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Balancing acts: the role of TGF- β in the mucosal immune system

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Abstract

The gastrointestinal mucosal immune system faces unique challenges in dealing not only with fed antigens but also both commensal and pathogenic bacteria. It is tasked with digesting, transporting and using nutritional antigens whilst protecting the host from pathogenic organisms. As such, mechanisms that mediate effective immunity and immune tolerance are active within the gut environment. To accomplish this, the mucosal immune system has evolved sophisticated mechanisms that safeguard the integrity of the mucosal barrier. Transforming growth factor- β (TGF- β) emerges as a key mediator, balancing the tolerogenic and immunogenic forces at play in the gut. In this review we discuss the role of TGF- β in the generation and functioning of gut lymphocyte populations. We highlight recent findings, summarize controversies, outline remaining questions and provide our personal perspectives.

TGF- β and gut immunity

The gut simultaneously encounters both harmless and harmful antigens on a constant basis. Thus, the mucosal immune system tackles challenges not faced at other sites in the body. Differentiated T cell populations have been identified within the healthy gut, and these, coupled with a degree of tonic inflammatory signaling, play a vital role in promoting barrier function, protecting from invading pathogenic organisms, as well as preventing unwanted autoimmunity and overt inflammation. In the steady-state, the gut is neither regulatory nor inflammatory but operates a balance of these two processes that play out to perform the vital function of maintaining gut barrier integrity. TGF- β plays an important role in mediating balanced responses within the gut mucosa, indeed, all gut responses occur in the presence of TGF- β . The gut is a TGF- β -rich environment in which most cell types can both produce and respond to this cytokine. Responses to TGF- β are pleiotropic, cell type and context dependent, yet canonical TGF- β -signaling pathways have been identified (Box 1).

An important role for TGF- β in the gut mucosa has been highlighted in a number of transgenic animals in which TGF- β -signaling is limited. Inhibiting canonical TGF- β -signaling through deletion of Smad3 or Smad4 or by increased expression of inhibitory Smad7 promotes gut inflammation (1-3). Additionally, the deletion of factors important in mediating the cleavage of latent TGF- β to the active form has been shown to lead to the development colitis (inflammation of the colon) (4, 5). In this review, we examine the role of TGF- β in maintaining gut immune homeostasis. The gut is a complex immune frontier in which all immune cells, some specific to the mucosal environment, play a role. Thus, although TGF- β mediates effects on a vast array of mucosal immune and indeed non-

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immune cell types, this review will specifically focus on the effects of TGF- β on lymphocyte populations.

CD4⁺Foxp3⁺ regulatory T cells

TGF- β and regulatory T cell generation—Regulatory T cells (Tregs) (Box 2), are vital mediators of intestinal homeostasis; in their absence gut pathology results. Indeed, the gut is a preferential site for the induction of Foxp3 in TCR $\alpha\beta$ ⁺CD4⁺ T cells (CD4⁺ T cells) (Table 1) (6, 7). It has been known for some time that induction of tolerance via the oral route leads to the generation of T cell populations with suppressive capacities (8), and this has been utilized in many animal models of autoimmune disease to alleviate symptoms. Importantly, suppression was shown to be TGF- β -dependent in a number of settings, as administration of anti-TGF- β could inhibit aspects of oral tolerance (9) (see Glossary). Increases in TGF- β were seen in the gut following oral feeding and T cells producing TGF- β (Th3) have been isolated from the gut associated lymphoid tissue (GALT) of orally tolerated mice (10). More recently, increased frequencies of Tregs have been found in the GALT compared with peripheral tissues (6). What is it about the gut that makes it an environment uniquely tailored for Treg generation and *foxp3*-induction, and why would it have developed to be so? *De novo* induction of Foxp3 in the gut is a sensible way to increase regulation in this dynamic environment. Additionally, it is a way to generate Tregs that are responsive to non-thymically derived antigens. Although estimates vary, and it is impossible to distinguish thymic-derived Tregs from those which are peripherally derived, conversion in the gut may represent a significant pathway of Treg generation. Perhaps the most important reason why the gut is an environment favoring Treg development is that it is TGF- β rich, and TGF- β is essential for induction of Foxp3 in naïve CD4⁺ T cells (11). The gut is also rich in a co-factor that synergizes with TGF- β to promote Foxp3 expression, the vitamin A metabolite retinoic acid (RA) (6, 7, 12). A tendency toward *de novo* Foxp3-induction is also favored by functionally specialized lamina propria (Lp) CD103⁺ dendritic cells (DC) (here called CD103⁺ DC), that induce Foxp3 in naïve T cells in a TGF- β - and RA-dependent manner. Why are these DC so good at inducing Foxp3? It is clear that CD103⁺ DC express enzymes which mediate the metabolism of vitamin A to RA (7), and it is possible that they express higher levels of these enzymes than other antigen presenting cells (APC). CD103⁺ DC have increased levels of TGF- β 2 mRNA (7), but as TGF- β requires cleavage from a latency associated protein (LAP) to be activated, it is possible that these DC are better at cleaving TGF- β -LAP. More recently it has been shown that in addition to supporting Treg induction, the gut also functions as a site of local Treg expansion, supported by gut dwelling CX3CR1⁺ macrophages (13).

