Review

Oral tolerance: intestinal homeostasis and antigen-specific regulatory T cells

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Mucosal surfaces, especially those of the gastrointestinal (GI) tract, are sites for tolerance induction to numerous exogenous antigens (Ags), and provide a microenvironment suitable for generating tolerogenic dendritic cells (DCs) that contribute to the functional maturation of regulatory T cells. During immune homeostasis in the GI tract, innate immune signals provided by innocuous or commensal bacteria play important roles in stabilizing this noninflammatory microenvironment and function of regulatory T cells. Thus oral tolerance consists of two phases of immune response: the maintenance of homeostasis and the suppression of immune responses mediated by Ag-specific regulatory T cells. Elucidating mechanisms for both phases should contribute to physiological intervention of local and systemic immunity, thereby improving homeostasis in both health and disease.

The gut is designed to tolerate harmless exogenous antigens

To accommodate the vast antigenic exposure to harmless food components and commensal bacteria, the gut has evolved a naturally anti-inflammatory environment. By means of this complex immunological network, the gut acts to tolerate harmless antigens (Ags) yet remains able to eliminate pathogens. This tolerance of food Ags is not dependent on the ability of the digestive system to break down food into components 'invisible' to the immune system; rather food Ags are presented to the mucosal immune system in a tolerogenic manner. Exogenous Ags are processed by the gut associated lymphoid tissue (GALT) system and utilized for an active suppression mechanism to render the host immunologically unresponsive to them. Recent knowledge gleaned from immunoregulatory cells suggests that specialist dendritic cells (DCs) in the gastrointestinal (GI) tract present food Ag to T cells as tolerogens thus granting them regulatory functions. Under normal physiological conditions, the GALT likewise employs mechanisms to tolerate, or exist in symbiosis with, a large innocuous community of gut microbes. Thus, the immune system of the gut must exhibit 'dominant tolerance' and an ability to generate a regulatory immune response is a key component of this.

Oral tolerance and regulatory T (Treg) cells

Oral tolerance is defined as the lack of a systemic immune response following parenteral immunization with an Ag to an animal that had been previously exposed to (or been immunized with) the same Ag through the GI tract [1]. In other words, oral tolerance is induced in an Ag-specific manner and its effects are not constrained to the local immunity within the gut. Indeed inflammatory immune responses induced in distal nonlymphoid organs, such as skin delayed-type hypersensitivity, allergic responses, or insulitis, can all be significantly down regulated by means of oral tolerance (or oral administration of Ags) [1,2]. This fact strongly suggests that immunoregulatory cells with suppressive function circulate in the entire body and perform the immunosurveillance responsible for maintaining this Ag-specific tolerance. It is now widely accepted that oral tolerance effector mechanisms include not only anergy or apoptosis (deletion) of Ag-specific T cells in the gut but also active suppression through the induction of Ag-specific regulatory T cells [2–5]. Suppressive T cell populations seem to overlap with anergic cells in some of their characteristics. For instance the naturally occurring regulatory T cells (nTreg) originally described by Sakaguchi *et al.* as $CD25^{hi}CD4^{+}$ cells, are generated through the thymus, express the transcription factor $Foxp3^{+}$ and produce little IL-2 [6]. Similarly, a majority of the Ag-experienced T cells in the gut not only arereluctant to proliferate or produce IL-2 but also function as suppressor cells against neighboring immune or inflammatory cells (see Box 1 detailing the various types of Treg cells).

The concept that a majority of T cells rendered anergic by Ag exposure in the GI tract share suppressive characteristics with induced regulatory T cells, particularly cells with a memory phenotype, seems to explain the long-term, Ag-specific systemic nonresponsiveness induced by oral tolerance. The presence of memory cells exhibiting Ag-specific regulatory functions could explain why established oral tolerance can be long-lasting (sometimes for the lifetime of the host) [7].

Ag-specific Treg are observed in the Peyer's patches (PP), and mesenteric lymph nodes (MLN) of the gut as early as 24–48 h after oral administration of Ag [8,9], suggesting that Ag- presenting cells (APCs) that trigger a tolerogenic response exist constitutively in the GALT and the draining lymphoid organs. Similarly, Ag-specific tolerance is observed in isolated lymphoid organs following oral immunization [10]. This begs the question of whether circulating T cells originating in the gut itself are entirely responsible for systemic immunoregulation or whether tolerogens and/or tolerogenic DCs themselves also enter isolated lymph nodes to generate Treg cells *in situ*. This

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Box 1. Regulatory T cell subsets involved in gut homeostasis and oral tolerance: Tr1, Th3, and nTreg

Regulatory T (Treg) cells appear to come in many different forms. IL-10-producing Tr1 cells were first described for splenocytes cultured and matured *in vitro* in the presence of IL-10. With the help of this regulatory cytokine, naïve $CD4^+$ cells develop into unique $CD4^+CD25^-Foxp3^-$ cells with suppressive functions attributed to IL-10 and TGF-β. It was subsequently shown that Tr1-like cells could also be generated from naïve T cells upon chronic exposure to antigen even in the absence of IL-10. Immunosuppressive drugs, IL-27, or complementation of IL-10 with IFN-α or TGF-β have been shown to be effective for the generation and *ex vivo* expansion of Tr1 cells. Although IL-10 is the predominant product of Tr1, both IL-10 and TGF-β are effective suppressive cytokines that inhibit cytokine production by activated T cells, the expression of co-stimulatory molecules on APCs, and antibody production.

Th3 cells are TGF-β-producing Ag-specific $CD4^+$ T cells originally isolated from MLN of orally tolerized mice. Th3 cells are considered to be a part of the LAP^+ T cell population that exert potent immunosuppressive properties via TGF-β and form effective anti-inflammatory cell populations for the maintenance of immune homeostasis. Because TGF-β alone induces expression of Foxp3, a definitive marker for Treg cells, Th3 cells can influence Treg cell development of neighboring cells (so-called infectious tolerance). In the gut, RA promotes the conversion of conventional T cells into $Foxp3^+$ Treg cells, suggesting significant roles for intestinal DCs that produce RA to form $Foxp3^+$ Treg cell populations.

nTreg develop in the thymus as $CD4^+CD25^+$ cells and express high levels of Foxp3. They are suppressive by nature and inhibit other effector cell functions *in vitro* and *in vivo*. Suppression mediators include inhibitory molecules such as CTLA-4 and immunoregulatory cytokines IL-10, TGF-β, or IL-35. nTreg are considered to maintain systemic homeostasis and prevent autoimmune diseases. All these regulatory T cells are involved in the mechanism of oral tolerance but a clear distinction of their contribution in the induction and stabilization of local and systemic tolerance remains to be determined.

question has been resolved, at least in part, by a study showing that the proximal draining lymph nodes (e.g. MLN for the intestinal lymphatic pathway) receive Ag-bearing DCs from the lamina propria (LP) in a CCR7-dependent manner where they play a substantial role in the induction of Ag-specific immune responses. However, distal lymph nodes such as inguinal lymph nodes, or the spleen, are isolated from the initiation of this tolerogenic process [11]. Systemic oral tolerance therefore seems to depend on the mobility of Ag-specific T cells that circulate in the body although the priming of these cells occurs in gut lymphoid tissue (i.e. PP and MLN) [reviewed in 12]. Similarly, we have observed Ag-specific *in vitro* active suppression in PP-derived $CD4^+$ T cells but no such active suppression in distal lymph node cells 1 week after feeding Ag to BALB/c mice, suggesting the generation of immunoregulatory cells in GALT *in situ* [13]. However, active suppression can also be observed in distal lymph node of orally tolerized mice following parenteral (i.e. via a route other than the gut) immunization with the same Ag [10]. This discrepancy can likely be explained by postulating that under steady-state conditions (i.e. in the absence of pathogen exposure) Treg cells induced in the gut reside in mucosal sites but once they sense inflammation (e.g. from infection, injury, or from the adjuvant supplied during immunization) they migrate to distal lymphoid tissues and exert their suppressive function. Accordingly, specific Ag is required for the induction and activation of Treg, whereas the suppressive effects themselves are mediated in an Ag-nonspecific manner (i.e. bystander suppression) [14,15]. Interestingly, there are studies that show oral tolerance and Treg cell suppression might be dysfunctional in some patients suspected of having, or diagnosed with, inflammatory bowel disease (IBD) [16,17].

