Tuning Microenvironments: Induction of Regulatory T Cells by Dendritic Cells

Yasmine Belkaid[1],* and Guillaume Oldenhove[1]
[1]Mucosal Immunology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20894, USA
*Correspondence: ybelkaid@niaid.nih.gov
DOI 10.1016/j.immuni.2008.08.005

The body requires the generation of regulatory T (Treg) cells to preserve its integrity. Each microenvironment is controlled by a specific set of regulatory elements that have to be finefrly and constantly tuned to maintain local homeostasis. These environments could be site specific, such as the gut environment, or induced by chronic exposure to microbes or tumors. Various populations of dendritic cells (DCs) are central to the orchestration of this control. In this review, we will discuss some new findings associating DCs from defined compartments with the induction of antigen-specific Treg cells.

Introduction

Several types of CD4$^+$ regulatory T (Treg) cells have been described on the basis of their origin, generation, and mechanism of action with two main origins identified: thymically derived Foxp3$^+$ Treg cells and inducible Treg cells, which are comprised of interleukin-10 (IL-10)-producing T regulatory 1 (Tr1) cells (Roncarolo et al., 2006), transforming growth factor-β (TGF-β)-producing T helper (Th) cells (Faria and Weiner, 2005), and inducible Foxp3$^+$ T cells (Chen et al., 2003). Both types of regulatory T cell, by virtue of their capacity to control the intensity of effector responses, play a major role in the control of peripheral homeostasis.

Although a large number of mechanisms, including natural Foxp3$^+$ Treg cell, contribute to the maintenance of peripheral tolerance, in defined situations or specific sites, the body requires the generation of Treg cells to preserve its integrity. Each microenvironment is controlled by a specific set of regulatory elements that have to be finely and constantly tuned to maintain local homeostasis. These environments could be site specific, such as the gut environment, or induced by chronic exposure to microbes or tumor (Figure 1). Dendritic cells (DCs) are central to the orchestration of this control (Figure 2). Under steady-state conditions, the default pathway of resident immature DCs is the induction of Treg cells. In the context of disturbance of the immune system, DCs manipulated by microbes or the tumor environment can acquire the capacity to induce new populations of antigen-specific Treg cells. These induced Treg cells are expected to have the antigen specificities and survival requirement appropriate for targeted control of immune responses.

Induction of Foxp3$^+$ Treg Cells by DCs

Although a role for Foxp3$^+$ Treg cells in the maintenance of immune tolerance has been demonstrated in both humans and mice, the origin of these cells is still not completely understood. Early neonatal thymectomy experiments in mice strongly suggested that Treg cells are generated in the thymus. Studies with Foxp3 reporter mice (Fontenot et al., 2005a) and transgenic mice that express foreign antigens in thymic tissue (Bensinger et al., 2001; Jordan et al., 2001) have also traced the development of Foxp3$^+$ cells to the thymus. Aside from evidence that natural Foxp3$^+$ Treg cells arise and mature in the thymus, there is mounting evidence that Foxp3$^+$ Treg cells can develop extra-

thymically under certain conditions. Both murine (Bettelli et al., 2006; Chen et al., 2003) and human (Fantini et al., 2004) naive T cells have been shown to express Foxp3 and acquire suppressive activity in vitro after T cell receptor (TCR) stimulation in the presence of TGF-β. In vivo, delivery of subimmunogenic doses of antigen (Kretschmer et al., 2005) as well as endogenous expression of foreign antigen in a lymphopenic environment (Knoechel et al., 2005) can also induce peripheral Foxp3$^+$ Treg cell development.

Despite a growing body of literature documenting a potential role for these converted cells in the control of autoimmune or inflammatory diseases (Curotto de Lafaille et al., 2008), the nature of the antigen-presenting cells (APCs) involved in this conversion process remains poorly understood. Several reports support the idea that immature DCs may be more efficient at inducing Foxp3$^+$ Treg cell development in the presence of TGF-β than activated DCs. For example, targeting of antigens to immature DCs via the regulatory receptor DEC205 can favor the induction of Foxp3$^+$ T cell development de novo (Knoechel et al., 2005). Although virtually all APCs at steady-state conditions may have the capacity to induce antigen-specific Treg cells, DCs appear to be more efficient at this process than other APCs (Yamazaki et al., 2007). Thus, spleen DCs are more potent than DC-depleted APCs for the induction of Treg cells and require lower doses of peptide antigen (Yamazaki et al., 2007). In the absence of exogenous IL-2, endogenous IL-2 production by T cells favoring Treg cell conversion can be efficiently triggered by DCs expressing CD80 and CD86 but not by other APCs (Yamazaki et al., 2007). However, another study has proposed that B cells are more efficient at inducing Foxp3$^+$ Treg cells than splenic DCs in the presence of TGF-β (Benson et al., 2007). This discrepancy is likely to be associated with the level of activation of the APCs in these different settings.