Such is the integral role for TGF- β in promoting Treg-generation within the gut that some pathogens have developed mechanisms to manipulate the TGF- β pathway. Excretory-secretory (ES) antigens from the intestinal helminth *Heligmosomoides polygyrus* have been shown to induce Foxp3 in naïve T cells through activation of TGF- β -signaling (14). This is the first demonstration that parasites themselves can amplify regulatory mechanism in the gut to promote their persistence through the targeted manipulation of TGF- β -signaling. Commensal bacteria also manipulate TGF- β -mediated induction of Foxp3; *Clostridium* spp. has been reported to promote gut Treg differentiation by enhancing TGF- β -signaling (15). As such, induction of Foxp3 provides a mechanism by which these bacteria suppress immune responses and promote regulation in a TGF- β -dependent manner.

Whether hijacked by parasitic and commensal organisms or not, it is clear that through high levels of TGF- β and RA the gut has hit upon a strategy to promote regulation within this dynamic environment. Such strategic insight however must be flexible and continue to function in the face of overt inflammation. Indeed, induction of Foxp3 in naïve CD4⁺ T cells

in the gut has been shown to still occur upon worm infection (14), thus maintaining regulation despite gut inflammation.

TGF- β as a mechanism of suppression—Insensitivity to TGF- β -signaling has been associated with dysregulated immune responses and a poorly controlled gut environment. Smad7 over-expression has been shown in the mucosa and mucosal T cells of patients with inflammatory bowel disease (IBD) (16). Crohns disease (CD) patients also exhibit TGF- β hypo-responsiveness, indicated by observations that inhibition of Smad7 restored TGF- β -signaling and inhibited inflammatory cytokine production from Lp mononuclear cells. Similarly, Tregs could not suppress colitogenic T cells from CD patients, however, knockdown of Smad7 in the responder population allowed Tregs to exert their suppressive function (2).

A frequently employed model of colitis involves the transfer of effector T cell populations into T cell-deficient mice; this colitis is prevented by co-transfer of Tregs. However, blockade of TGF- β overcomes Treg-mediated colitis suppression (17). Additionally, if effector T cell populations could not respond to TGF- β they escaped Treg control and colitis resulted (18). Whether Tregs themselves are the important source of TGF- β remains a contentious issue. A number of groups have transferred *TGF- β 1^{-/-}* Tregs in this model of colitis and noted conflicting results. *TGF- β 1^{-/-}* Tregs have been shown to be both incapable (19, 20) and capable of suppression (18, 21) in this disease model. Interestingly, although one report showing *TGF- β 1^{-/-}* Tregs could suppress colitis, Fahlen *et al* also showed that the *TGF- β 1^{-/-}* Tregs were no longer able to do this when anti-TGF- β was co-administered (18). Thus, although the role of Treg-derived TGF- β in colitogenic pathways remains to be determined, it is clear that impaired responsiveness to TGF- β promotes inflammatory processes in the gut.