Ag-nonspecific mode of oral tolerance: intestinal homeostasis

We would like to define two phases of oral tolerance each of which shows physiological benefits. One phase is the generation and maintenance of local (i.e. the gut) immunosuppressive conditions, preventing unwanted inflammation and exhaustion of immune cells *in situ*, and consequently providing a suitable immunological environment for generating Ag-specific immune responses. The other phase is the establishment of Ag-specific anergy or Treg that can maintain systemic immune quiescence (Figure 1). Because APCs in the gut, particularly DCs, are responsible for both the production of regulatory cytokines and presentation of Ags to T cells, intestinal DCs are likely to play key roles in both phases of oral tolerance. Their multiple roles include (1) the generation of an immunosuppressive or anti-inflammatory microenvironment via regulatory cytokines, (2) the generation of Ag-specific regulatory T cells via Ag presentation, chemical mediators [e.g. the vitamin A metabolite retinoic acid (RA), or indoleamine 2,3 dioxygenase (IDO)], and possibly providing inhibitory co-stimulatory molecules (such as ligands for inducible T cell co-stimulation ligand, ICOS and/or programmed death 1, PD-1).

Moreover, to initiate and stabilize the interaction between T cells and DC, the localization of tolerogenic DCs and Treg seems to be controlled by chemokines. Intestinal DCs influence T cells to express $\alpha_4\beta_7$ and CCR9 via (RA signaling so that imprinted Treg have the ability to home to mucosal sites (also see the article by Agace in this issue). LP DCs produce the CCR4 ligands CCL17 or CCL22 so that they can attract $CCR4^+$ Tregand retain them at mucosal sites [18]. Recently, the function of $Foxp3^+$ Treg to prevent unwanted inflammation was observed not only at the late stages of infection but also at the initiation of the protective immune response [19]. This suggests that the function of $Foxp3^+$ Treg is to avoid unfocused inflammation to innocent neighbors and to localize immunological defenses at the site of inflammation. Treg are therefore responsible for avoiding activation-induced exhaustion of effector T cells and enable effective immune defence against pathogens.

Ag-specific mode of oral tolerance and regulatory T cells

De novo generation of Ag (OVA)-specific $Foxp3^+$ cells is observed in PP and MLN after feeding OVA to mice in nontransfer system [8], reflecting efficient Ag-uptake via M cells in PP and possibly via villous M cells [20,21]. Soluble Ag can also be taken up directly from the intestinal lumen via epithelial cells (transcytosis) or possibly by DCs that form tight-junction-like structures with intestinal epithelial cells [22]. LP DCs load Ag and carry them to MLN through a continuous steady-state cell trafficking and present Ag to T cells, whereas PP DCs migrate to MLN or interact with PP T cells *in situ*. The most prominent Ag-specific suppressive activity can be observed in

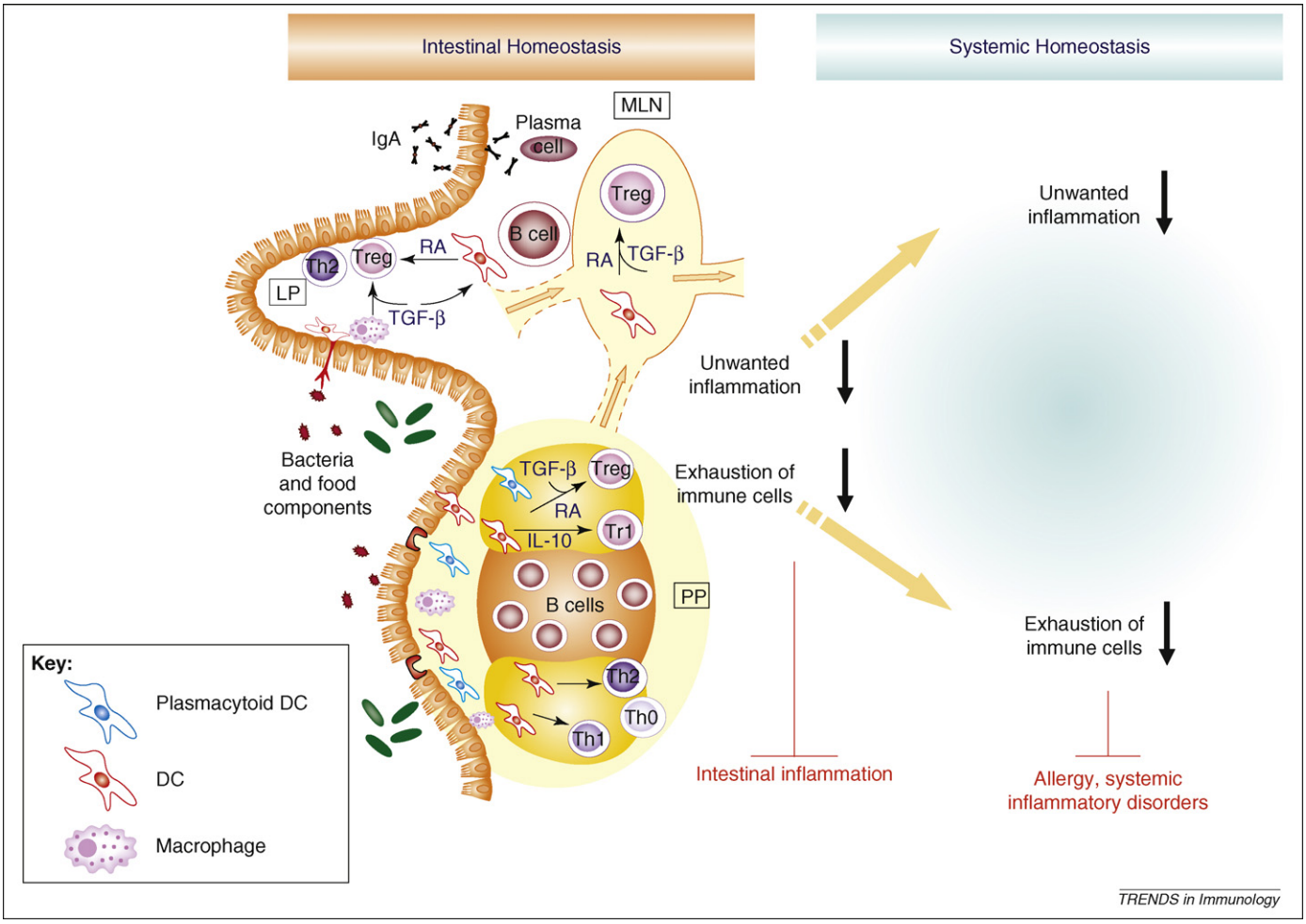

Figure 1. Oral tolerance: a physiological avenue towards intestinal and systemic homeostasis by means of regulatory T cells. Ag-specific and Ag-nonspecific regulatory mechanisms of intestinal immunity contribute to both local and systemic immune responses. During intestinal responses, regulatory cells protect the intestine from unwanted inflammation (immunopathology) and exhaustion of immune cells thereby preventing food allergy or inflammatory bowel disease. IL-10 and TGF-β-producing regulatory T cells play important roles in maintaining intestinal homeostasis. During systemic immune responses, observations in experimental oral tolerance suggest that gut-induced Ag-specific T cells are able to migrate to distal inflammatory sites and regulate unwanted inflammation such as allergic hypersensitivity and inflammatory disorders.