Several reports suggest that the status of activation of DCs as well as inflammatory mediators modulates the capacity of these cells to induce Treg cells de novo (Bettelli et al., 2006; Korn et al., 2007; Nurieva et al., 2007; Stumhofer et al., 2006; Veldhoen et al., 2006; Zhou et al., 2007). For example, IL-6 and TGF-β in tandem can direct the production of IL-17-secreting T cells (Th17 cells) over Treg cells (Bettelli et al., 2006; Veldhoen et al., 2006), and Th1 and Th2 cell effector cytokines have an antagonistic effect
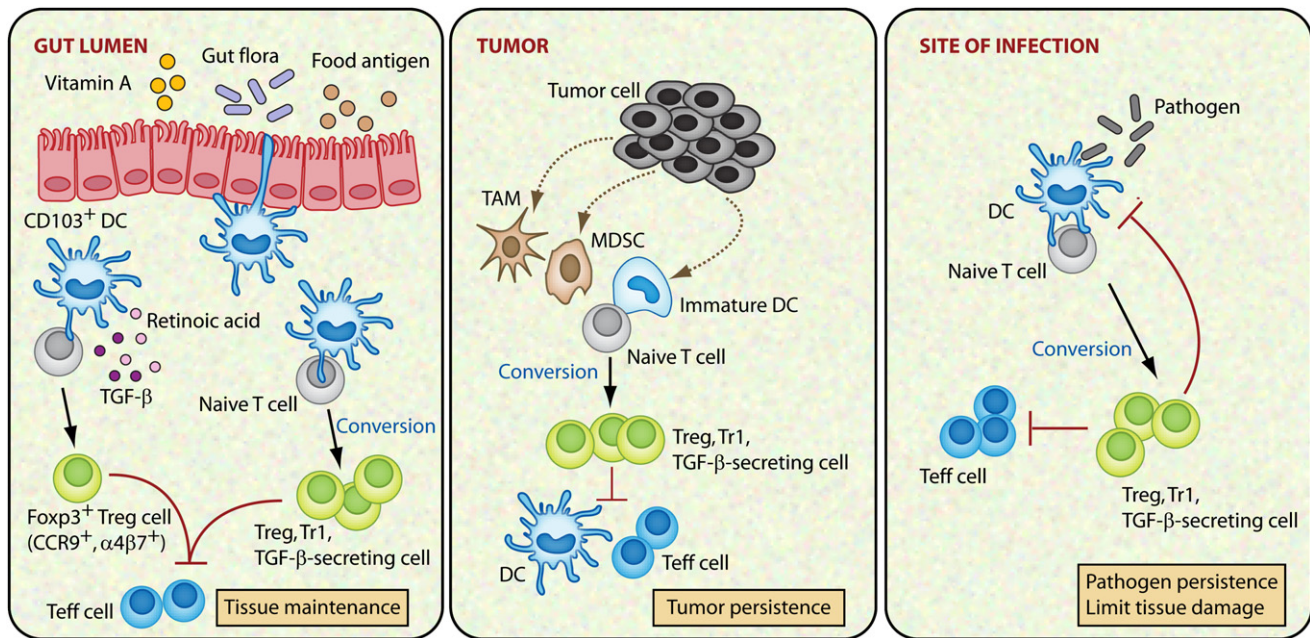
Figure 1. Induction of Regulatory T Cells in Defined Microenvironnements
In intestine lamina propria, several subsets of DCs reside and are in close contact with lumen antigen, like gut flora and food-derived antigens. To maintain local homeostasis, these DCs can induce the differentiation of Tr1 cells, TGF-β-secreting T cells, and Foxp3 Treg cells. In particular, CD103$^+$ DCs can induce the neo-conversion of Foxp3$^+$ Treg cells via their capacity to release TGF-β and retinoic acid. These de novo-induced Foxp3$^+$ Treg cells express the gut-homing receptors CC-chemokine receptor 9 (CCR9) and α4β7-integrin. In the tumor microenvironment, tumor cells produce suppressive factors that license DCs, myeloid-derived suppressor cells, or tumor-associated macrophages to induce Treg cells. The consequence of this induction is enhanced local immune suppression. In chronic infections, Treg cells can be induced. The consequence of this induction is maintenance of microbial persistence and limitation of collateral tissue damages.

on Treg cell conversion (Wei et al., 2007). Through the use of an APC-free system in vitro, it has been suggested that strong cos-timulation provided by extensive CD28 signaling can inhibit Foxp3 induction (Benson et al., 2007). In vivo, efficient induction of Foxp3$^+$ Treg cells is also abolished in the presence of strong costimulation (Kretschmer et al., 2005). DCs deficient in CD80 and CD86 induce higher expression of Foxp3 on naive T cells than control DCs (Benson et al., 2007). Furthermore, when ex vivo spleen DCs were activated with an agonist anti-CD40 or lipopolysaccharide, induction of Foxp3$^+$ Treg cells was impaired (Wang et al., 2008). Thus, activated DCs are poor inducers of Foxp3$^+$ Treg cells in favor of the induction of effector responses. This is in contrast with the observation that activated DCs are more efficient at inducing the proliferation of natural Treg cells than immature DCs (Tarbell et al., 2004; Yamazaki et al., 2003). This could suggest that in some defined situations, Treg cells could play a sequential and complementary role. For instance, the endogenous population may preferentially control highly inflammatory settings, whereas converted Treg cells may play a more important role during the downstream of the inflamma-tory process or in situation of chronic infections.

The demonstration that some DCs subsets from lymphoid tis-sues could be more efficient at inducing Treg cells than others came from a study showing that CD8$^+$ DCs induce higher conversion than other spleen DC subsets in the presence of TGF-β (Wang et al., 2008). Several molecules contribute to the induction of Foxp3$^+$ Treg cell development. For instance, the B7-CTLA-4 axis is important to favor the induction of these cells (Belghith et al., 2003; Liang et al., 2005; Zheng et al., 2006). A role

for PD-L1 expressed by DCs in the induction of Treg cells has been recently reported (Wang et al., 2008), adding another po-tential role of this molecule in the control of peripheral tolerance (Latchman et al., 2004).

Previous work demonstrates that CD3 antibody treatment transiently depletes large numbers of T cells and subsequently induces long-term immune tolerance (Belghith et al., 2003). A re-cent study provides evidence that the mechanism underlying this regulatory effect is an enhanced production of TGF-β by macrophages and immature DCs after engulfment of apoptotic T cells (Perruche et al., 2008). This increase in TGF-β induces the development of Foxp3$^+$ Treg cells and contributes to immune tolerance (Perruche et al., 2008). Clearance of apoptotic bodies has been shown to lead to the development of Treg cells (Lacy-Hulbert et al., 2007). The relative contribution of this pathway in the constitutive generation of Treg cells at sites with a high amount of remodeling (e.g., gut or uterus) remains to be addressed.

