROR γ t.^{62–64} These ROR γ t⁺ Foxp3⁺ cells were shown to be very stable and to strongly express immunosuppressive molecules, such as CTLA-4, LAG-3, TIM3 and ICOS.⁶⁴ Mice lacking ROR γ t expression, specifically in Foxp3-expressing cells, do not develop spontaneous colitis but are more susceptible to chemically induced colitis and T-cell-driven colitis.^{62–64} The development of ROR γ t-expressing Tregs is also dependent on the wide-range of commensal bacterial species, including the butyrate-expressing *Clostridia* species, but also SFB and *B. thetaiotaomicron*, as well as on the levels of vitamin A, IL-6 and IL-23. Although not shown in these studies, we could hypothesize that TGF- β , described to induce the co-expression of Foxp3 and ROR γ t in CD4 T cells⁶⁵ is also one of the key cytokines involved in the development of ROR γ t-expressing Tregs.

In conclusion, bacteria and bacteria-derived products from the gut microbiota can influence TGF- β levels both under healthy and pathological conditions. This process modulates local Treg homeostasis, thus altering peripheral tolerance thresholds. Whether the imbalance in gut bacteria composition (dysbiosis), observed in patients with IBD, similarly affects TGF- β production and its corresponding alterations on the immune cell tolerance remains to be addressed.

TGF- β SIGNALING, MICROBIOTA AND TYPE 17 HELPER CELLS

The type 17 helper (Th17) cells constitute a subset of CD4⁺ T cells that express the transcription factor ROR γ t and produce cytokines, such as IL-17A/F, IL-22, often in association with other cytokines, including IL-10, GM-CSF and IFN γ , depending on local inflammatory conditions.⁶⁶ Th17 cells are commonly associated with the development of chronic inflammatory diseases, particularly autoimmunity and tumorigenesis.^{67,68} However, tissue-protective roles have also been attributed to Th17 cells in the gut. Neutralization of IL-17A with antibodies increases tissue damage in patients with Crohn's disease,⁶⁹ and IL-17A injection into mice maintains the tight junctions in the epithelium and therefore the intestinal barrier integrity.^{70,71}

Th17-cell differentiation from naive CD4⁺ T cells requires antigen stimulation and was largely reported to be influenced by TGF- β .^{18,72} Th17 cells generated with TGF- β and IL-6 produce IL-17A but fail to be pathogenic without further exposure to IL-23.⁷³ Moreover, Th17 cells can be generated in the absence of TGF- β signaling but in the presence of IL-23, IL-6 and IL-1 β .⁷⁴ These TGF- β -independently generated Th17 cells were described as strongly pathogenic,⁷⁴ reinforcing the importance of TGF- β in the control of pathogenic features of

Th17 cells. In CD4⁺ T cells, in the presence of IL-6, TGF- β signaling promotes the expression of both *Rorc* and *Rora* encoding for ROR γ t and ROR α , respectively, two transcriptional factors essential for Th17 differentiation.⁷³ Interestingly, the induction of *Rorc* and *Rora* by TGF- β is independent of SMAD4, and an opposite role for SMAD2 and SMAD3 in the regulation of ROR γ t transcriptional activity was described.^{75,76} Using mice with specific ablation of TGF- β in CD4⁺ T cells, it has been proposed that the autocrine production of TGF- β by CD4⁺ T cells is essential for Th17-cell differentiation.⁴⁴ However, a recent study revealed that effector CD4⁺ T cells are unable to convert TGF- β into its bioactive form²⁹ suggesting that some other cells, able to activate the TGF- β , are mandatory for Th17-cell differentiation. Thus, within the gut, it is likely that IECs and DCs, which express large amounts of TGF- β and can lead to its activation, contribute to Th17-cell differentiation.

Alongside its ability to enhance pTreg-cell differentiation, RA was observed to prevent Th17-cell differentiation.⁷⁷ RA was shown to inhibit IL-6R and IL-23R upregulation induced by TGF- β and IL-6, respectively.⁵⁸ However, RA was also proposed to work in concert with microbial-driven signals through TLR-5 on DCs of the lamina propria to enhance T-cell differentiation into Th17 cells.⁷⁸ These observations suggest that depending on the accessibility of microbial-derived products for immune cell actors and thus on the gut barrier integrity of the host, RA can differently influence Th17-cell differentiation.