PP-derived cells, when intestinal and spleen cells are monitored after feeding Ag [13]. Accordingly, mice lacking PP were shown to be more susceptible to disease development than PP-bearing mice in a food allergy model. These protective effects were related to Ag-specific IL-10 producing $CD4^+CD25^+$ cells [23]. PPs are thus one of the major inductive sites of acquired immunity in GALT, including regulatory immune responses. Unfortunately, information regarding the physiological APC(s) responsible for generating Treg cells remains limited.

Among the various types of APCs, DCs exhibit the most potent stimulatory capacity for Ag-specific T cells by virtue of their high levels of MHC class II and co-stimulatory molecule expression. There are various DC ($CD11c^+$) subsets in PPs (e.g. $CD11b^+$, $CD8a^+$, and $CD4^-CD8a^-CD11b^-$) with each subset involved in a specific type of immune response such as the induction of Treg and/or elimination of pathogens. For instance, $CD11b^+$ PP DCs observed at the PP subepithelial dome (SED) produce IL-10 and induce IL-10-producing Th2 cells from naïve T cells, whereas $CD8a^+$ DCs at the T-cell zone (interfollicular region, IFR), and $CD4^-CD8a^-CD11b^-$ DCs at both the SED and IFRs, produce IL-12 and induce IFN-γ-producing Th1 cells and are likely responsible for pathogen clearance [24].

Plasmacytoid DCs ($CD11c^{int}B220^+$) are also observed in PPs at the IFR region. $CD8a^+CD11c^+B220^+$ ($CD8a^+$ plasmacytoid DC) are expanded after flt3-ligand treatment of animals. These DC are reported to express IDO and can induce IL-10 producing Treg*in vitro* [25]. Likewise the expanded population of plasmacytoid DC in flt3-treated animals were found to produce less type I interferon compared to plasmacytoid DC in spleen, probably due to the effect of regulatory cytokines in PPs (e.g. IL-10, TGF-β) [26]. This cell population also produces prostaglandin E2 (PGE2), which might be relevant to the effect observed in the humans that PGE2 can convert $CD4^+CD25^-$ T cells into $FOXP3^+$ Treg [27]. studies using PP DCs from mice without flt3-ligand treatment described that $CD11c^+CD11b^+$ cells [28] or IDO^+ $CD11c^+$ cells [29] from orally tolerized mice can induce IL-10 producing $CD4^+CD25^+$ T cells.

Several mechanisms involved in Ag-induced oral tolerance occurring at the DC-T cell interface have been described using gene-deficient mice, although none of these mechanisms appears to be unique to PP DCs. The inhibitory co-stimulatory molecules cytotoxic T lymphocyte antigen 4 (CTLA-4) and ICOS seem to play distinct roles in tolerance induction; namely, CTLA-4 is suggested to be required in high-dose tolerance (anergy and deletion),

Table 1. Defined characteristics of intestinal DCs

Organ	Phenotype	Function	Refs
PP	$CD11b^+$ $CD11c^+$ cells	Produce IL-6 and induce IgA secretion	[97]
	$CD11b^+$ $CD11c^+$ cells	Produce high levels of IL-10 and low levels of IL-12, induce IL-4 and IL-10-producing CD4 T cells	[37]
	$CD8\alpha^+$ $CD11c^+$ and $CD11b^-$ $CD8\alpha^-$ $CD11c^+$ cells	Produce IL-12p70	[37]
	$CD11c^+$ cells	Induce $\alpha_4\beta_7$ and CCR9 expression on B and T cells via RA	[80,81,98]
	IDO^+ $CD11c^+$ cells (from orally tolerized mice)	Induce $CD4^+CD25^+Foxp3^+$ T cells	[29]
	$CD11b^+$ $CD11c^+$ cells (from orally tolerized mice)	Generate $CD4^+CD25^+$ T cells	[28]
	pDC ($B220^+$ $Gr1^+$ $CD11c^{int}$ cells)	Produce low levels of type I IFNs	[26]
MLN	$CD103^+$ $CD11c^+$ cells	Promote the generation of $Foxp3^+$ T cells via TGF-β and RA	[78]
	$CD103^+$ $CD11c^+$ cells	Induce $\alpha_4\beta_7$ and CCR9 expression on T cells	[100,101]
	pDC (induced by Flt3L: $CD8\alpha^+$ $B220^+$ $CD11c^{lo}$ cells)	Induce Tr1-like cells	[25]
	$PDL2^+$ ($CD8\alpha^-$) $CD11c^+$ cells	Induce oral tolerance	[32]
	$CD103^+$ $CD11c^+$ cells (induced by cholera toxin)	Promote Th2 skewing via OX40	[101]
	$CD11c^+$ cells	Enhance TGF-β-dependent $Foxp3^+$ T cell differentiation by RA	[79]
	$CD103^-$ $CD11c^+$ cells	Promote the differentiation of IFN-γ-producing cells	[99]
LP	$CD8\alpha^-$ $CD11b^-$ $CD11c^+$ cells	Produce IL-12p40	[102]
	$CD11b^+$ $CD11c^+$ cells	Induce IL-17 production by CD4 T cells	[36]
	$CD11c^+$ cells (induced by Flt3L)	Express significant levels of IL-10 mRNA and low levels of IL-12p40 mRNA	[103]
	MHC class II^+ $CD103^+$/- $CD11c^+$ cells	Promote the generation of $Foxp3^+$ T cells via TGF-β and RA	[53]
	$\alpha_v\beta_8$-expressing $CD11c^+$ cells	Induce $Foxp3^+$ T cells	[77]
	$iNOS^+$ $CD11c^+$ cells	Induce IgA production	[104]
	TLR5-expressing CD11c**hi** CD11b**hi** cells	Induce IgA production	[41]
Colon (human)	TSLP-treated $CD11c^+$ cells	Induce Th2-type responses	[105]

whereas ICOS is important for low-dose oral tolerance, suggesting the ICOS-ICOS-L system is related to generating IL-10 producing Tr1 cells [30,31]. Similar to the ICOS-ICOS-l pathway, the PD-1-PD-L co-stimulatory pathway is known to be involved in inhibitory mechanisms. Particularly PD-L2 expressed exclusively on activated DC might play a substantial role in tolerance induction [32,33]. $CD40L^{-/-}$ mice also fail to establish oral tolerance, suggesting that CD40-CD40L interaction is required for limiting immune responses or induction of regulatory cells during the course of tolerance induction [34]. Ablation of the CD11b molecule resulted in enhanced IL-6 production by APCs and preferential differentiation of naïve T cells into Th17 cells, and consequently abrogated the development of oral tolerance [35]. This could be related to the finding that $CD11b^+$ LP-macrophages limit Th17 cell differentiation [36], or $CD11b^+$ PP DCs induce IL-10 producing T cells [37]. In terms of the cytokine milieu, IL-18 has also been shown to have a role in establishing oral tolerance via its ability to enhance IL-10-production from PP-$CD11c^+$ cells and generation of Ag-specific suppressive T cells [38]. Additionally, local IgG seems to be important in both oral and nasal tolerance by limiting DC activation via its interaction with the inhibitory Fc receptor (FcγRIIB) [39]. The role of Toll-like receptor (TLR) ligation on intestinal DC and modulation of gut immune responses has begun to be intensively examined [40,41]. Further studies on the specific tolerogenic mechanisms employed by the various mucosal DC subsets are keenly awaited (summarized in Table 1).