Role of Tissue-Specific DCs in the Induction of Treg Cells

No other tissue is subjected to more antigenic pressure than the gut. For instance, the adult human intestine contains up to 10^{14} microorganisms (Backhed et al., 2005). It is therefore a highly regulated immunologic site that must generate both tolerogenic and immunogenic responses. On one hand, immune reactivity against nonpathogenic gut elements is not only wasteful but also dangerous to the host and is known to lead to severe tissue damages (e.g., inflammatory bowel disease). On the other hand,
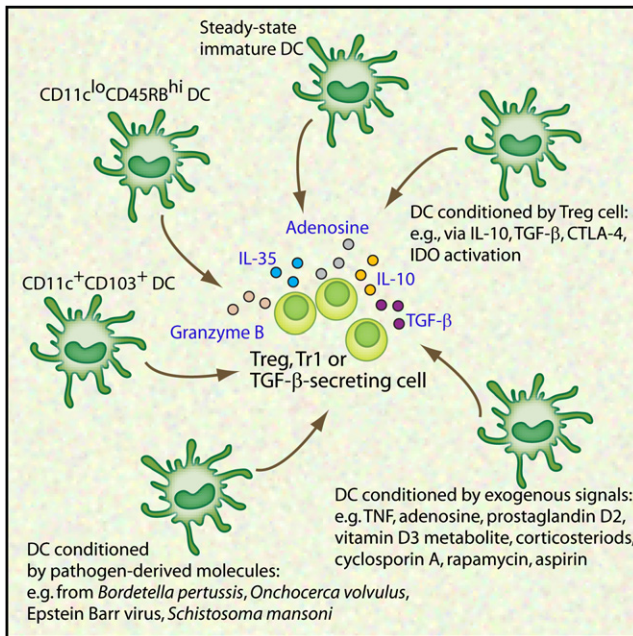
Figure 2. Various Populations of DCs Can Induce Antigen-Specific Treg Cells
Steady-state immature DCs can induce Treg cells. Defined subsets of gut DCs such as CD11c^loCD45RB^hi or CD103^+ can induce new populations of Treg cells. In some cases, DCs conditioned by Foxp3^+ Treg cells, pathogen-derived molecules (e.g., filamentous haemagglutinin [FHA], adenylate cyclase toxin [CyaA]) or exogenous signals [e.g., TNF-α, adenosine, prostaglandin D(2), or immunosuppressive drugs like Vitamin D3 metabolite 1a,25-(OH)2D3, corticosteroids] can induce new population of Treg cells. Various populations of Treg cells are induced to control local homeostasis. Foxp3^+ Treg cells can control local responses via mechanisms including the production of IL-10, TGF-β, cAMP, Granzyme B, adenosine, IL-35, or CTLA-4 (Vignali, 2008). Tr1 cells can control immune responses via their capacity to release TGF-β and IL-10. TGF-β producing Th cells can control local responses via their production of TGF-β.

the development of active immunity is required to protect the host against invasive pathogens. Different subsets of Treg cells have been shown to be instrumental in the maintenance of this complex homeostasis. Additionally, several subsets of DCs with regulatory properties have been described with the capacity to induce IL-10 secretion from T cells or induce oral tolerance at steady-state conditions (Chirdo et al., 2005; Coombes and Powrie, 2008; Iwasaki and Kelsall, 2001; Mowat, 2003). Some of these features may have been influenced by conditioning signals received from noninflammatory cytokines constitutively produced by the intestinal epithelia. These cytokines include TGF-β and the Th2 cell response driving thymic stromal lymphopoietin (Barnard et al., 1993; Coombes and Powrie, 2008; Rimoldi et al., 2005).

It has recently been demonstrated that the gut-associated lymphoid tissue is a preferential site for the peripheral induction of Foxp3^+ Treg cells (Coombes et al., 2007; Mucida et al., 2005, 2007; Sun et al., 2007). A role for local DCs in this conversion process is supported by the observation that DCs from the lamina propria of the small intestine and from the mesenteric lymph node (MLN) are noticeably better than splenic DCs at inducing the expression of Foxp3 in naive T cells in the presence of exogenous TGF-β (Coombes et al., 2007; Sun et al., 2007). Similarly,

lamina propria macrophages can efficiently induce Foxp3^+ T cells (Denning et al., 2007). In particular, DCs expressing CD103^+ in these two compartments can induce Foxp3^+ T cells in the absence of any exogenous factors (Coombes et al., 2007; Sun et al., 2007). This conversion process was associated with their capacity to release bioactive TGF-β, which could be linked with their capacity to activate latent TGF-β (Coombes et al., 2007). This hypothesis is supported by the observation that DCs lacking the TGF-β-activating integrin αvβ8 or αv fail to induce Foxp3^+ Treg cells in vitro (Lacy-Hulbert et al., 2007; Travis et al., 2007). Furthermore, mice in which myeloid cells do not express αv or DCs that do not express αvβ8 have reduced colonic Foxp3^+ Treg cells and develop colitis (Lacy-Hulbert et al., 2007; Travis et al., 2007). Loss of v-integrin expression by myeloid cells led to the development of intestinal inflammation, probably through the combined effects of a failure to remove apoptotic cells and a loss of TGF activation (Lacy-Hulbert et al., 2007).

Although DCs derived from the lamina propria and MLN can efficiently induce Treg cells, the exact sites in which these events take place remains unclear. We cannot exclude that Treg cell conversion may occur in the lamina propria, because a small but sizeable population of naive cells can be found in this tissue. However, early after oral feeding with antigen, most of the converted cells can be found in the MLN preceding their accumulation in the lamina propria (Y.B., unpublished data). Gut DCs constitutively transport apoptotic epithelial cells to the MLN (Huang et al., 2000). Recent evidence also suggest that the majority of CD103^+ DCs represent a tissue-derived migratory population that plays an important role in presenting orally derived soluble antigen to T cells (Jaensson et al., 2008). Thus, as predicted by previous studies showing a central role for the MLN in the acquisition of oral tolerance (Spahn et al., 2002), most of the conversion may occur in this compartment in response to tissue-migrating CD103^+ DCs. A likely hypothesis would be that these gut-converted Treg cells could become part of the peripheral Treg cell pool. So, over time, the gut flora, oral pathogens, or food may have an important role in shaping the repertoire of peripheral Foxp3^+ Treg cells. The relative contribution of these converted Treg cells to peripheral tolerance and the outcome of infections, as well as how pathogens can utilize or interfere with this pathway to favor their own survival, remains to be addressed. Currently, in absence of definitive markers to distinguish endogenous versus converted Foxp3^+ Treg cells, these questions will remain difficult to answer.