TGF- β and Th17 cells

Th17 differentiation—The pluripotency of TGF- β allows it to support the generation of a number of cell populations important in maintaining gut integrity. In addition to induction of Tregs, TGF- β promotes the differentiation of naïve T cells to the T helper-17 (Th17) phenotype (22). TGF- β achieves this indirectly by suppressing naïve T cell differentiation into either Th1 or Th2 cells. Importantly however, TGF- β , in the presence of inflammatory cytokines such as IL-6, directly promotes differentiation of Th17 cells. Activation of naïve CD4⁺ T cells in the presence of TGF- β (with or without IL-6) leads to the up-regulation of both Foxp3 and the Th17-defining transcription factor ROR γ t. Expression of either Foxp3 or ROR γ t is then extinguished (23). How TGF- β mediates the preference of one transcription factor and cell fate over the other is an intriguing question, and many factors have been shown to influence the resultant cell fate. The outcome is sensitive to the concentration of TGF- β (23), with high doses favoring acquisition of Treg fate and low doses favoring Th17 differentiation. The presence of other cytokines affects cell fate, with the STAT3 activating cytokines IL-21 and IL-23 amplifying ROR γ t induction and Th17 differentiation (24) and IL-2 (a STAT5 activating cytokine) inhibiting Th17 cell fate (25). Competition has been demonstrated between STAT3 and STAT5 at the IL-17 locus, providing a molecular basis for these cytokine mediated effects (26). Also at the molecular level E2A, a member of the helix-loop-helix protein family, plays a role in TGF- β -mediated *Rorc* gene transcription (27). What is interesting is that E2A is also vital for proper induction of Foxp3 expression.

The Treg-Th17 balance—Foxp3⁺ Tregs and Th17 cells are both enriched in the gut, and the balance between these two populations is tightly controlled and critical for maintaining barrier integrity. The outcome of the Treg-Th17 balance is complex and influenced by many factors (some are discussed above). Recent data suggests that the presence of activated

Tregs can promote Th17 differentiation through uptake of IL-2 (28, 29). In the oral mucosa during *Candida albicans* infection, the presence of Tregs was shown to promote Th17 responses and fungal clearance (29). Although not examined in the gut, it would be interesting to determine if this mechanism functions at this mucosal site and whether Tregs contribute to promoting the gut barrier function mediated by Th17 cells (Box 3).

The presence of microbiota and food metabolites can also affect the Th17-Treg balance. The microbiome is important in Th17 generation in the gut as Th17 cells are dramatically reduced in germ free (GF) mice (30). Yet not all bacteria are equal in their ability to drive Th17 differentiation in the gut. Segmented filamentous bacteria (SFB) appear to be fully sufficient to reconstitute Th17 cells following colonization (30). But what is the point of having gut Th17 cells? SFB colonization confers protection from *Citrobacter rodentium* infection (30). So, as can be imagined, the presence of mucosal Th17 cells leads to better protection from pathogenic organisms. Such promotion of Th17 cells is not unique to SFB, general ATP-producing bacteria can promote Th17 cell accumulation in the Lp (31). Yet, there could be downsides to this protection, the presence of Th17 cells in the gut could impact diseases mediated by the peripheral immune system. Indeed, it has been demonstrated that the presence of SFB-induced Th17 cells exacerbates disease in a mouse model of arthritis. Mice housed in GF conditions have attenuated arthritis due to reduced Th17 cells, and add back of SFB was seen to provoke arthritis in the GF mice (32). In contrast to the Th17-promoting SFB, the commensal Gram-positive bacteria *Clostridium* spp. has been reported to promote Treg-generation in the gut mucosa (15). Increased levels of TGF- β and enzymes that cleave LAP-TGF β , were seen in the colon of *Clostridium*-colonized mice. Thus, it appears that constituents of the commensal microbiota can alter the make-up of the mucosal T cell compartment.

RA has been proposed to be a key factor in the TGF- β -mediated Treg-Th17 balance as, in addition to its ability to promote Treg induction, it has also been reported to inhibit Th17 differentiation (12). Collectively, these data suggest a simple model of the Treg-Th17 balance, mediated under a blanket of TGF- β in the steady-state gut: CD103⁺ DC promote Treg induction through the secretion of RA, and CD103⁻ DC (33, 34), in the presence of the proinflammatory cytokines, promote Th17 differentiation. But we are forgetting for a moment the layers of tweaking this model experiences by commensal bacteria and their products, as well as a network of diverse APC populations, other Tregs and Th17 cells, and even TGF- β itself.

We must therefore concede that mediating the balance between effector and regulatory responses in the gut can never be simple, but rather over-simplified. Take for example recent data showing that RA is not just an anti-inflammatory factor good for promoting Treg induction, but is also needed for the development of inflammatory T cell populations and robust T cell responses (35). In addition, although TGF- β is an established component of Th17 differentiation, Th17 differentiation has been shown to occur in its absence (36). Without TGF- β , a combination of IL-6, IL-1 β and IL-23 can mediate the differentiation of an IL-17-producing Th cell that is more pathogenic than conventional TGF- β -derived Th17 cells. Whether such cells would differentiate in the TGF- β -rich environment of the gut is unknown, but they would certainly mediate important functions at other peripheral sites. In addition, Foxp3⁺ IL-17-producing cells have been observed in the guts of CD patients (37), implying a degree of plasticity to T cell phenotypes.