Role of TGF-β and IL-10 in the generation of regulatory T cells in oral tolerance

Progress in the study of $Foxp3^+$ Treg cells has accelerated our understanding of the specialized microenvironment necessary for the physiological maturation of different subsets of regulatory T cells [6,42]. The immunosuppressive cytokine TGF-β is abundantly expressed in the gut and plays an important role in maintaining immune homeostasis. TGF-β is produced by both stroma and T cells in the gut. In addition to its immunosuppressive properties, TGF-β is also a critical factor for IgA class switching that helps generating the IgA-predominated immunoglobulin milieu characteristic to the intestine [43,44] (also see the article by Fagarasan and colleagues in this issue). An intriguing connection between TGF-β and Foxp3 has now been widely reported whereby TGF-β signals induce Foxp3 expression in conventional T cells (i.e. $CD4^+CD25^-$) and cause them to become regulatory in nature [45–48]. In turn, Foxp3 downregulates the expression of Smad7 that blocks TGF-β signaling, thereby rendering responding T cells more susceptible to TGF-β [49]. Because Foxp3 works as a negative regulator of IL-2 transcription [50,51], its expression limits T cell proliferation unless there is an exogenous source of IL-2. Not only does TGF-β trigger Foxp3 expression, it also inhibits the differentiation of Th1 and Th2 cells by blocking the expression of the lineage specific transcription factors T-bet and GATA-3, respectively [52].

Cell transfer studies have revealed that the conversion of conventional T cells into $Foxp3^+$ Treg cells in the gut is an efficient process, reflecting the TGF-β-rich milieu. Mice orally tolerized to specific Ag show the highest numbers of converted $Foxp3^+$ T cells in PP, followed by LP, and finally the MLN [53]. Thus, the intestinal microenvironment seems to be essential for the extra-thymic generation of $Foxp3^+$ T cells and probably also the maintenance of their Foxp3 expression levels (Figure 2). Although it is not yet known if such a conversion system is involved in the development and maintenance of Ag-specific oral tolerance, one can see how it would be important for the maintenance of intestinal immune homeostasis.

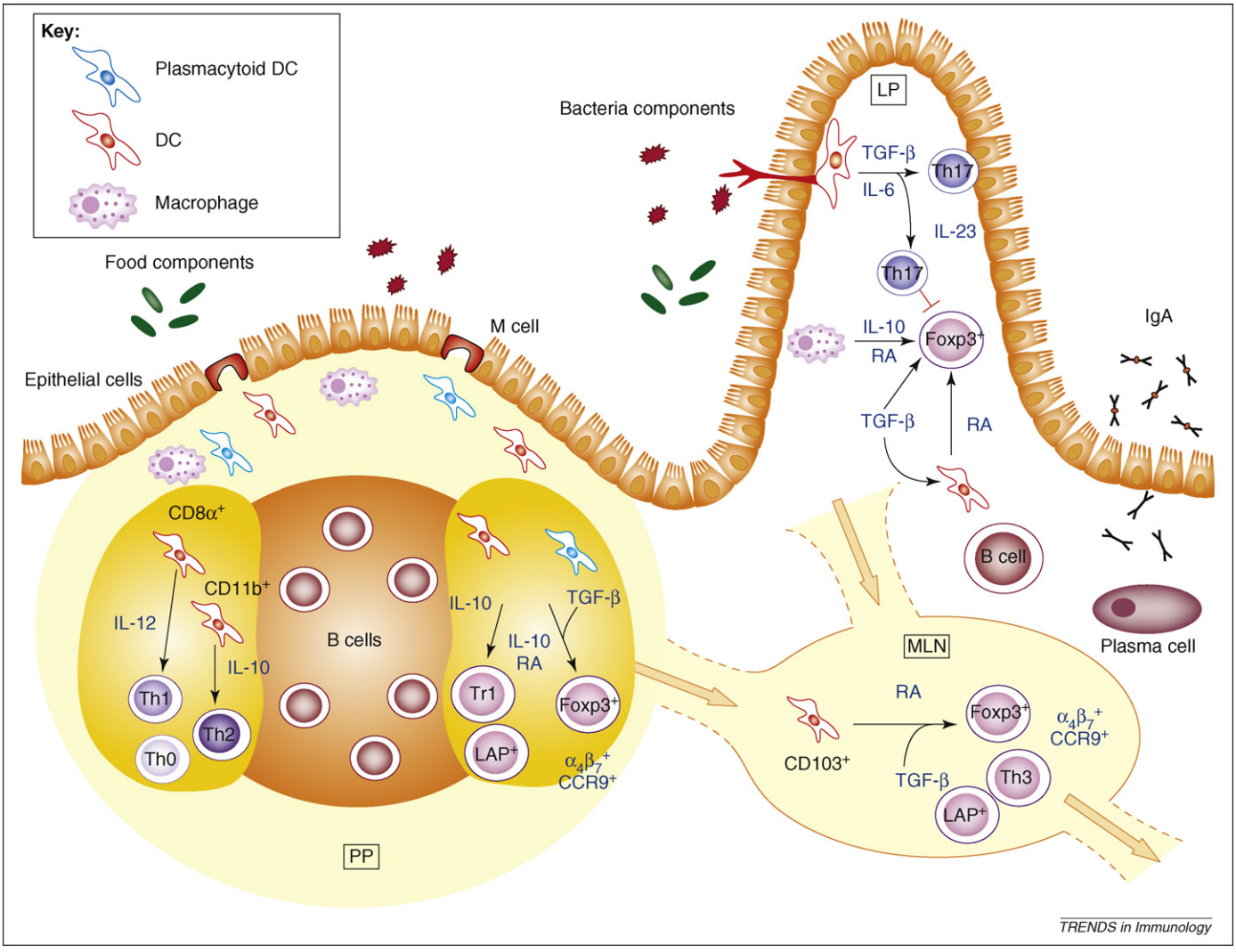

Figure 2. A simplified scheme depicting the generation and functional maturation of intestinal effector cells including regulatory T cells. The intestinal cytokine milieu such as IL-10, TGF-β, and/or RA modifies local DCs and T cells, resulting in the generation of regulatory T cells (Foxp3$^+$, LAP$^+$ and IL-10-producing cells). Ag-uptake via M cells in the PP and possibly via villous M cells enables intestinal APCs such as DC (CD8α$^+$, CD11b$^+$), macrophages, and possibly B cells to present Ag to T cells in the GALT (PP, LP) or the draining lymph nodes (i.e. the MLN). RA generated by DC in GALT and MLN enhances expression of Foxp3 in T cells, facilitates IgA class switching in B cells, and imprints gut-homing ability to immune cells by inducing expression of the appropriate homing receptors ($\alpha_4\beta_7$ and CCR9). TGF-β and IL-6 generate IL-17-producing Th17 cells. IL-23 promotes pathological features of this cell population, which can disturb intestinal homeostasis and cause inflammatory disorders. LP DCs load Ag and carry them to MLN through a continuous steady-state cell trafficking and present Ag to T cells, whereas PP DCs migrate to MLN or interact with PP T cells *in situ*. Ag carriage to MLN by LP DC is CCR7-dependent. Ag, antigen; DC, dendritic cell; Foxp3, forkhead box P3; LP, lamina propria; LAP, latency associated peptide; MLN, mesenteric lymph nodes; PP, Peyer's patch; RA, retinoic acid.