Role of DC-Derived Products in Imprinting Homing and Regulatory Properties
Gut DCs also have an important role in dictating the homing potential of lymphocytes. DCs isolated from the Peyer's patches, small-intestinal lamina propria, and MLNs promote the expression of the gut-homing receptors α4β7-integrin and CCR9 by CD4^+ and CD8^+ T cells (Johansson-Lindbom et al., 2003, 2005; López-Bravo and Ardavín, 2008, in this issue of *Immunity*; Mora et al., 2003; Stagg et al., 2002). The molecule CCR9 binds to CCL25 produced by epithelial cells of the small intestine, and α4β7-integrin binds to mucosal vascular addressin cell-adhesion molecule 1 (MADCAM1), which is expressed by the vascular endothelium of the gastrointestinal tract. It is also becoming clear that nutrient status can impact an individual's susceptibility to

intestinal pathologies (Ziegler et al., 2003). In the case of vitamin A and, in particular, its transcriptionally active metabolite, retinoic acid (RA), prolonged insufficiency not only disrupts the integrity of the intestinal epithelial barrier but also prevents the proper deployment of effector lymphocytes into the gut-associated lymphoid tissue (GALT) after priming. Indeed, the capacity of GALT DCs to imprint gut-homing receptors to lymphocytes is associated with their capacity to release retinoic acid (Iwata et al., 2004; Mora et al., 2006; Ziegler et al., 2003). More recently, it was demonstrated that RA and cytokines produced by DCs in the Peyer's Patches synergized to promote IgA secretion by gut-activated B cells (Mora et al., 2006). Importantly, the addition of RA to naturally occurring Treg cells in vitro can promote their expression of gut tropism receptors and subsequently favor their migration to the GALT (Siewert et al., 2007). Another effect of RA on the immune regulation of the gastrointestinal (GI) tract is associated with its capacity to enhance the TGF-β-mediated generation of Foxp3$^+$ Treg cells from naive T cells by gut DCs (Benson et al., 2007; Coombes et al., 2007; Denning et al., 2007; Elias et al., 2008; Kang et al., 2007; Mucida et al., 2007; Schambach et al., 2007; Sun et al., 2007) (Figure 1). The induction of Foxp3 observed in the presence of small-intestinal lamina propria DCs and CD103$^+$ MLN DCs can be inhibited by a retinoic-acid receptor (RAR) antagonist (Coombes et al., 2007; Sun et al., 2007). Conversely, incubation of splenic or CD103$^-$ MLN DCs with both TGF-β and RA enhanced their capacity to induce Foxp3 Treg cells (Coombes et al., 2007; Mucida et al., 2007; Sun et al., 2007). RA produced by intestinal macrophages can also synergize with TGF-β to induce Foxp3$^+$ Treg cells (Denning et al., 2007). Importantly, RA can induce the conversion of naive CD4$^+$ T cells purified from human cord blood into Foxp3$^+$ Treg cells (Kang et al., 2007). Reciprocally, RA can inhibit the generation of Th17 cells (Elias et al., 2008; Kang et al., 2007; Mucida et al., 2007; Schambach et al., 2007), suggesting that RA may play an important role in maintaining the balance between effector and regulatory populations in the GI tract. The mechanism by which RA produced by DCs can enhance the capacity of TGF-β to induce Foxp3 on naive T cells remains unclear but is likely due to a conjunction of effects on both T cells and DCs. One possible role of RA would be via its capacity to enhance TGF-β signaling, e.g., RA can increase the expression of TGF-β receptor subunit (Balmer and Blomhoff, 2002). Another possibility could be associated with the capacity of RA to suppress effector cytokines known to suppress the induction of Foxp3 by T cells (Cantorna et al., 1996; Wei et al., 2007).

In addition to the gut, other compartments could be involved in the generation of Foxp3$^+$ T cells. For instance, a recent report demonstrated that liver plasmacytoid DCs can contribute to the acquisition of tolerance against oral antigens (Goubier et al., 2008, in this issue). Of importance, the liver and in particular Ito cells can store up to 80% of total body retinol (Geerts, 2004). How liver DCs can acquire retinol form these cells and convert it to RA remains to be addressed.

Although the capacity of GALT DCs or macrophages to imprint gut-homing receptors and induce Foxp3$^+$ Treg cells is associated with their capacity to release RA, it remains unclear whether these cells are the main producer of this metabolite in the gut. Synthesis of RA from stored or dietary retinol depends on the expression of the appropriate enzymes, which can be expressed directly by GALT DCs. DCs from Peyer's patches and MLNs express *Aldh1a1* and *Aldh1a2*, respectively (Coombes et al., 2007; Iwata et al., 2004). DCs from the lamina propria express a large array of this family of enzymes, such as ADH1, ADH4, ADH5, Adh1a1, Adh1a2, and Adh1a3 (Y.B., unpublished data). Supporting the idea of a role for these cells as producers of RA, Peyer's patch and MLN DCs can directly convert retinol to RA in culture (Iwata et al., 2004). However other cells, including epithelial cells, can express enzymes associated with vitamin A metabolism (Saurer et al., 2007), suggesting that DCs may also acquire RA from other sources and store it. A recent study demonstrates that monocyte-derived DCs pretreated with RA can acquire several attributes characteristic of mucosal DCs, such as secretion of TGF-β and IL-6 and the capacity to augment mucosal-homing receptor expression and IgA production. In this particular study, these gut-derived features acquired by DCs were associated with the capacity of DCs to become carriers and not producers of RA (Saurer et al., 2007). The precise factors that govern the activation of some of these enzymes and how inflammation or infections modify the metabolism of vitamin A remain to be explored. Another important question would be to understand the timing necessary for DCs migrating in the GALT to acquired RA from epithelial cells and how these processes can be modified during inflammatory responses. How RA contributes to oral tolerance and at the same time protective immunity in the GI tract also remains to be addressed. One possibility would be that RA could favor the induction of Treg cells in the absence of secondary signals but enhances effector response after exposure to inflammatory mediators. Indeed, lamina propria DCs stimulated with flagellin can induce the differentiation of Th17 cells in an RA-dependent manner, suggesting that at physiological doses RA does not inhibit but rather promotes this pathway (Vijay-Kumar et al., 2008). This observation is consistent with the fact that the gut enriched in RA is home to a large number of IL-17-producing T cells at steady-state condition (Ivanov et al., 2006). After exposure to CpG, lamina propria dendritic cells can prevent the induction of Treg cells while inducing effector T cells expressing a high amount of RA-dependent gut-homing receptors (Y.B., unpublished data).