IgA production and the Treg-IgA axis

An important mechanism at play in the mucosa to promote immunity without causing inflammation is the production of IgA. While many cytokine signals have been shown to be involved in the generation of IgA, the major cytokine signal is TGF- β -mediated (38). IgA

binds polymeric Ig receptors (pIgR) on the basolateral surface of intestinal epithelial cells and is then translocated across the epithelial cells to the gut lumen. IgA mediates protection at the mucosal barrier by two mechanisms: high affinity antibodies neutralize toxins and pathogens and low affinity antibodies inhibit the adhesion of commensal bacteria to epithelial cells. Collectively, these mechanisms aid in keeping bacteria in the gut lumen without inducing inflammation that would invariably damage the mucosal barrier.

IgA production is induced by a combination of TGF- β -signaling and CD40 ligation, or binding of BAFF or APRIL (both structurally related to CD40). The importance of TGF- β in IgA production is demonstrated in mice in which TGF- β -signaling is specifically extinguished in B cells. This resulted in the complete absence of serum IgA in both naïve and immunized mice, and a lack of IgA⁺ cells in the spleen and Payer's Patches (PP) (39). Later studies further demonstrated that no IgA was found in the gut following nasal or oral immunization of these mice (40). *Smad7*^{-/-} mice exhibit increased isotype switching to IgA (41). Conversely, deletion of *Smad2* specifically in B cells led to a reduction in IgA⁺ B cells, IgA secreting cells in the PP and decreased IgA responses following immunization (42).

The presence of other immune cells can affect the development of IgA⁺ B cells. Certain APC populations have been shown to exhibit enhanced capabilities at driving IgA production (43, 44). Again, these APC mediate IgA production through the combined effects of TGF- β and RA. Depletion of Tregs from the gut has been shown to coincide with the loss of post-class switch recombination IgA⁺ mucosal B cells and a decrease in intestinal IgA (45). *In vitro* culture of Tregs with IgD⁺ B cells led to increased levels of IgA, which was inhibited by anti-TGF- β . Intriguingly, Tsuji *et al* have shown that Foxp3⁺ cells can differentiate into T follicular helper cells (T_{FH}) in PP and then support IgA production (46). In further differentiating into T_{FH}, Tregs promote germinal center formation in PP and the differentiation of IgA producing cells. Continuing these studies to further define the Treg-IgA axis is important, as is determining whether Th17 cells can influence IgA production, which is particularly relevant given recent reports of Th17-derived TGF- β (47).

TGF- β retains lymphocytes in the gut

Following activation in lymph nodes, T cells traffic to tissues associated with the lymph node in which they were primed. For the gut, this means that cells activated in the GALT up-regulate the gut homing molecules $\alpha_4\beta_7$ and CCR9 and these direct gut tropism. Imprinting of $\alpha_4\beta_7$ and CCR9 on both T and B cells is achieved by GALT APC that induce expression of homing molecules in an RA-dependent manner (44, 48). Again, we see that a specific subset of DC are associated with this function; CD103⁺ DC are particularly capable of imprinting gut homing receptors (49).

Once in the gut, expression of $\alpha_E\beta_7$ is important for the retention of lymphocytes in the gut environment. Importantly, expression of α_E (also called CD103) on T cells is dependent on TGF- β -signaling (50). CD8⁺ T cells which accumulate in the gut in a mouse model of graft-verses-host disease (GVHD), fail to up-regulate CD103 upon gut entry when they express a dominant negative TGF- β receptor (T β R) (51). Subsequently, TGF- β -signaling defective T cells were retained in the gut less efficiently. This provides yet another example of how TGF- β , together with RA, is able to regulate the lymphocyte compartment of the gut, ensuring that lymphocytes traffic to, and are retained in, the gut. Further dissection of the APC network and the effects of TGF- β on these cell types is now needed. Indeed, given that TGF- β drives CD103 expression on T cells it would be interesting to determine whether it also influences the development of CD103⁺ DC.