The physiological role of TGF-β and induced Treg cells (or iTreg) in oral tolerance was originally described by Weiner *et al.* through the discovery of Ag-specific Th3 cells [43]. Further studies revealed that cells bearing the membrane-bound form of TGF-β (latency-associated peptide, LAP), are potent regulatory T cells [54,55]. The existence of these LAP$^+$ regulatory T cell populations are described in both CD25$^+$CD4$^+$ and CD25$^-$CD4$^+$ cells. The LAP$^+$ CD4$^+$CD25$^-$ cells exhibit a similar pattern of cytokine secretion to Th3 cells, except that they also produce a significant amount of IFN-γ. The effector molecule for *in vivo* suppression seems to be TGF-β, whereas IL-10 is required at the early phase for these cells to function. CD4$^+$CD25$^+$LAP$^+$ cells show higher expression levels of Foxp3 and more potent suppressive activity than CD4$^+$CD25$^+$LAP$^-$ cells [56]. Maintenance of high levels of expression of Foxp3 is dependent on TGF-β, and this in turn ensures Foxp3 cells to be potent regulatory T cells [57]. Since CD4$^+$ cell-specific modification or ablation of TGF-βRII causes a severe phenotype of colitis and organ inflammation [58,59], the observation that high levels of TGF-βRII is expressed preferentially on LAP$^+$ T cells (especially on CD4$^+$CD25$^+$LAP$^+$ cells) is important, although it needs be confirmed if the receptor signaling is fully effective in creating an immunosuppressive microenvironment for LAP$^+$ and neighboring T cells [56].

Recently, TGF-β was also shown to play an important role for the induction of IL-10 secreting regulatory cells, in an IL-27 [60,61] or IL-6 [62]-dependent manner, suggesting DCs that produce IL-27 or IL-6 have the ability to induce IL-10-producing T cells (i.e. Tr1 cells, IL-10 producing Th1 and/or Th17 cells). IL-27 producing DCs are generated by interaction with Foxp3$^+$ cells, whereby TGF-β augments such cellular communication [60]. Because both IL-27 and IL-6 are proinflammatory cytokines mostly produced by innate immune cells including

Table 2. Ag-stimulated regulatory T cells observed in the intestine

Organ	Phenotype	Cytokine produced	Refs
PP	Ag-induced, $CD4^+$, $CD4^+CD25^+$	TGF-β > IL-10	[1,10]
	Ag-induced, $CD4^+$, $CD4^+CD25^+$(Foxp3?)	IL-10 > TGF-β	[13,28]
	Ag-induced, $CD4^+CD25^+Foxp3^+$	IL-10 > TGF-β	[23,29]
	anti-TCR injected[a]	IL-10 (TGF-β?)	[63]
	Ag-induced[b], $CD4^+CD25^+$(Foxp3?), $CD4^+CD25^-$(Foxp3?)	Not applicable	[8]
	Ag-induced[b], $CD4^+Foxp3^+$	Not applicable	[53]
MLN	Ag-induced, $CD4^+$	TGF-β > IL-10	[1]
	oral anti-TCR induced, LAP^+CD25^+ and LAP^+CD25^-	TGF-β > IL-10	[107]
	Ag-induced[c], $CD4^+Foxp3^+$	Not applicable	[77]
	Ag-induced[b], $CD4^+Foxp3^+$	Not applicable	[53]
LP[d]	Ag-induced[b], $CD4^+Foxp3^+$	Not applicable	[53]
IEL	Anti-TCR injected[a]	IL-10 (TGF-β?)	[63]

[a]IL-10 reporter mouse model.
[b]TCR-transgenic cell transfer model.
[c]TCR-transgenic mouse model.
[d]Resident regulatory T cells in LP bear anti-inflammatory property via IL-10, TGF-β and CTLA-4 [106].

DCs and macrophages upon TLR ligation, commensal flora and food components might greatly affect such an immunological feedback (repression) system in the GALT environment through induction of IL-27 and/or IL-6 production. Indeed IL-10-producing T cells are highly abundant in the intestine and most frequently observed in PP and intraepithelial lymphocytes (IEL) in the small and large intestine [63]. IL-10-producing T cells can be induced from both $Foxp3^+$ and $Foxp3^-$ T cells when IL-10 gene-sufficient T cells are transferred into $IL\text{-}10^{-/-}$ mice, suggesting that supply of IL-10 itself from other cells is not an essential condition for the generation of Tr1 and other IL-10-producing T cells [64]. It was also shown that the IL-10-producing $Foxp3^+$ cells are necessary to protect animals from colitis [65]. Therefore, IL-10 can be induced in T cells without IL-10 from other cell types and T cell-produced IL-10 is important for the maintenance of immunological homeostasis. However, the direct effect of exogenous IL-10 on the induction of Ag-specific Tr1 is also well established [66]. The physiological role of IL-10 produced from T cells and other cell types in the gut on the generation of intestinal $Foxp3^+$ and $Foxp3^-$ IL-10-producing cells needs to be further elucidated. Table 2 summarizes the phenotypes and cytokine production of Ag-induced (or TCR-stimulated) intestinal regulatory T cells observed under homeostatic conditions. Many of these studies suggest that IL-10-producing T cells are physiological in PP whereas TGF-β-producing T cells are more easily observed in MLN.

APC microenvironment in the GI tract

TGF-β does not just play an anti-inflammatory role. It is now well described that TGF-β, in concert with proinflammatory cytokines (IL-1β, IL-6, IL-21, and/or IL-23), induces Th17 cells, which express the transcription factor ROR-γt and produce the inflammatory cytokines IL-17A, IL-17F and IL-22 [67,68]. Th17 cells have been shown to cause pathogenesis in experimental allergic encephalomyelitis and rheumatoid arthritis, as well as colitis. It has been suggested that IL-21 and IL-23 stimulation of Th17 cells expands these cells and elicits their effector function to cause pathology in mouse models *in vivo* [69,70]. Moreover, IL-23 is a key factor that breaks intestinal homeostasis because this cytokine can alone control regulatory T cell induction and overrides their immunosuppressive abilities [71]. It still needs to be confirmed whether TGF-β plus IL-6 is a sufficient combination to alone induce 'IL-10-producing' Th17 in the setting of the gut. In relation to this question, one study showed using $IL\text{-}6^{-/-}$ mice that IL-6 is essential *in vivo* to promote IL-17-producing cells but not IL-10-producing ROR γt^+ T(αβ) [72]. The combination of TGF-β and IL-6 inhibits the generation of $Foxp3^+$ cells [73] and in turn Foxp3 antagonizes ROR-γt function to inhibit Th17 cell differentiation [74,75]. Therefore, to understand the functional maturation of $Foxp3^+$, LAP^+ and IL-10-producing cells, and their mutual relationship *in vivo,* it will be important to understand how active TGF-β can be provided to T cells both spatially and temporally. Further evidence for the role of TGF-β comes from mice with a DC-specific ablation of β_8 (a component of the integrin $\alpha_v\beta_8$) that develop colitis and autoimmunity [76]. Interestingly, the inflammatory phenotype of these mice resembles that of CD4-specific depletion of TGF-βRII or TGF-β1. Integrin $\alpha_v\beta_8$ acts on LAP to release the bioactive function of TGF-β; therefore the interaction between DCs and LAP^+ T-cells is of particular interest in the generation of regulatory T cells in the GI tract. Other LAP-activating factors such as thrombospondin-1 and $\alpha_v\beta_6$ also exist, but their physiological roles in the GI tract have yet to be demonstrated.