The shared feature by local mediators to imprint homing and regulatory properties is also observed in another defined microenvironment. Vitamin D3 is generated as an inactive form in the skin in response to sunlight and is converted to the active form, 1,25-dihydroxyvitamin D$_3$ [1,25(OH)$_2$D$_3$], by an enzymatic cascade involving 25-hydroxylases and 1-hydroxylase. The D vitamins have many effects on immune cells. It has been shown that 1,25(OH)$_2$D$_3$ can inhibit the differentiation and maturation of DCs (Griffin et al., 2000; Penna and Adorini, 2000; Piemonti et al., 2000), leading to decreased IL-12 and enhanced IL-10 production and decreased T cell activation. Several experimental evidences support the regulatory function of vitamin D. Mouse models revealed that that 1,25(OH)$_2$D$_3$ can inhibit several autoimmune-disease models in mice (Cantorna and Mahon, 2005). Vitamin D3 in combination with dexamethasone can also favor the induction of cells able to produce IL-10 with strong regulatory properties (O'Garra et al., 2008). Vitamin D3 was also shown to confer T cell tropism in the skin (Sigmundsdottir et al., 2007). CCL27 is a skin-specific chemokine ligand expressed by keratinocytes in the epidermis, and the expression of its receptor CCR10 allows T cells to migrate toward this chemokine. Vitamin

D3 increases the expression of this receptor. Furthermore, active vitamin D3 suppressed the expression of the gut-homing receptors α4β7-integrin and CCR9 by T cells. Gene-expression analysis of skin DCs showed that they express both 25-hydroxylase and 1-hydroxylase, produce active vitamin D₃, and induce CCR10 expression on T cells (Sigmundsdottir et al., 2007). Vitamin D can also enhance the suppressive capacity of Foxp3⁺ T cells from regional lymph node when delivered topically (Gorman et al., 2007).

Defined microenvironments may have evolved self-containing strategies in which local mediators can imprint homing properties while at the same time favoring the induction or function of Treg cells. Site-specific cells or factors such as neurons or hormones can also favor the induction of Foxp3⁺ Treg cells (Liu et al., 2006; Tai et al., 2008). It is therefore tempting to speculate that a link between homing and regulatory function induction may represent a more general mechanism. Such strategy could allow the constant generation and migration of Treg cells to defined compartments. These Treg cells are expected to have the prerequisite antigen specificities (e.g., flora antigens), status of activation, and survival requirement, allowing them to regulate a defined microenvironment.

Treg Cells Induced by Microbe-Manipulated DCs

In order to sustain their transmission and/or reproduction, a large number of microbes have to establish long-term interactions with their host. During this coexistence, the microbe must avoid elimination by the host immune response and sustain its life cycle, while at the same time delaying or preventing host destruction. Microbe-mediated modulation of innate and acquired immune responses of the persistently infected host has to meet these requirements and restore a homeostatic environment. Failure to establish or maintain homeostatic conditions usually causes disease. This is clearly the case of microflora that invade our gut or our skin, as well as for pathogenic microbes that establish chronic infections. All persistent microbes obey the same principle: the immune system constitutes their ecological niche, and they have coevolved with their host to learn how to manipulate APC function in order to dictate an immune response appropriate to insure their survival. For instance, microbes have been shown to induce a large array of regulatory cells to insure their own survival (Belkaid, 2007). Surviving an infection requires the generation of a controlled immune response in the host that recognizes and controls the invading pathogen while limiting collateral damage to self-tissues that may result from an exuberant immune response. This implies that induction of Treg cells also arises as a result of the host response to the infectious process in a bid to maintain or restore a homeostatic environment and/or that it can be actively induced by the pathogen to promote pathogen survival (Belkaid, 2007) (Figure 1).

The role of IL-10 as an immunoregulatory cytokine in infection has been mainly documented in the context of chronic infections (Moore et al., 2001). IL-10 can inhibit the immune responses (by both Th1 cells and Th2 cells) to many pathogens in experimental models (Gazzinelli et al., 1992; Hoffmann et al., 2000; Li et al., 1999) and in human infectious diseases, such as tuberculosis, malaria, hepatitis C, filariasis, leishmaniasis, and schistosomiasis (Boussiotis et al., 2000; Carvalho et al., 1994; King et al., 1996; MacDonald et al., 2002; Mahanty et al., 1997; Plebanski

et al., 1999). The most remarkable example of this control is illustrated by its crucial role during acute infection of mice with *Toxoplasma gondii*. In this model, IL-10 production by T cells is the key regulator of effector cell responses, because IL-10-deficient mice can control parasite number, but they succumb to lethal immunopathology driven by unrestrained effector cell responses (Gazzinelli et al., 1996). During Th2 cell-dominated helminths infection, the majority of IL-10 is produced by the Th2 cells (Moore et al., 2001). Besides T cells, IL-10 can also be produced by numerous cell types, including B cells, natural killer (NK) cells, macrophages, and DCs (reviewed in Moore et al., 2001). In acute *Plasmodium yoelii* infection, a subset of regulatory DCs expressing CD11cˡᵒCD45RBʰⁱ and inducing IL-10-secreting T cells becomes the predominant DC population in the spleen (Wong and Rodriguez, 2008). IL-10 can also be produced by natural Treg cells and, in some cases, is associated with their function; however, in most cases, the inducible Tr1 cell population is the relevant source of this cytokine during infection. During various infections, Tr1 cells develop from conventional T cells after encountering with certain signals, such as exposure to deactivated or immature APCs, repeated exposure to antigen, or IL-10 itself (reviewed in O'Garra et al., 2004; Roncarolo et al., 2006). Of note, these conditions prevail during chronic infection in which APC functions are often targeted by the pathogen and cells of the immune system are chronically exposed to microbial antigens. Consistent with a role for these cells in human disease, Tr1 cell clones can be isolated from patients who are chronically infected with hepatitis C virus (HCV) (MacDonald et al., 2002). Interestingly, these regulatory clones had similar viral antigen specificity to protective Th1 cell clones isolated from the same patient (MacDonald et al., 2002). Defined microbial products can manipulate DCs in a way that induces Treg cell populations (Mills and McGuirk, 2004) (Figure 1). For example, filamentous haemagglutinin (FHA) from *Bordetella pertussis* was shown to induce IL-10 production by DCs; these DCs favor the differentiation of naive T cells into Tr1 cells (McGuirk et al., 2002). Similarly, Tr1 cells can be generated from naive T cells in the presence of DCs stimulated with phosphatidylserine from *Schistosoma mansoni* (Van der Kleij et al., 2002).