Specific bacteria, such as ATP-producing bacteria, or IEC-adhering bacteria like SFB, were described to induce intestinal Th17 cells.^{79,80} Although most intestinal Th17 cells bear a TCR specific for SFB antigens,^{81,82} SFB antigens alone do not drive Th17 differentiation⁸² and TLR signaling is not required for Th17 cells, as MyD88-deficient mice display a normal number of intestinal Th17 cells compared with control littermates.^{42,79} The binding of SFB to IECs directly induces the expression of the serum amyloid A1 and 2 (SAA1/2) and reactive oxygen species, which in turn promote Th17-cell differentiation.^{80,83} Whether SFB promote the expression of TGF- β by IECs and/or DCs has so far not been addressed. However, a source of bioactive TGF- β is required to induce Th17-cell differentiation in response to the commensal bacteria.⁷⁹

Thus, within the gut, both microbiota and TGF- β signaling in CD4⁺ T cells promote the differentiation of Th17 cells. The contribution of these two key actors is largely influenced by the inflammatory conditions, which in turn influence the profile of cytokines secreted by Th17 cells and their functions.

TGF- β SIGNALING, MICROBIOTA AND ILCs

ILCs are the most recently described immune cells to be associated with the regulation of gut homeostasis. ILCs share common features with CD4⁺ T cells but lack T-cell lineage markers and have been categorized into at least three subsets ILC1, ILC2 and ILC3, based on differential transcription factor expression patterns.⁸⁴ ILC1 express the transcription factor t-Bet, the surface marker CD127, Nkp44 (or Nkp46 in mice) and produce cytokines, such as TNF- α and IFN- γ . In the human gut, ILC1s have a strong tropism for the epithelium where they produce IFN- γ in response to IL-12 and IL-15. This intraepithelial location of ILC1s seems to be dependent on TGF- β signaling. ILC1s express hallmarks of TGF- β imprinting, including CD103, CD9 and NEDD9.⁸⁵ A recent study, targeting *Tgfb2* in ILC1, showed that TGF- β , in a SMAD4-independent manner, is required for ILC1 development in the salivary gland.⁸⁶ So far, the role of TGF- β signaling on intestinal ILC1 development and function has not been tested and needs further investigations. ILC2s are identified as lineage

negative GATA-3⁺ ST2⁺ CRTH2⁺. In response to the IL-33, an IEC-produced cytokine, gut ILC2s produce amphiregulin (Areg), involved in tissue repair, and Areg-deficient mice are more susceptible to dextran sodium sulfate, a microbiota-dependent colitis model.⁸⁷ *In vitro*, TGF-β was shown to be a potent inducer of Areg in human lung adenocarcinoma.⁸⁸ Whether TGF-β, a microbiota-derived cytokines expressed by IECs, could be involved in Areg production by intestinal ILC2s remains to be elucidated. ILC3s are the main ILC subset in the gut. ILC3s express the transcription factor RORγt and secrete cytokines, such as IL-17A, and IL-22, in association with IFN-γ, GM-CSF or alone. ILC3s have been divided into three subsets: LTi (Lymphoid tissue inducer), Nkp46⁻ ILC3 and Nkp46⁺ ILC3. In the adult colon, the role of the microbiota on both the development and functions of ILC3s remains controversial.^{89,90} Through their production of IL-22, both Nkp46⁺ and Nkp46⁻ ILC3s have a redundant function with an important role in microbiota segregation, pathogen clearance, maintenance of the intestinal barrier integrity, mucosal healing.⁹¹ Interestingly, TGF-β signaling was recently proposed to control the balance between Nkp46⁺ and Nkp46⁻ ILC3;⁹² however, the source of TGF-β and the actors of the TGF-β signaling pathway involved remain unknown. Thus several recent studies have established a link between the ILC biology, TGF-β and microbiota. Regarding the role of ILCs in gut homeostasis, and the protective role of TGF-β in the gut, future investigations should address whether TGF-β induced by the microbiota could allow ILCs to regulate the intestinal barrier integrity and in turn protect against bacterial and parasitic entry in the deeper layers of the gut.