TGF- β dependent generation of TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ intra-epithelial lymphocytes

Nestled between epithelial cells of the gut wall are intestinal intra-epithelial lymphocytes (IEL). Due to their location, IEL contribute to barrier function and the integrity of epithelial cells. As well as producing cytokines, total IEL have been shown to express junctional molecules important in preserving the epithelial barrier during infection (52). IEL also form the first line of defense against invading pathogens, and it is suggested that this function accounts for the “activated but resting” phenotype of most IEL. Yet IEL are a phenotypically diverse population of cells and although some must function to defend the mucosa, others could function to regulate ongoing immune responses. IEL can be characterized into two groups. Conventional IEL are made up of TCR $\alpha\beta^+$ cells expressing either CD4 or CD8 $\alpha\beta$ heterodimers, these are conventional T cells primed systemically and trafficked to the gut. Unconventional IEL are defined by the expression of CD8 $\alpha\alpha$ homodimers, bear either TCR $\gamma\delta^+$ or TCR $\alpha\beta^+$, and derive from separate developmental pathways to conventional T cells (for review see (53)). Unconventional TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL are an IEL population tentatively suggested to have a regulatory role. Micro-array analysis has shown that these cells express high levels of CTLA-4, PD-1, TGF- β , Lag-3, and inhibitory natural killer receptors, molecules associated with a regulatory phenotype (54). TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL could also suppress colitis in the T cell transfer model of disease (55) and, although they showed signs of activation upon antigen encounter, unlike conventional TCR $\alpha\beta^+$ CD8 $\alpha\beta^+$ IEL they did not become cytotoxic or secrete inflammatory cytokines (56).

TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL are suggested to develop in the thymus and, similar to Tregs, require a thymic agonist-selection process whereby they must encounter a high-affinity ligand to drive their differentiation (53). Similar to the developmental pathway of Tregs, it has been demonstrated that TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL development is TGF- β -dependent (57). TGF- β 1 $^{-/-}$ mice and mice with a T cell specific deletion of T β RI both lacked TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL. This was due to a number of factors. Firstly, TGF- β -signaling prevented the death of TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL precursors in the thymus. Secondly, TGF- β induced the expression of CD8 α on TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL thymic precursors. Strikingly, regulation of CD8 α expression by TGF- β was a more general phenomenon and was not limited to IEL generation, as TGF- β treatment of lineage-committed peripheral CD4 $^+$ T cells could induce CD8 α expression on these cells. TGF- β -induced CD8 α expression on peripheral CD4 $^+$ T cells involved altered expression of two transcription factors important in lineage commitment, Runx3 and Thpok. Interactions between the TGF- β -signaling pathway and Runx proteins have been previously described in IgA CSR, Foxp3-induction and CD103 expression (58-60), subjects already discussed in this review. Further examination of the effects of TGF- β on Thpok expression now needs to occur.

Induction of CD8 α on CD4 $^+$ T cells raises the interesting possibility that TCR $\alpha\beta^+$ CD4 $^+$ CD8 α^+ lymphocytes in the gut arise from conventional CD4 $^+$ T cells. Transfer of CD4 $^+$ T cells into T cell-deficient recipients has been shown to give rise to CD4 $^+$ CD8 α^+ T cells specifically in the gut (61). Examination of the TCR usage of IEL populations has shown CD4 $^+$ and CD4 $^+$ CD8 $^+$ cells have an overlapping repertoire, with the CD4 $^+$ CD8 $^+$ repertoire being more restricted, suggesting the two populations are developmentally related (62), and that TCR $\alpha\beta^+$ CD4 $^+$ CD8 α^+ cells in the gut develop from CD4 $^+$ T cells in a TGF- β dependent manner.

We anticipate that these observations will function as a starting point to examine the effects of TGF- β on T cell biology in the gut. What is the function of TCR $\alpha\beta^+$ CD4 $^+$ CD8 α^+ cells? If TCR $\alpha\beta^+$ CD8 $\alpha\alpha^+$ IEL hold a regulatory role, do TCR $\alpha\beta^+$ CD4 $^+$ CD8 α^+ cells? And is the expression of CD8 α on CD4 $^+$ T cells exclusively responsible for this? Indeed, all these questions can be boiled down into one which desperately needs addressing: what is function of CD8 α expression on CD4 $^+$ T cells? Posing this question is even more important in light

of data detailing the presence of $\text{TCR}\alpha\beta^+\text{CD4}^+\text{CD8}\alpha^+$ cells in mice, rats, pigs, primates and humans in the periphery, in the gut, and under both normal and pathological conditions. Determining whether all these arise in a TGF- β -dependent manner is important. What role $\text{TCR}\alpha\beta^+\text{CD4}^+\text{CD8}\alpha^+$ cells play in gut immunity and/or homeostasis also waits to be explored.