An intriguing role for specialized APCs in the GI tract was recently demonstrated. The induction of Foxp3 in T cells by TGF-β is enhanced in the presence of intestinal DCs via RA [53,77–79]. Intestinal DCs express retinal dehydrogenase that catalyses retinal to RA, which not only affects the generation of $Foxp3^+$ T cells but also induces the expression of gut-homing receptors for T cells ($\alpha_4\beta_7$ and CCR9) [80], as well as supporting the mechanisms to enrich IgA production in the gut microenvironment [81]. Thus several mechanisms such as generation of $Foxp3^+$ cells, IgA production, and the tropism of gut lymphocytes (T cells and B cells) are mobilized in a coordinated manner by RA. These observations consolidate a central role of intestinal DC in tuning the immunity of the gut. There are several other subpopulations and distinct functions of intestinal DCs, and they are summarized in Table 1 together with the DC subpopulations mentioned above.

Specialist macrophage-APCs in the GALT also show related immunoregulatory activities. $CD11b^+CD11c^-$ LP

macrophages induce the generation of Foxp3$^+$ T cells more efficiently than DC populations in the presence of TGF-β [36]. The ability of these LP macrophages to induce Foxp3$^+$ T cells is possibly associated with their ability to produce a large amount of IL-10. This would have the effect of limiting the local production of inflammatory cytokines such as IL-6 and thereby enhance TGF-β's ability to induce Foxp3 expression.

Conclusion: oral tolerance as an avenue leading to intestinal and systemic homeostasis with Ag-specific regulatory T cells

Induction of tolerogenic DCs and Treg does not result in suppression of the total immune response. Rather, it can be accomplished in such a way that immunological homeostasis is stabilized, unwanted inflammation is reduced, and the potential of immunological activities to focus on harmful agents is increased (also see an analogous process related to the eye in the article by Stein-Streilein). Physiologically, such dynamic modulation of immunological activity and the abilities of GALT to achieve this are introduced at birth by exposure to environmental microorganisms and especially by acquisition of commensal microflora. Such early modulation of intestinal immunity ensures efficient and specific immune quiescence to food components (i.e. oral tolerance) and robust responses to pathogens encountered via mucosal surfaces (i.e. protective immunity including IgA production). Establishment of the two phases of oral tolerance (i.e. local and systemic) is critical for effective immune homeostasis and necessary to be maintained throughout life. When the body fails to establish this intestinal immune homeostasis and peripheral immune response at the early life, it might not be able to establish the systemic immune response in a sufficient manner later in life. For instance, congenitally germ-free animals not only fail to establish oral tolerance but also show poor Ag-specific immune responses [82,83]. Similarly, in humans the breakdown of either phase of oral tolerance might result in intestinal inflammatory disorders and/or food allergy in infancy, and consequently more disseminated defects in immunological homeostasis later in life. Therefore, it is essential for the body to encounter commensal bacteria at an early stage of life to achieve appropriate condition of the intestinal microenvironment (also see the article by Nanno and colleagues in this issue).

Co-opting the immune system towards avoiding unwanted inflammation and stabilizing its homeostasis is an ideal approach for the treatment of immune dysfunction. To this end, induction of Ag-specific tolerance is an attractive solution whereby promising strategies for induction of Ag-specific regulatory T cells might include usage of soluble peptides, tolerogenic adjuvants and/or cytokines, as well as direct application of Ag via the mucosal route [84–96].

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References

1 Faria, A.M. and Weiner, H.L. (2005) Oral tolerance. *Immunol. Rev.* 206, 232–259
2 Faria, A.M. and Weiner, H.L. (2006) Oral tolerance: therapeutic implications for autoimmune diseases. *Clin. Dev. Immunol.* 13, 143–157
3 Kraal, G. *et al.* (2006) The importance of regional lymph nodes for mucosal tolerance. *Immunol. Rev.* 213, 119–130
4 Tsuji, N.M. (2006) Antigen-specific CD4$^+$ regulatory T cells in the intestine. *Inflamm. Allergy Drug Targets* 5, 191–201
5 Dubois, B. *et al.* (2005) Oral tolerance and regulation of mucosal immunity. *Cell. Mol. Life Sci.* 62, 1322–1332
6 Sakaguchi, S. *et al.* (2008) Regulatory T cells and immune tolerance. *Cell* 133, 775–787
7 Hauet-Broere, F. *et al.* (2005) New cohorts of naive T cells exacerbate ongoing allergy but can be suppressed by regulatory T cells. *Allergy* 60, 1530–1536
8 Hauet-Broere, F. *et al.* (2003) Functional CD25- and CD25$^+$ mucosal regulatory T cells are induced in gut-draining lymphoid tissue within 48 h after oral antigen application. *Eur. J. Immunol.* 33, 2801–2810
9 Zinselmeyer, B.H. *et al.* (2005) In situ characterization of CD4$^+$ T cell behavior in mucosal and systemic lymphoid tissues during the induction of oral priming and tolerance. *J. Exp. Med.* 201, 1815–1823
10 Tsuji, N.M. *et al.* (2003) Antigen-specific, CD4$^+$CD25$^+$ regulatory T cell clones induced in Peyer's patches. *Int. Immunol.* 15, 525–534
11 Worbs, T. *et al.* (2006) Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J. Exp. Med.* 203, 519–527
12 Worbs, T. and Förster, R. (2007) A key role for CCR7 in establishing central and peripheral tolerance. *Trends Immunol.* 28, 274–280
13 Tsuji, N.M. *et al.* (2001) Interleukin-10-secreting Peyer's patch cells are responsible for active suppression in low-dose oral tolerance. *Immunology* 103, 458–464
14 Bäckström, N.F. and Dahlgren, U.I. (2004) Bystander suppression of collagen-induced arthritis in mice fed ovalbumin. *Arthritis Res. Ther.* 6, R151–R160
15 Millington, O.R. *et al.* (2004) Induction of bystander suppression by feeding antigen occurs despite normal clonal expansion of the bystander T cell population. *J. Immunol.* 173, 6059–6064
16 Kraus, T.A. *et al.* (2006) Evidence for a genetic defect in oral tolerance induction in inflammatory bowel disease. *Inflamm. Bowel Dis.* 12, 82–88
17 Nagata, S. *et al.* (2000) Human Peyer's Patch T Cells are sensitized to dietary antigen and display a Th cell Type 1 cytokine profile. *J. Immunol.* 165, 5315–5321
18 Guo, Z. *et al.* (2008) CD4$^+$CD25$^+$ regulatory T cells in the small intestinal lamina propria show and effector /memory phenotype. *Int. Immunol.* 20, 307–315
19 Lund, J.M. *et al.* (2008) Coordination of early protective immunity to viral infection by regulatory T cells. *Science* 320, 1220–1224
20 Mach, J. *et al.* (2005) Development of intestinal M cells. *Immunol. Rev.* 206, 177–189
21 Jang, M.H. *et al.* (2004) Intestinal villous M cells: an antigen entry site in the mucosal epithelium. *Proc. Natl. Acad. Sci. U. S. A.* 101, 6110–6115
22 Rescigno, M. *et al.* (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* 2, 361–367
23 Takayama, N. *et al.* (2007) Regulatory role of Peyer's patches for the inhibition of OVA-induced allergic diarrhea. *Clin. Immunol.* 123, 199–208
24 Kelsall, B.L. and Leon, F. (2005) Involvement of intestinal dendritic cells in oral tolerance, immunity to pathogens, and inflammatory bowel disease. *Immunol. Rev.* 206, 132–148
25 Bilsborough, J. *et al.* (2003) Mucosal CD8α$^+$ DC, with a plasmacytoid phenotype, induce differentiation and support function of T cells with regulatory properties. *Immunology* 108, 481–492
26 Contractor, N. *et al.* (2007) Peyer's patch plasmacytoid dendritic cells (pDC) produce low levels of type I interferons: possible role for IL-10, TGFβ, and prostaglandin E2 in conditioning a unique mucosal pDC phenotype. *J. Immunol.* 179, 2690–2694
27 Bryn, T. *et al.* (2008) LPS-activated monocytes suppress T-cell immune responses and induce FOXP3+ T cells through a COX-2-PGE2-dependent mechanism. *Int. Immunol.* 20, 235–245