IGA⁺ B CELLS: A LINK BETWEEN TGF-β AND BACTERIAL SEQUESTRATION

Immunoglobulin A (IgA) can uniquely pass through the epithelium and additionally modulate luminal bacterial composition. In the gut, IgA secreted from B cells can bind to polymeric Ig receptors on the surface of IECs and subsequently translocate into the lumen. Several grams of IgA are thus secreted into the lumen daily. IgA can be categorized into two subsets: high-affinity IgAs, which neutralize pathogens, and low-affinity IgAs, which prevent bacterial adhesion

by aggregating them together.⁹³ It has been proposed that specific commensal bacteria have a crucial role in IgA production. SFB, but not *Escherichia coli*, are capable of inducing IgA secretion in Peyer's Patches and in isolated lymphoid follicles.⁹⁴ So far, the mechanisms linking SFB and IgA production remain unclear and require further investigations. TGF-β is a master regulator of IgA production among all of the other IgA-modulating immune cell factors and cytokines, with B-cell-specific deletion of TGFβR resulting in the loss of IgA-producing B cells.⁹⁵ TGF-β-induced IgA production is dependent on canonical pathways, as evidenced by the fact that SMAD2, SMAD3 and SMAD4 deficiency results in the decrease in IgA levels.^{36,96} Interestingly, TGF-β can directly and indirectly control IgA production. Indeed, in addition to its effect on B cells, TGF-β also regulates T-follicular-helper-cell development, a CD4⁺ T-cell subset involved in the control of isotype-switched antibody production.⁹⁷ *Alcaligenes* species, gut-associated lymphoid tissue-resident bacteria, as well as *Lactobacillus gasseri* SBT2055, a probiotic bacterium, were shown to induce TGF-β production by small intestinal DCs in a TLR2-dependent manner and in turn induce IgA production by B cells.⁹⁸ Thus by influencing TGF-β production in the gut, the microbiota regulates IgA secretion and thus the strength of the intestinal barrier.

CONCLUDING REMARKS

Largely overlooked until the last decade, the interplay between the microbiota and the mammalian host organism is now widely regarded as essential for host homeostasis and health. In this review, we have highlighted the complexity of this interplay within the gut and the central role of TGF-β in regulating these interactions. TGF-β levels in the gut are directly and indirectly modulated by the gut microbiota, which impacts the development and functions of immune cell subsets, which in turn regulates microbiota sequestration within the mammalian lumen (Table 1 and Figure 2).

Overall, the roles of TGF-β in shaping microbiota composition require further examination. This area of research should contribute to fully understanding the effects of TGF-β on microbiota homeostasis and the risks of utilizing either neutralizing TGF-β antibodies or TGF-β analogs in human patients.

Table 1 Relationship between microbiota, intestinal lymphocytes and TGF-β

Immune cells	Link with TGF-β	Relationship with microbiota
Th17	TGF-β induces nonpathogenic Th17 at low concentrations in addition to IL-6. TGF-β represses IL-22 expression within T cells via c-maf.	Th17 induce AMP expression in IECs. SFB induce Th17 in a SAA-dependent manner. Microbiota-induced IL-25 inhibits Th17.
Treg	TGF-β induces Foxp3 expression in naive T cells. Tregs activate TGF-β under inflammatory conditions.	Tregs are induced by the microbiota (e.g., <i>Clostridia</i> cluster IV, XIVa) and <i>Bacteroides fragilis</i> . Control microbiota-induced T-cell activation.
TFH	TGF-β prevents accumulation of TFH in the germinal center.	SFB induce TFH differentiation and drive migration to inflammatory sites. TFH through PD-1 regulates IgA production.
ILC1	TGFβ allows ILC1 localization within the gut epithelium.	ND
ILC3	TGF-β balances the Nkp46 ⁺ ILC3/Nkp46 ⁻ ILC3 equilibrium.	ILC3s induce AMP expression in IECs through the secretion of IL-22. ILC3s inhibit microbiota-induced CD4 ⁺ T-cell activation.
IgA ⁺ B cells	TGF-β controls IgA ⁺ production.	IgAs contribute to bacterial sequestration into the lumen.