CONCLUDING REMARKS

In this review, we have discussed the most profound effects of TGF- β on lymphocytes in the dynamic immune environment of the gut. What is yet to be considered is how the TGF- β -mediated balance of Tregs and Th17 cells, Tregs and IgA production, lymphocyte gut trophism and IEL generation affect the systemic immune system. It is well established that the mucosal immune system of the gut can affect systemic immune functioning (for example in oral tolerance), so it is possible that TGF- β checks and balances set in place in the gut could affect the immune system at large. It may be easiest to examine those balances affected by commensal bacteria, and a number of studies have shown altered disease status following changes to the gut microbiome in mouse models of arthritis, diabetes and multiple sclerosis (32, 63, 64).

Clearly, TGF- β promotes and regulates the development of a variety of different cell types in the gut. In this review, we have been primarily concerned with lymphocytes, but what of the APC network? For instance, it would of interest to examine whether TGF- β can affect the development of either CD103^+ DC or, considering the TGF- β -mediated induction of CD8 α on CD4^+ T cells, any APC population bearing CD8 α molecules. Exciting areas to explore involve the role of TGF- β in gut APC populations and TGF- β regulation of interactions between immune cells and non-immune cells of the gut.

Ultimately, the immediate outcome of functional TGF- β -signaling in the mucosa is that gut integrity is maintained. What we are now beginning to uncover is how the balanced responses of the gut can affect immune responses outside of the mucosal world. So, how TGF- β maintains populations of Tregs, Th17 cells, B cells, IELs and APC could well influence susceptibility and pathogenesis of autoimmunity, chronic inflammation and cancer as well as determine how well one can deal with infection.

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Glossary

CD8$\alpha\alpha$	Homodimer of transmembrane glycoproteins expressed on the surface of some T cells (mainly IEL populations). CD8 $\alpha\alpha$ homodimers do not appear to act like the more typical T cell co-receptors, CD4 or CD8 $\alpha\beta$, which promote T cell activity.
Colitis	Inflammation of the colon.
Colitogenic T cell	Colitis-causing populations of T cells.
Commensal bacteria	Bacteria that colonize the gastrointestinal tract but which cause no harm and instead confer advantages to the host.

Germ free mice	Mice which are completely free of all micro-organisms, including bacteria, exogenous viruses, fungi, and other parasites. These mice are born and raised in sterile environments and have never been exposed to micro-organisms.
Germinal Center	Microscopically distinguishable areas of lymph nodes, spleen or Payer's Patches that are sites of B cell maturation
Gut trophism	Trafficking of immune cells towards the gut.
Intra-epithelial lymphocyte (IEL)	Lymphocytes (both T cells and B cells) located in the epithelial cell layer. In this review, we are specifically referring to lymphocytes in the epithelial layer of the mucosa.
IgA	The antibody isoform with type α heavy chains. IgA is enriched at mucosal surfaces and in secretions owing to the concerted action of polymeric IgA (pIgA) producing plasma cells and mucosal epithelial cells that take up pIgA and process it for secretion into tears, saliva, and gut fluids. IgA can exist in secreted dimeric or polymeric form and binds to the cell surface receptor CD89 on effector cells.
Lamina Propria	A layer of connective tissue that underlies the epithelial cell layer. Collectively these two layers constitute the mucosa.
Oral tolerance	Suppression of systemic immune response to specific antigens by first administering the antigen orally (i.e. via feeding).
Payer's Patches	Organized lymphoid structures or nodules located in the small intestine.
Retinoic acid	Vitamin A metabolite that mediates changes in gene transcription by binding to nuclear retinoic acid receptors (RARs). RARs form dimers with retinoid X receptors (RXRs) and function as ligand-dependent transcription factors. When RA is not bound to RAR, the RAR/RXR complexes are thought to function as transcriptional repressors.
TCR$\gamma\delta$	A small subset of T cells express this distinct T cell receptor. These are referred to as $\gamma\delta$ T cells and are enriched in the gut mucosa. Although they have a TCR, $\gamma\delta$ T cells exhibit innate cell characteristics.
TGF-β1, 2, 3	Separate isoforms of TGF- β . TGF β -1 is thought to be the most important in immune system functioning, however this does not preclude roles for TGF β -2 and/or TGF β -3.

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Text Box 1**Canonical TGF- β signaling pathway**

TGF- β mediates both positive and negative effects on cells of the immune system, though is generally considered to be immunosuppressive. TGF- β profoundly inhibits lymphocyte proliferation, cytokine production and differentiation of T cells into either the Th1 or Th2 phenotype. It also promotes the generation of immune-suppressive regulatory T cells. However, TGF- β can prevent T cell apoptosis and, conversely, promotes the differentiation of T cells to the Th17 phenotype.