28 Min, S.Y. *et al.* (2006) Antigen-induced, tolerogenic CD11c$^+$,CD11b$^+$ dendritic cells are abundant in Peyer's patches during the induction of oral tolerance to type II collagen and suppress experimental collagen-induced arthritis. *Arthritis Rheum.* 54, 887–898

29 Park, M.J. *et al.* (2008) Indoleamine 2,3-dioxygenase-expressing dendritic cells are involved in the generation of CD4$^+$CD25$^+$ regulatory T cells in Peyer's patches in an orally tolerized, collagen-induced arthritis mouse model. *Arthritis Res. Ther.* 10, R11

30 Miyamoto, K. *et al.* (2005) The ICOS molecule plays a crucial role in the development of mucosal tolerance. *J. Immunol.* 175, 7341–7347

31 Ito, T. *et al.* (2007) Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulatory ligand. *J. Exp. Med.* 204, 105–115

32 Zhang, Y. *et al.* (2006) Regulation of T cell activation and tolerance by PDL2. *Proc. Natl. Acad. Sci. U. S. A.* 103, 11695–11700

33 Keir, M. *et al.* (2007) PD-1 and its ligands in T-cell immunity. *Curr. Opin. Immunol.* 19, 309–314

34 Kweon, M.N. *et al.* (1999) Mucosally induced systemic T cell unresponsiveness to ovalbumin requires CD40 ligand-CD40 interactions. *J. Immunol.* 162, 1904–1909

35 Ehirchiou, D. *et al.* (2007) CD11b facilitates the development of peripheral tolerance by suppressing Th17 differentiation. *J. Exp. Med.* 204, 1519–1524

36 Denning, T.L. *et al.* (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat. Immunol.* 8, 1086–1094

37 Iwasaki, A. and Kelsall, B.L. (2001) Unique functions of CD11b$^+$, CD8α^+, and double-negative Peyer's patch dendritic cells. *J. Immunol.* 166, 4884–4890

38 Tsuji, N.M. and Nowak, B. (2004) IL-18 and antigen-specific CD4+ regulatory T cells in Peyer's patches. *Ann. N. Y. Acad. Sci.* 1029, 413–415

39 Samsom, J.N. *et al.* (2005) FcgRIIB regulates nasal and oral tolerance: a role for dendritic cells. *J. Immunol.* 174, 5279–5287

40 Monteleone, I. *et al.* (2008) IL-10-dependent partial refractoriness to Toll-like receptor stimulation modulates gut mucosal dendritic cell function. *Eur. J. Immunol.* 38, 1533–1547

41 Uematsu, S. *et al.* (2008) Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat. Immunol.* 9, 769–776

42 Zheng, Y. and Rudensky, A.Y. (2007) Foxp3 in control of the regulatory T cell lineage. *Nat. Immunol.* 8, 457–462

43 Faria, A.M. and Weiner, H.L. (2006) Oral tolerance and TGF-β-producing cells. *Inflamm. Allergy Drug Targets* 5, 179–190

44 Macpherson, A.J. and Slack, E. (2007) The functional interaction of commensal bacteria with intestinal secretory IgA. *Curr. Opin. Gastroenterol.* 23, 673–678

45 Chen, W. *et al.* (2003) Conversion of peripheral CD4$^+$CD25$^+$ naïve T cells to CD4$^+$CD25$^+$ regulatory T cells by TGF-β induction of transcription factor Foxp3. *J. Exp. Med.* 198, 1875–1886

46 Pyzik, M. and Piccirollo, C.A. (2007) TGF-β1 modulates Foxp3 expression and regulatory activity in distinct CD4$^+$ T cell subsets. *J. Leukoc. Biol.* 82, 335–346

47 Selvaraj, R.K. and Geiger, T.L. (2007) A kinetic and dynamic analysis of Foxp3 induced in T cells by TGF-β. *J. Immunol.* 178, 7667–7677

48 Zheng, S.G. *et al.* (2004) Natural and induced CD4$^+$CD25$^+$ cells educate CD4$^+$CD25- cells to develop suppressive activity: The role of IL-2, TGF-b, and IL-10. *J. Immunol.* 172, 5213–5221

49 Fantini, M.C. *et al.* (2004) Cutting edge: TGF-β induces a regulatory phenotype in CD4$^+$CD25- T cells through Foxp3 induction and down-regulation of Smad7. *J. Immunol.* 172, 5149–5153

50 Ono, M. *et al.* (2007) Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature* 446, 685–689

51 Wu, Y. *et al.* (2006) FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* 126, 375–387

52 Wan, Y.Y. and Flavell, R.A. (2007) 'Yin-Yang' functions of transforming growth factor-beta and T regulatory cells in immune regulation. *Immunol. Rev.* 220, 199–213

53 Sun, C.M. *et al.* (2007) Small intestine lamina propria dendritic cells promote *de novo* generation of Foxp3 T reg cells via retinoic acid. *J. Exp. Med.* 204, 1775–1785

54 Nakamura, K. *et al.* (2001) Cell contact-dependent immunosuppression by CD4$^+$CD25$^+$ regulatory T cells is mediated by cell surface-bound transforming growth factor β. *J. Exp. Med.* 194, 629–644

55 Oida, T. *et al.* (2003) CD4$^+$CD25- T cells that express latency-associated peptide on the surface suppress CD4$^+$CD45RBhigh-induced colitis by a TGF-β-dependent mechanism. *J. Immunol.* 170, 2516–2522

56 Chen, M.L. *et al.* (2008) Latency-associated peptide identifies a novel CD4$^+$CD25$^+$ regulatory T cell subset with TGFβ-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. *J. Immunol.* 180, 7327–7337

57 Wan, Y.Y. and Flavell, R.A. (2007) Regulatory T cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature* 445, 766–770

58 Li, M.O. *et al.* (2006) Transforming growth factor-β controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and –independent mechanisms. *Immunity* 25, 455–471