Abbreviations: AMP, antimicrobial peptide; IECs, intestinal epithelial cell; IgA, immunoglobulin A; ILC, innate lymphoid cell; ND, not determined; SAA, serum amyloid A; SFB, segmented filamentous bacteria; TFH, T follicular helper cell; TGF, transforming growth factor; Th17, type 17 helper cell; Treg, regulatory T cell.

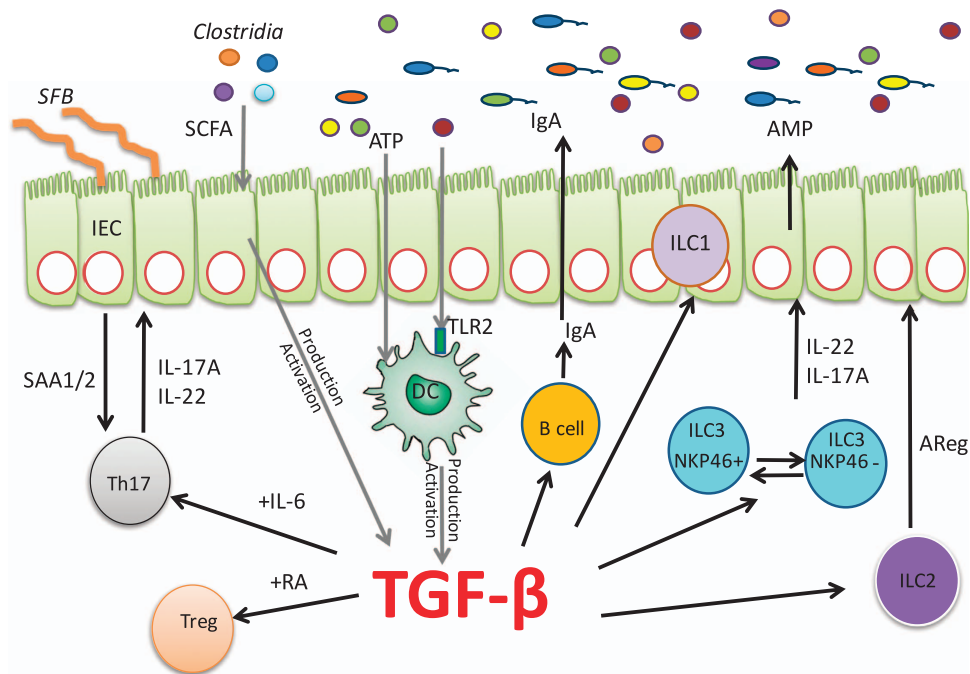


Figure 2 TGF- β linking the gut microbiota and immune cells. TGF- β is mainly expressed and activated by intestinal epithelial cells (IECs) upon short-chain fatty acid (SCFA) stimulation or by dendritic cells (DCs) after bacterial contact. TGF- β in combination with various cytokines or factors drives the development and/or function of lymphocytes, which reinforce the gut barrier.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- Ottman N, Smidt H, de Vos WM, Belzer C. The function of our microbiota: who is out there and what do they do? *Front Cell Infect Microbiol* 2012; **2**: 104.
- Pfeiffer JK, Virgin HW. Viral immunity. Transkingdom control of viral infection and immunity in the mammalian intestine. *Science* 2016; **351**: 6270.
- Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016; **14**: e1002533.
- Brown EM, Sadarangani M, Finlay BB. The role of the immune system in governing host-microbe interactions in the intestine. *Nat Immunol* 2013; **14**: 660–667.
- Ericsson AC, Hagan CE, Davis DJ, Franklin CL. Segmented filamentous bacteria: commensal microbes with potential effects on research. *Comp Med* 2014; **64**: 90–98.
- Schroeder BO, Backhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med* 2016; **22**: 1079–1089.
- Arrieta MC, Finlay BB. The commensal microbiota drives immune homeostasis. *Front Immunol* 2012; **3**: 33.
- Longman RS, Diehl GE, Victorio DA, Huh JR, Galan C, Miraldi ER *et al*. CX3CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. *J Exp Med* 2014; **211**: 1571–1583.
- Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T *et al*. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J Immunol* 2007; **179**: 8098–8104.
- Buonocore S, Ahern PP, Uhlig HH, Ivanov II, Littman DR, Maloy KJ *et al*. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* 2010; **464**: 1371–1375.
- Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q *et al*. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat Med* 2008; **14**: 282–289.
- Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK *et al*. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J Clin Invest* 2008; **118**: 534–544.
- Goto Y, Obata T, Kunisawa J, Sato S, Ivanov II, Lamichhane A *et al*. Innate lymphoid cells regulate intestinal epithelial cell glycosylation. *Science* 2014; **345**: 1254009.
- Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012; **336**: 1268–1273.