Although a large number of mediators of TGF- β -signaling have been identified, for simplicity the canonical TGF- β -signaling is outlined below and in Figure I.

1. TGF- β homodimers are cleaved from latency associated proteins (LAP) allowing active TGF- β to bind TGF- β receptors. Cleavage can be mediated by a number of mechanisms, including but not limited to proteases, integrins, and changes in pH.
2. Active TGF- β homodimers bind TGF- β receptor II which recruits and phosphorylates TGF- β receptor I.
3. The activated receptor complex can then phosphorylate a receptor-regulated Smad (R-Smad), Smad2 or Smad3.
4. Once activated the R-Smad associates with the Common Smad (Co-Smad), Smad4, and translocates to the nucleus.
5. In the nucleus the Smad complex binds DNA-binding partners, then subsequently binds the DNA and activates transcription.

Inhibitory Smads such as Smad7 are negative regulators of TGF- β signaling. Smad7 has been shown to inhibit TGF- β signaling at least at steps 3, 4 and 5.

Text Box 2**Foxp3 and regulatory T cells**

Foxp3 is a transcription factor which belongs to the forkhead/winged-helix family. It is described as the “master regulator” of Tregs, in that it is both necessary and sufficient for Treg function. The critical role of Foxp3 in the development and function of Tregs, and the consequent role of Tregs in the maintenance of a healthy immune system are plainly demonstrated in mice and humans with mutations in the *Foxp3* gene. The human disease immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) and the scurfy phenotype in mice both arise from Foxp3 mutations and cause severe lymphoproliferative diseases and consequential morbidity and mortality (for review see (65)).

Induction of Foxp3 in naïve CD4⁺ T cells drives differentiation to the Treg cell fate, and indeed, Tregs are defined as CD4⁺Foxp3⁺ T cells. Once expressing Foxp3, these T cells are now endowed with the Treg signature and are capable of suppressing other immune cell types both *in vitro* and *in vivo*. Treg-mediated suppression has been shown to occur via a plethora of mechanism, some mediated by cytokines and factors released by the Treg, others that require direct contact between the Treg and its target cell.

Text Box 3**Th17 cells and barrier function**

Th17 cells are not the only population of immune cells capable of making Th17 cytokines; CD8⁺ T cells, NK, NK-T and $\gamma\delta$ T cells all make the Th17 signature cytokines IL-17A, IL-17F, IL-22 and, in humans, IL-26. Despite this, it is important to consider the vital role played by these cytokines in maintaining the mucosal barrier even though development of these other cell types is not necessarily regulated by TGF- β . Treatment of epithelial cells with IL-17, IL-22 or IL-26 leads to increased proinflammatory cytokine, chemokine, and iNOS production, migration of intestinal epithelial cells and anti-microbial peptide production. *In vivo* the anti-microbial peptides RegIII γ and RegIII β and defensins 1, 3 and 4 (66, 67) are induced by IL-22 and IL-17, respectively. How important are Th17 cytokines to the protection of the gut environment? Considering first the anti-microbial peptides, IL-22-deficient mice are susceptible to *Citrobacter rodentium* infection, yet treatment of IL-22-deficient mice with RegIII γ increased the survival of these mice (67). Indeed, Th17 cells have been shown by a number of investigators to be vital for protection against *Citrobacter rodentium* infection (22, 66, 67). Further evidence for the vital role of Th17 in gut barrier function comes from work with simian immunodeficiency virus (SIV), where a lack of Th17 cells accounts for impaired barrier function and increased bacterial translocation (68).

Thus, although only briefly outlined here, Th17 cytokines employ many mechanisms to promote barrier function. Without the TGF β -induced Th17 cells barrier breaches could occur more frequently. Therefore, to complicate matters further, one could think that Th17 cells, by maintaining barrier integrity, and thereby intestinal homeostasis, also play a regulatory role within the gut environment. Indeed, IL-17A production has, in fact, been shown to be protective in models of colitis (69, 70).