59 Marie, J.C. *et al.* (2006) Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. *Immunity* 25, 441–454

60 Awasthi, A. *et al.* (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat. Immunol.* 8, 1380–1389

61 Stumhofer, J.S. *et al.* (2007) Interleukins 27 and 6 induce STAT-3-mediated T cell production of interleukin 10. *Nat. Immunol.* 8, 1363–1371

62 McGeachy, M.L. *et al.* (2007) TGF-β and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T_H-17 cell-mediated pathology. *Nat. Immunol.* 8, 1390–1397

63 Kamanaka, M. *et al.* (2006) Expression of Interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knock in tiger mouse. *Immunity* 25, 941–952

64 Maynard, C.L. *et al.* (2007) Regulatory T cells expressing interleukin 10 develop from Foxp3$^+$ and Foxp3- precursor cells in the absence of interleukin 10. *Nat. Immunol.* 8, 931–941

65 Rubtsov, Y.P. *et al.* (2008) Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28, 546–558

66 Roncarolo, M.G. *et al.* (2006) Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol. Rev.* 212, 28–50

67 McGeachy, M.J. and Cua, D.J. (2008) Th17 cell differentiation: The long and winding road. *Immunity* 28, 445–453

68 Manel, N. *et al.* (2008) The differentiation of human T_H-17 cells requires transforming growth factor-β and induction of the nuclear receptor RORγt. *Nat. Immunol.* 9, 641–649

69 Monteleone, G. *et al.* (2008) Interleukin-21: a critical regulator of the balance between effector and regulatory T-cell responses. *Trends Immunol.* 29, 290–294

70 Hue, S. *et al.* (2006) Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J. Exp. Med.* 203, 2473–2483

71 Izcuel, A. *et al.* (2008) Interleukin-23 restrains regulatory T Cell activity to drive T Cell- dependent colitis. *Immunity* 28, 559–570

72 Lochner, M. *et al.* (2008) *In vivo* equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. *J. Exp. Med.* 205, 1381–1393

73 Bettelli, E. *et al.* (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441, 235–238

74 Zhou, L. *et al.* (2008) TGF-β-induced Foxp3 inhibits T_H17 cell differentiation by antagonizing ROR γt function. *Nature* 453, 236–240

75 Ichiyama, K. *et al.* (2008) Foxp3 inhibits ROR{gamma}t-mediated IL-17A mRNA transcription through direct interaction with ROR{gamma}t. *J. Biol. Chem.* 283, 17003–17008

76 Travis, M.A. *et al.* (2007) Loss of integrin αvβ8 on dendritic cells causes autoimmunity and colitis in mice. *Nature* 449, 361–366

77 Coombes, J.L. *et al.* (2007) A functionally specialized population of mucosal CD103$^+$ DCs induces Foxp3$^+$ regulatory T cells via a TGF-β and retinoic acid-dependent mechanism. *J. Exp. Med.* 204, 1757–1764

78 Mucida, D. *et al.* (2007) Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 317, 256–260

79 Benson, M.J. *et al.* (2007) All-trans retinoic acid mediates enhanced T regulatory cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *J. Exp. Med.* 204, 1765–1774

80 Iwata, M. *et al.* (2004) Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 21, 527–538
81 Mora, J.R. *et al.* (2006) Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 314, 1157–1160
82 Ishikawa, H. *et al.* (2008) Effect of intestinal microbiota on the induction of regulatory $CD25^+CD4^+$ T cells. *Clin. Exp. Immunol.* 153, 127–135
83 Tanaka, K. and Ishikawa, H. (2004) Role of intestinal bacterial flora in oral tolerance induction. *Histol. Histopathol.* 19, 907–914
84 Verginis, P. *et al.* (2008) Induction of antigen-specific regulatory T cells in wild-type mice: visualization and targets of suppression. *Proc. Natl. Acad. Sci. U. S. A.* 105, 3479–3484
85 Kang, Y. *et al.* (2008) Immunosuppressant as adjuvant for tolerogenic immunization. *J. Immunol.* 180, 5172–5176
86 Battaglia, M. *et al.* (2006) Rapamycin and interleukin-10 treatment induces T regulatory type 1 cells that mediate antigen-specific transplantation. *Diabetes* 55, 40–49
87 Roncarolo, M.G. and Battaglia, M. (2007) Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nat. Rev. Immunol.* 7, 585–598
88 Hawrylowicz, C.M. and O'Garra, A. (2005) Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat. Rev. Immunol.* 5, 271–283
89 Xystrakis, E. *et al.* (2006) Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J. Clin. Invest.* 116, 146–155
90 Sun, J.B. *et al.* (2006) Oral tolerance induction with antigen conjugated to cholera toxin B subunit generates both $Foxp3^+CD25^+$ and Foxp3-CD25-$CD4^+$ regulatory cells. *J. Immunol.* 177, 7634–7644
91 Huibregtse, I.L. *et al.* (2007) Induction of ovalbumin-specific tolerance by oral administration of Lactococcus lactis secreting ovalbumin. *Gastroenterology* 133, 517–528
92 Verhasselt, V. *et al.* (2008) Brest milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma. *Nat. Med.* 14, 170–175
93 Akdis, C.A. *et al.* (2006) Immunological mechanisms of sublingual immunotherapy. *Allergy* 61, 11–14
94 Nishimura, E. *et al.* (2004) Induction of antigen-specific immunologic tolerance by *in vivo* and *in vitro* antigen-specific expansion of naturally arising $Foxp3^+CD25^+CD4^+$ regulatory T cells. *Int. Immunol.* 16, 1189–1201
95 Quintana, F.J. *et al.* (2008) Control of T_{reg} and T_H17 cell differentiation by the aryl hydrocarbon receptor. *Nature* 453, 65–71
96 Hauben, E. *et al.* (2008) Activation of the aryl hydrocarbon receptor promotes allograft specific tolerance through direct- and DC-mediated effects on regulatory T cells. *Blood* 112, 1214–1222
97 Sato, A. *et al.* (2003) $CD11b^+$ Peyer's patch dendritic cells secrete IL-6 and induce IgA secretion from naive B cells. *J. Immunol.* 171, 3684–3690
98 Mora, J.R. *et al.* (2003) Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 424, 88–93
99 Annacker, O. *et al.* (2005) Essential role for CD103 in the T cell-mediated regulation of experimental colitis. *J. Exp. Med.* 202, 1051–1061
100 Johansson-Lindbom, B. *et al.* (2005) Functional specialization of gut $CD103^+$ dendritic cells in the regulation of tissue-selective T cell homing. *J. Exp. Med.* 202, 1063–1073
101 Blázquez, A.B. and Berin, M.C. (2008) Gastrointestinal dendritic cells promote Th2 skewing via OX40L. *J. Immunol.* 180, 4441–4450
102 Becker, C. *et al.* (2003) Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J. Clin. Invest.* 112, 693–706
103 Chirdo, F.G. *et al.* (2005) Immunomodulatory dendritic cells in intestinal lamina propria. *Eur. J. Immunol.* 35, 1831–1834
104 Tezuka, H. *et al.* (2007) Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. *Nature* 448, 929–933
105 Rimoldi, M. *et al.* (2005) Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat. Immunol.* 6, 507–551
106 Ochi, H. *et al.* (2006) Oral CD3-specific antibody suppresses autoimmune encephalomyelitis by inducing $CD4^+CD25\text{-}LAP^+$ T cells. *Nat. Med.* 12, 627–635
107 Izcue, A. *et al.* (2006) Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. *Immunol. Rev.* 212, 256–271