- Flavell RA, Sanjabi S, Wrzesinski SH, Licona-Limon P. The polarization of immune cells in the tumour environment by TGFbeta. *Nat Rev* 2010; **10**: 554–567.

- Letterio JJ, Roberts AB. Regulation of immune responses by TGF-beta. *Annu Rev Immunol* 1998; **16**: 137–161.
- Massague J. TGFbeta signalling in context. *Nat Rev* 2012; **13**: 616–630.
- Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M *et al*. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; **441**: 235–238.
- Descargues P, Sil AK, Sano Y, Korchymskyi O, Han G, Owens P *et al*. IKKalpha is a critical coregulator of a Smad4-independent TGFbeta-Smad2/3 signaling pathway that controls keratinocyte differentiation. *Proc Natl Acad Sci USA* 2008; **105**: 2487–2492.
- Doisne JM, Bartholin L, Yan KP, Garcia CN, Duarte N, Le Luëdec JB *et al*. iNKT cell development is orchestrated by different branches of TGF-beta signaling. *J Exp Med* 2009; **206**: 1365–1378.
- Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; **425**: 577–584.
- Massague J. Integration of Smad and MAPK pathways: a link and a linker revisited. *Genes Dev* 2003; **17**: 2993–2997.
- Kashiwagi I, Morita R, Schichita T, Komai K, Saeki K, Matsumoto M *et al*. Smad2 and Smad3 inversely regulate TGF-beta autoinduction in Clostridium butyricum-activated dendritic cells. *Immunity* 2015; **43**: 65–79.
- Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J *et al*. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 1999; **96**: 319–328.
- Worthington JJ, Fenton TM, Czajkowska BI, Klementowicz JE, Travis MA. Regulation of TGFbeta in the immune system: an emerging role for integrins and dendritic cells. *Immunobiology* 2012; **217**: 1259–1265.
- Yang X, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H *et al*. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *Embo J* 1999; **18**: 1280–1291.
- Munoz NM, Upton M, Rojas A, Washington MK, Lin L, Chytil A *et al*. Transforming growth factor beta receptor type II inactivation induces the malignant transformation of intestinal neoplasms initiated by Apc mutation. *Cancer Res* 2006; **66**: 9837–9844.
- Travis MA, Reizis B, Melton AC, Masteller E, Tang Q, Proctor JM *et al*. Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice. *Nature* 2007; **449**: 361–365.
- Worthington JJ, Kelly A, Smedley C, Bauche D, Campbell S, Marie JC *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**: 903–915.
- Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M *et al*. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992; **359**: 693–699.
- Marie JC, Liggitt D, Rudensky AY. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. *Immunity* 2006; **25**: 441–454.
- Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity* 2006; **25**: 455–471.