Figure 1. Pluripotency of TGF-β in the gut mucosa

TGF-β drives the development of CD4⁺Foxp3⁺ Tregs, Th17 cells, IgA⁺ B cells and TCRαβ⁺CD8αα⁺ IEL which act to promote gut barrier integrity. The generation of Foxp3⁺ Tregs in the gut mucosa is supported by high concentrations of TGF-β. Treg generation is also favored by CD103⁺ DC, which promote the development of both Tregs and IgA⁺ B cells via TGF-β- and RA-dependent mechanisms that remain loosely-defined. The microbiome can also influence Treg generation, with the bacteria *Clostridium* spp. shown to enhance the gut Treg population. Present at high frequencies in the gut, Tregs can in turn promote the development of IgA⁺ B cells in a TGF-β-dependent manner, and it is possible that Tregs could enhance Th17 differentiation through the uptake of IL-2, which is inhibitory to Th17 development. Th17 generation is dependent on inflammatory cytokines such as IL-6 that act with TGF-β to drive Th17 differentiation. Th17 development is also sensitive to the commensal bacteria population, and Th17 cells are enhanced in guts colonized by ATP-producing commensal bacteria, for example segmented filamentous bacteria.

Figure I.
Canonical TGF- β signaling; Smad-dependent pathway downstream of TGF- β receptors.

Table 1

Key cell types.

Cell type	Markers	Description	Role of TGF- β ?
T cells	TCR $\alpha\beta$ ⁺	Mediators of adaptive immunity that express unique receptors on their cell surface called T cell receptors (TCR), along with either the co-receptor CD4 (T helper cells) or CD8 (cytotoxic T cells).	
Naïve T cells	TCR $\alpha\beta$ ⁺	A T cell which has yet to encounter its cognate antigen in the periphery and become activated.	
Tregs	TCR $\alpha\beta$ ⁺ CD4 ⁺ Foxp3 ⁺	Vital mediators of immune-suppression. Tregs suppress ongoing immune responses and induction of immune responses to prevent collateral damage to tissues. This is achieved via a plethora of suppressive mechanisms.	<ol style="list-style-type: none"> 1 The master regulator of Tregs is the transcription factor Foxp3. Induction of Foxp3 in naïve T cells occurs in a TGF-β-dependent manner. 2 Tregs can suppress immune responses by secreting TGF-β.
Th1 cells	TCR $\alpha\beta$ ⁺ CD4 ⁺ T-bet ⁺	Subset of T helper cells that produce IFN γ and are important in activating macrophages and memory T cells and clearing intracellular pathogens.	TGF- β inhibits differentiation of naïve T cells to the Th1 phenotype.
Th2 cells	TCR $\alpha\beta$ ⁺ CD4 ⁺ GATA3 ⁺	Subset of T helper cells that predominantly produce IL-4, IL-5 and IL-13. Are important in stimulating B cells and clearing extracellular pathogens.	TGF- β inhibits differentiation of naïve T cells to the Th2 phenotype.
Th17 cells	TCR $\alpha\beta$ ⁺ CD4 ⁺ ROR γ t ⁺	Subset of T helper cells that produce IL-17 and IL-22. Th17 cells are pro-inflammatory and important in clearing fungal infections.	The master regulator of Th17 cells is the transcription factor ROR γ t, induction of which is dependent on the presence of TGF- β and IL-6.
Th3 cells	TCR $\alpha\beta$ ⁺ CD4 ⁺	Subset of T helper cells that predominantly produce TGF- β .	Production of TGF- β by these cells is thought to mediate suppression.
T follicular helper cells (TFH)	TCR $\alpha\beta$ ⁺ CD4 ⁺ CXCR5 ⁺	Subset of T helper cells that are found in the B cell follicles of lymph nodes, spleen and Payer's patches. TFH aid antigen specific B cell activation.	
TCR$\alpha\beta$⁺ CD8$\alpha\beta$⁺ IEL	TCR $\alpha\beta$ ⁺ CD8 α ⁺ CD8 β ⁺	Conventional CD8 ⁺ cytotoxic T cells which have trafficked to the gut and reside within the epithelial layer of the gut wall.	TGF- β maintains CD8 α expression.
TCR$\alpha\beta$⁺ CD8$\alpha\alpha$⁺ IEL	TCR $\alpha\beta$ ⁺ CD8 α ⁺ CD8 β ⁻	Unconventional CD8 ⁺ T cells which reside within the epithelial layer of the gut wall.	Develop in a TGF- β dependent manner.
CD103⁺ DC	CD11c ⁺ CD103 ⁺	DC enriched in the gut and GALT. Promote differentiation of Tregs.	Enriched in transcripts for TGF- β .
CXCR1⁺ macrophage	CXCR1 ⁺	Gut resident macrophage involved in expansion of Tregs in the gut.	