One promising therapeutic approach has emerged from the observation that microbial products can favor the induction of Tr1 cell populations in vivo. Exposure of mice to *S. mansoni* antigen prevents development of type 1 diabetes in NOD mice (Zaccone et al., 2003), as well as experimental colitis (Elliott et al., 2003). The use of single microbial molecules as therapeutic agents has been recently shown as FHA of *Bordetella pertussi* can efficiently treat experimental colitis (Braat et al., 2006).

Although Tr1 cells define a population of T cells that can produce IL-10 and/or TGF-β, some IL-10-producing T cells can also produce IFN-γ. The autocrine regulation by IL-10 of Th1 and Th2 cells was initially described in human clones (Del Prete et al., 1993). In the context of an infectious disease, IFN-γ and IL-10 double producers were first described in the bronchoalveolar lavage of patients with tuberculosis (Gerosa et al., 1999) and in individuals chronically infected with *Borrelia burgdorferi* (Pohl-Koppe et al., 1998). Indeed, in many chronic infections, in humans and experimental animals, the presence of CD4⁺ T cells that produce high amounts of both IL-10 and IFN-γ have been documented (reviewed in Trinchieri, 2001).

Recently it was shown that IFN-γ- and IL-10-producing CD4$^+$ T cells emerge during experimental infection with *T. gondii* and in a model of nonhealing Leishmaniasis (Anderson et al., 2007; Jankovic et al., 2007) and that these cells share many features with Th1 cells and were the main source of protective IL-10. These T cells were identified as activated T-bet$^+$ Th1 cells and were distinct from Th2 cells, natural Treg cells, or other subsets of inducible regulatory T cells. Unlike IFN-γ production, IL-10 production was transient, observed in only a fraction of the IFN-γ-producing cells, and was produced more rapidly by recently activated T cells than by resting T cells (Jankovic et al., 2007). The instability of IL-10 synthesis, which was observed only when the Th1 cells were fully activated, is probably necessary to prevent sustained suppression of effector functions. Thus, it appears that, in some cases, cells with regulatory properties could arise from fully differentiated Th1 cells as a negative-feedback loop. It is likely that numerous previous studies of Tr1 cells were in fact incriminating similar populations. These IFN-γ- and IL-10-producing T cells may represent a dominant regulatory response to infections that induce highly polarized Th1 cell responses.

The nature of the APC or status of activation required for the imprinting of IL-10 on these Th1 cells remains poorly understood, but some evidence suggests that the cytokines produced by DCs could contribute to this phenotype. For instance, repetitive exposure to IL-12 could induce IL-10 on IFN-γ-producing cells (Meyaard et al., 1996). Several recent reports suggested that IL-27 might be an important determinant for the induction of IL-10 on Th1 cells (Awasthi et al., 2007; Fitzgerald et al., 2007; Stumhofer et al., 2007). A role for this cytokine as a regulatory mediator has recently emerged. IL-27 can limit Th1, Th2, and Th17 cell responses in various models of infection and autoimmunity (Kastelein et al., 2007). DCs from the spleen modified by exposure to TGF-β-producing Treg cells acquire a plasmacytoid phenotype and release TGF-β and IL-27, which in turn allow the induction of IL-10-producing T cells (Awasthi et al., 2007). How IL-27 could contribute to the induction of IL-10 in the GI tract and how this pathway could contribute to the maintenance of gut homeostasis remains to be addressed.

Induction of Foxp3$^+$ Treg Cells by DCs during Infections

Acute infection with *Listeria monocytogenes* in mice failed to induce Foxp3 by conventional CD4$^+$ T cells (Fontenot et al., 2005b). Thus, highly inflammatory environments that will prevail in acute infection may not favor the emergence of Foxp3$^+$ T cells. This hypothesis is supported by the observation that Th1 or Th2 cell-polarizing cytokines can interfere with the induction of these cells (Wei et al., 2007). However, chronic infections may require an additional layer of regulation, which would be provided by converted Foxp3$^+$ Treg cells. This hypothesis is supported by the observation that during infection, the downstream effects of inflammatory responses are also often associated with anti-inflammatory processes, including TGF-β production. Furthermore, some pathogens target sites in which TGF-β is highly produced, such as the GI tract, the skin, and the eye, which may assist in the conversion in vivo. TGF-β can be also produced by infected cells or by cells the microorganisms are in contact with, or arise as a result of an inflammatory process. For example, the trypomastigote stage of *Trypanosoma cruzi* induced TGF-β and IL-10 secretion by DCs (Poncini et al., 2008). Compel-

ling data in a mouse model of malaria suggest that TGF-β and Treg cells are central regulators of immunopathology and parasite expansion (Walther et al., 2005). During late infection with *Plasmodium yoelii* infection, DCs migrate to the spleen of infected mice and secrete TGF-β together with IL-10 and PGE2 (Ocana-Morgner et al., 2007). After experimental malaria infection of human volunteers, enhanced TGF-β and Foxp3$^+$ Treg cell responses in peripheral blood mononucleated cells correlate with a faster parasitic growth rate (Walther et al., 2005). Cells with natural Treg cell characteristics are rapidly induced after blood-stage infection and are associated with a decrease of proinflammatory cytokines and antigen-specific responses. Monocytes are a likely source of the early TGF-β production in this infection (Walther et al., 2005). Some nematodes can themselves express homologs of TGF-β (Gomez-Escobar et al., 1997). Compelling experimental data support the idea that Foxp3$^+$ Treg cells can be induced during *Heligmosomoides polygyrus* infection (Finney et al., 2007). This pathway may not be limited to the GI tract, because we found that the Bacillus Calmette-Guerin (BCG) can induce new populations of Foxp3$^+$ T cells in vivo that accumulate at the dermal site of infection (Y.B., unpublished data). The relative contribution of these converted Treg cells to peripheral tolerance and the outcome of infections, as well as how pathogens can utilize or interfere with this pathway to favor their own survival, remains to be addressed. Currently, in absence of definitive markers to distinguish endogenous versus converted Foxp3$^+$ regulatory T cells, these questions will remain difficult to answer.