- 33 Liu Y, Zhang P, Li J, Kulkarni AB, Perruche S, Chen W. A critical function for TGF- β signaling in the development of natural CD4⁺CD25⁺Foxp3⁺ regulatory T cells. *Nat Immunol* 2008; **9**: 632–640.
- 34 Gorelik L, Constant S, Flavell RA. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. *J Exp Med* 2002; **195**: 1499–1505.
- 35 Zorzi F, Angelucci E, Sedda S, Pallone F, Monteleone G. Smad7 antisense oligonucleotide-based therapy for inflammatory bowel diseases. *Dig Liver Dis* 2013; **45**: 552–555.
- 36 Klein J, Ju W, Heyer J, Wittek B, Haneke T, Knaus P *et al*. B cell-specific deficiency for Smad2 *in vivo* leads to defects in TGF- β -directed IgA switching and changes in B cell fate. *J Immunol* 2006; **176**: 2389–2396.
- 37 Ihara S, Hirata Y, Serizawa T, Suzuki N, Sakitani K, Kinoshita H *et al*. TGF- β signaling in dendritic cells governs colonic homeostasis by controlling epithelial differentiation and the luminal microbiota. *J Immunol* 2016; **196**: 4603–4613.
- 38 Dignass AU, Podolsky DK. Cytokine modulation of intestinal epithelial cell restitution: central role of transforming growth factor beta. *Gastroenterology* 1993; **105**: 1323–1332.
- 39 Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H *et al*. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 2013; **500**: 232–236.
- 40 Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y *et al*. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 2011; **331**: 337–341.
- 41 Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M *et al*. ATP drives lamina propria T(H)17 cell differentiation. *Nature* 2008; **455**: 808–812.
- 42 Boucard-Jourdin M, Kugler D, Endale Ahanda ML, This S, De Calisto J, Zhang A *et al*. β 8 Integrin expression and activation of TGF- β by intestinal dendritic cells are determined by both tissue microenvironment and cell lineage. *J Immunol* 2016; **197**: 1968–1978.
- 43 Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol* 2007; **8**: 277–284.
- 44 Gutcher I, Donkor MK, Ma Q, Rudensky AY, Flavell RA, Li MO. Autocrine transforming growth factor- β 1 promotes *in vivo* Th17 cell differentiation. *Immunity* 2011; **34**: 396–408.
- 45 Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF- β 1 maintains suppressor function and Foxp3 expression in CD4⁺CD25⁺ regulatory T cells. *J Exp Med* 2005; **201**: 1061–1067.
- 46 Haribhai D, Williams JB, Jia S, Nickerson D, Schmitt EG, Edwards B *et al*. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. *Immunity* 2011; **35**: 109–122.
- 47 Ouyang W, Beckett O, Ma Q, Li MO. Transforming growth factor-beta signaling curbs thymic negative selection promoting regulatory T cell development. *Immunity* 2010; **32**: 642–653.
- 48 Zheng Y, Josefowicz S, Chaudhry A, Peng XP, Forbush K, Rudensky AY. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature* 2010; **463**: 808–812.
- 49 Schlenner SM, Weigmann B, Ruan Q, Chen Y, von Boehmer H. 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**: 1529–1535.
- 50 Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y *et al*. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* 2012; **482**: 395–399.
- 51 Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N *et al*. Peripheral education of the immune system by colonic commensal microbiota. *Nature* 2011; **478**: 250–254.
- 52 Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL *et al*. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. *Nature* 2013; **497**: 258–262.
- 53 Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG *et al*. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* 2016; **351**: 858–863.
- 54 Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D *et al*. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013; **504**: 446–450.
- 55 Arpaia N, Campbell C, Fan X, Dikiy S, van der Veen J, deRoos P *et al*. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 2013; **504**: 451–455.
- 56 Kumari R, Ahuja V, Paul J. Fluctuations in butyrate-producing bacteria in ulcerative colitis patients of North India. *World J Gastroenterol* 2013; **19**: 3404–3414.
- 57 Del Zotto B, Mumolo G, Pronio AM, Montesani C, Tersigni R, Boirivant M. TGF- β 1 production in inflammatory bowel disease: differing production patterns in Crohn's disease and ulcerative colitis. *Clin Exp Immunol* 2003; **134**: 120–126.
- 58 Xiao S, Jin H, Korn T, Liu SM, Oukka M, Lim B *et al*. Retinoic acid increases Foxp3⁺ regulatory T cells and inhibits development of Th17 cells by enhancing TGF- β -driven Smad3 signaling and inhibiting IL-6 and IL-23 receptor expression. *J Immunol* 2008; **181**: 2277–2284.
- 59 Schambach F, Schupp M, Lazar MA, Reiner SL. Activation of retinoic acid receptor- α favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation. *Eur J Immunol* 2007; **37**: 2396–2399.
- 60 Hall JA, Cannons JL, Grainger JR, Dos Santos LM, Hand TW, Naik S *et al*. Essential role for retinoic acid in the promotion of CD4⁺ T cell effector responses via retinoic acid receptor alpha. *Immunity* 2011; **34**: 435–447.