However, the interaction with persistent microbes does not always lead to the induction of Treg cells. As discussed above, activation of DCs that can be mediated by TLR ligands impairs Treg conversion. We found that gut flora-derived DNA (gfDNA), but not other TLR agonists, strongly constrained the capacity of lamina propria dendritic cells to induce Treg cell conversion in vitro and can act as a natural adjuvant for priming intestinal responses via modulation of Treg-Teff (T effector) cell equilibrium (Y.B., unpublished data). This would suggest that in some highly regulated environment, persistent microbes might limit peripheral conversion by modulating APC function.

DCs as Targets of Treg Cell Regulation: The Concept of Infectious Tolerance

Treg cells directly interact with DCs in vivo (Tang et al., 2006). Treg cells, which are more mobile than naive T cells in vitro, out compete the latter in aggregating around DCs via a mechanism dependent on LFA-1 (Onishi et al., 2008). After forming aggregates, Treg cells specifically downregulate the expression of CD80 and CD86, but not CD40 or class II MHC, on DCs in both a CTLA-4- and LFA-1-dependent manner (Onishi et al., 2008). Treg cells exert this CD80- and CD86-downmodulating effect even in the presence of strong DC-maturing stimuli. A consequence of this interaction is the induction of infectious tolerance, which is believed to allow the expansion of the regulatory environment in a bystander manner. The interaction of Treg cells with DCs impairs the capacity of the antigen-presenting cell to establish lasting interaction with effector T cells (Tadokoro et al., 2006), and CTLA-4 appears to play an important role in this process (Oderup et al., 2006). Importantly, Treg cells via CTLA-4 interaction can initiate the immunoregulatory pathway

of tryptophan catabolism in DCs. The mechanisms by which indoleamine 2,3-dioxygenase (IDO) downregulates immune responses are still elusive and may involve multiple pathways. In particular, CD4$^+$ T cells exposed to low tryptophan and kynurenin produced increased amounts of IL-10 and TGF-β but little IFN-γ and IL-4 (Fallarino et al., 2006).

In a model of *Aspergillus conidia* infection in mice, control of allergic immunopathology induced by the fungus requires the sequential activity of various populations of Treg cells (Montagnoli et al., 2006). Early in infection, inflammation is controlled by the expansion and local recruitment of natural Treg cells that are capable of limiting innate immune responses through the combined action of IL-10 and CTLA-4 to induce the production of IDO by APCs. This control of innate responses, in particular of DCs, leads to the subsequent activation and expansion of Tr1 cells that produce both IL-10 and TGF-β. In turn, Tr1 cells can inhibit Th2 cells, which are responsible for the allergic response to the fungus (Montagnoli et al., 2006). This sequential role for various populations of regulatory T cells orchestrated by DCs may not be an exception but the rule, as most infections proceed through various stages and therefore require various layers of regulation.

Immune regulation has to be shaped in a manner specific to the microenvironment targeted. Because a large number of studies evaluating the mechanism of Treg cell induction were done with mitogenic stimuli or DCs from lymphoid organs, our understanding of tissue-specific DCs, the complexity of the local subsets, and the peculiarity of their function remains sparse and clearly requires further exploration. An additional challenge to a better understanding of local regulation is associated with the extreme plasticity of both DC and Treg cell populations. Evidence suggests that Treg cells may not always be stable and that some interactions with some stimulatory signals can deprogram them (Degauque et al., 2008). Furthermore, even regulatory DCs conditioned by a specific microenvironment can become potent inducers of immune responses upon inflammatory settings. We have only just begun to grasp the complexity of the dialog between tissue-specific DCs and regulatory T cells, and a better understanding of this local regulation is clearly needed for the design of rational and targeted approaches to either disturb (as in the case of tumors) or restore local homeostasis (as in the case of gut inflammatory diseases).

ACKNOWLEDGMENTS

REFERENCES

Anderson, C.F., Oukka, M., Kuchroo, V.J., and Sacks, D. (2007). CD4(+)CD25(−)Foxp3(−) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. J. Exp. Med. *204*, 285–297.

Awasthi, A., Carrier, Y., Peron, J.P., Bettelli, E., Kamanaka, M., Flavell, R.A., Kuchroo, V.K., Oukka, M., and Weiner, H.L. (2007). A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat. Immunol. *8*, 1380–1389.

Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I. (2005). Host-bacterial mutualism in the human intestine. Science *307*, 1915–1920.

Balmer, J.E., and Blomhoff, R. (2002). Gene expression regulation by retinoic acid. J. Lipid Res. *43*, 1773–1808.

Barnard, J.A., Warwick, G.J., and Gold, L.I. (1993). Localization of transforming growth factor beta isoforms in the normal murine small intestine and colon. Gastroenterology *105*, 67–73.

Belghith, M., Bluestone, J.A., Barriot, S., Megret, J., Bach, J.F., and Chatenoud, L. (2003). TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. Nat. Med. *9*, 1202–1208.

Belkaid, Y. (2007). Regulatory T cells and infection: A dangerous necessity. Nat. Rev. Immunol. *7*, 875–888.

Bensinger, S.J., Bandeira, A., Jordan, M.S., Caton, A.J., and Laufer, T.M. (2001). Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4(+)25(+) immunoregulatory T cells. J. Exp. Med. *194*, 427–438.