- 61 Bhattacharya N, Yuan R, Prestwood TR, Penny HL, DiMaio MA, Reticker-Flynn NE *et al*. Normalizing microbiota-induced retinoic acid deficiency stimulates protective CD8⁺ T cell-mediated immunity in colorectal cancer. *Immunity* 2016; **45**: 641–655.
- 62 Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y *et al*. MUCOSAL IMMUNOLOGY. The microbiota regulates type 2 immunity through ROR γ t⁺ T cells. *Science* 2015; **349**: 989–993.
- 63 Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM *et al*. MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct population of ROR γ t⁺ regulatory T cells. *Science* 2015; **349**: 993–997.
- 64 Yang BH, Hagemann S, Mamareli P, Lauer U, Hoffmann U, Beckstette M *et al*. Foxp3⁺ T cells expressing ROR γ t represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol* 2015; **9**: 444–457.
- 65 Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD *et al*. TGF- β -induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing ROR γ t function. *Nature* 2008; **453**: 236–240.
- 66 McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. *Immunity* 2008; **28**: 445–453.
- 67 Grivninkov SI, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D *et al*. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 2012; **491**: 254–258.
- 68 Troncone E, Marafini I, Pallone F, Monteleone G. Th17 cytokines in inflammatory bowel diseases: discerning the good from the bad. *Int Rev Immunol* 2013; **32**: 526–533.
- 69 Hueber W, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W, Higgins PD *et al*. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut* 2012; **61**: 1693–1700.
- 70 Lee JS, Tato CM, Joyce-Shaikh B, Gulen MF, Cayatte C, Chen Y *et al*. Interleukin-23-dependent IL-17 production regulates intestinal epithelial permeability. *Immunity* 2015; **43**: 727–738.
- 71 Maxwell JR, Zhang Y, Brown WA, Smith CL, Byrne FR, Fiorino M *et al*. Differential roles for interleukin-23 and interleukin-17 in intestinal immunoregulation. *Immunity* 2015; **43**: 739–750.
- 72 Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF β in the context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. *Immunity* 2006; **24**: 179–189.
- 73 Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A *et al*. Induction and molecular signature of pathogenic TH17 cells. *Nat Immunol* 2012; **13**: 991–999.
- 74 Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE *et al*. Generation of pathogenic T(H)17 cells in the absence of TGF- β signalling. *Nature* 2011; **467**: 967–971.
- 75 Martinez GJ, Zhang Z, Chung Y, Reynolds JM, Lin X, Jetten AM *et al*. Smad3 differentially regulates the induction of regulatory and inflammatory T cell differentiation. *J Biol Chem* 2009; **284**: 35283–35286.
- 76 Martinez GJ, Zhang Z, Reynolds JM, Tanaka S, Chung Y, Liu T *et al*. Smad2 positively regulates the generation of Th17 cells. *J Biol Chem* 2010; **285**: 29039–29043.
- 77 Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M *et al*. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007; **317**: 256–260.
- 78 Uematsu S, Fujimoto K, Jang MH, Yang BG, Jung YJ, Nishiyama M *et al*. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol* 2008; **9**: 769–776.
- 79 Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB *et al*. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008; **4**: 337–349.
- 80 Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S *et al*. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. *Cell* 2015; **163**: 367–380.
- 81 Goto Y, Panea C, Nakato G, Cebula A, Lee C, Diez MG *et al*. Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. *Immunity* 2014; **40**: 594–607.
- 82 Yang Y, Torchinsky MB, Gobert M, Xiong H, Xu M, Linehan JL *et al*. Focused specificity of intestinal TH17 cells towards commensal bacterial antigens. *Nature* 2014; **510**: 152–156.
- 83 Sano T, Huang W, Hall JA, Yang Y, Chen A, Gavzy SJ *et al*. An IL-23R/IL-22 circuit regulates epithelial serum amyloid A to promote local effector Th17 responses. *Cell* 2015; **163**: 381–393.
- 84 Robinette ML, Fuchs A, Cortez VS, Lee JS, Wang Y, Durum SK *et al*. Transcriptional programs define molecular characteristics of innate lymphoid cell classes and subsets. *Nat Immunol* 2015; **16**: 306–317.
- 85 Fuchs A, Vermi W, Lee JS, Lonardi S, Gilfillan S, Newberry RD *et al*. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN- γ -producing cells. *Immunity* 2013; **38**: 769–781.
- 86 Cortez VS, Cervantes-Barragan L, Robinette ML, Bando JK, Wang Y, Geiger TL *et al*. Transforming growth factor-beta signaling guides the differentiation of innate lymphoid cells in salivary glands. *Immunity* 2016; **44**: 1127–1139.
- 87 Monticelli LA, Osborne LC, Noti M, Tran SV, Zaiss DM, Artis D. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc Natl Acad Sci USA* 2015; **112**: 10762–10767.
- 88 Bennett KL, Plovman GD, Buckley SD, Skonier J, Purchio AF. Regulation of amphiregulin mRNA by TGF- β in the human lung adenocarcinoma cell line A549. *Growth Factors* 1992; **7**: 207–213.

- 89 Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C *et al.* ROR γ t and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46⁺ cells. *Nat Immunol* 2009; **10**: 83–91.
- 90 Reynders A, Yessaad N, Vu Manh TP, Dalod M, Fenis A, Aubry C *et al.* Identity, regulation and *in vivo* function of gut NKp46⁺ROR γ t⁺ and NKp46⁺ROR γ t⁻ lymphoid cells. *EMBO J* 2011; **30**: 2934–2947.
- 91 Song C, Lee JS, Gilfillan S, Robinette ML, Newberry RD, Stappenbeck TS *et al.* Unique and redundant functions of NKp46⁺ ILC3s in models of intestinal inflammation. *J Exp Med* 2015; **212**: 1869–1882.
- 92 Viant C, Rankin LC, Girard-Madoux MJ, Seillet C, Shi W, Smyth MJ *et al.* Transforming growth factor-beta and Notch ligands act as opposing environmental cues in regulating the plasticity of type 3 innate lymphoid cells. *Sci Signal* 2016; **9**: ra46.
- 93 Mantis NJ, Rol N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. *Mucosal Immunol* 2011; **4**: 603–611.
- 94 Lecuyer E, Rakotobe S, Lengline-Garnier H, Lebreton C, Picard M, Juste C *et al.* Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. *Immunity* 2014; **40**: 608–620.
- 95 Cazac BB, Roes J. TGF-beta receptor controls B cell responsiveness and induction of IgA *in vivo*. *Immunity* 2000; **13**: 443–451.
- 96 Park SR, Lee JH, Kim PH. Smad3 and Smad4 mediate transforming growth factor-beta1-induced IgA expression in murine B lymphocytes. *Eur J Immunol* 2001; **31**: 1706–1715.
- 97 McCarron MJ, Marie JC. TGF-beta prevents T follicular helper cell accumulation and B cell autoreactivity. *J Clin Invest* 2015; **124**: 4375–4386.
- 98 Obata T, Goto Y, Kunisawa J, Sato S, Sakamoto M, Setoyama H *et al.* Indigenous opportunistic bacteria inhabit mammalian gut-associated lymphoid tissues and share a mucosal antibody-mediated symbiosis. *Proc Natl Acad Sci USA* 2010; **107**: 7419–7424.



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