Benson, M.J., Pino-Lagos, K., Rosemblatt, M., and Noelle, R.J. (2007). Alltrans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. J. Exp. Med. *204*, 1765–1774.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235–238.

Boussiotis, V.A., Tsai, E.Y., Yunis, E.J., Thim, S., Delgado, J.C., Dascher, C.C., Berezovskaya, A., Rousset, D., Reynes, J.M., and Goldfeld, A.E. (2000). IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. J. Clin. Invest. *105*, 1317–1325.

Braat, H., McGuirk, P., Kate, F.T., Huibregtse, I., Dunne, P., Hommes, D., Deventer, S., and Mills, K. (2006). Prevention of experimental colitis by parenteral administration of a pathogen-derived immunomodulatory molecule. Gut *56*, 351–357.

Cantorna, M.T., and Mahon, B.D. (2005). D-hormone and the immune system. J. Rheumatol. Suppl. *76*, 11–20.

Cantorna, M.T., Nashold, F.E., Chun, T.Y., and Hayes, C.E. (1996). Vitamin A down-regulation of IFN-gamma synthesis in cloned mouse Th1 lymphocytes depends on the CD28 costimulatory pathway. J. Immunol. *156*, 2674–2679.

Carvalho, E.M., Bacellar, O., Brownell, C., Regis, T., Coffman, R.L., and Reed, S.G. (1994). Restoration of IFN-gamma production and lymphocyte proliferation in visceral leishmaniasis. J. Immunol. *152*, 5949–5956.

Chen, W., Jin, W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., and Wahl, S.M. (2003). Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J. Exp. Med. *198*, 1875–1886.

Chirdo, F.G., Millington, O.R., Beacock-Sharp, H., and Mowat, A.M. (2005). Immunomodulatory dendritic cells in intestinal lamina propria. Eur. J. Immunol. *35*, 1831–1840.

Coombes, J.L., and Powrie, F. (2008). Dendritic cells in intestinal immune regulation. Nat. Rev. Immunol. *8*, 435–446.

Coombes, J.L., Siddiqui, K.R., Arancibia-Carcamo, C.V., Hall, J., Sun, C.M., Belkaid, Y., and Powrie, F. (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-{beta}- and retinoic acid-dependent mechanism. J. Exp. Med. *204*, 1757–1764.

Curotto de Lafaille, M.A., Kutchukhidze, N., Shen, S., Ding, Y., Yee, H., and Lafaille, J.J. (2008). Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. Immunity *29*, 114–126.

Degauque, N., Mariat, C., Kenny, J., Zhang, D., Gao, W., Vu, M.D., Alexopoulos, S., Oukka, M., Umetsu, D.T., DeKruyff, R.H., et al. (2008). Immunostimulatory Tim-1-specific antibody deprograms Tregs and prevents transplant tolerance in mice. J. Clin. Invest. *118*, 735–741.

Del Prete, G., De Carli, M., Almerigogna, F., Giudizi, M.G., Biagiotti, R., and Romagnani, S. (1993). Human IL-10 is produced by both type 1 helper (Th1) and

type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J. Immunol. 150, 353–360.

Denning, T.L., Wang, Y.C., Patel, S.R., Williams, I.R., and Pulendran, B. (2007). Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. Nat. Immunol. 8, 1086–1094.

Elias, K.M., Laurence, A., Davidson, T.S., Stephens, G., Kanno, Y., Shevach, E.M., and O'Shea, J.J. (2008). Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. Blood 111, 1013–1020.

Elliott, D.E., Li, J., Blum, A., Metwali, A., Qadir, K., Urban, J.F., Jr., and Weinstock, J.V. (2003). Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G385–G391.

Fallarino, F., Grohmann, U., You, S., McGrath, B.C., Cavener, D.R., Vacca, C., Orabona, C., Bianchi, R., Belladonna, M.L., Volpi, C., et al. (2006). The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J. Immunol. 176, 6752–6761.

Fantini, M.C., Becker, C., Monteleone, G., Pallone, F., Galle, P.R., and Neurath, M.F. (2004). Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25- T cells through Foxp3 induction and down-regulation of Smad7. J. Immunol. 172, 5149–5153.

Faria, A.M., and Weiner, H.L. (2005). Oral tolerance. Immunol. Rev. 206, 232–259.

Finney, C.A., Taylor, M.D., Wilson, M.S., and Maizels, R.M. (2007). Expansion and activation of CD4(+)CD25(+) regulatory T cells in Heligmosomoides polygyrus infection. Eur. J. Immunol. 37, 1874–1886.

Fitzgerald, D.C., Ciric, B., Touil, T., Harle, H., Grammatikopolou, J., Das Sarma, J., Gran, B., Zhang, G.X., and Rostami, A. (2007). Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector phase of experimental autoimmune encephalomyelitis. J. Immunol. 179, 3268–3275.

Fontenot, J.D., Dooley, J.L., Farr, A.G., and Rudensky, A.Y. (2005a). Developmental regulation of Foxp3 expression during ontogeny. J. Exp. Med. 202, 901–906.

Fontenot, J.D., Rasmussen, J.P., Williams, L.M., Dooley, J.L., Farr, A.G., and Rudensky, A.Y. (2005b). Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity 22, 329–341.

Gazzinelli, R.T., Oswald, I.P., James, S.L., and Sher, A. (1992). IL-10 inhibits parasite killing and nitrogen oxide production by IFN- gamma-activated macrophages. J. Immunol. 148, 1792–1796.

Gazzinelli, R.T., Wysocka, M., Hieny, S., Scharton-Kersten, T., Cheever, A., Kuhn, R., Muller, W., Trinchieri, G., and Sher, A. (1996). In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. J. Immunol. 157, 798–805.

Geerts, A. (2004). On the origin of stellate cells: Mesodermal, endodermal or neuro-ectodermal? J. Hepatol. 40, 331–334.

Gerosa, F., Nisii, C., Righetti, S., Micciolo, R., Marchesini, M., Cazzadori, A., and Trinchieri, G. (1999). CD4(+) T cell clones producing both interferon-gamma and interleukin-10 predominate in bronchoalveolar lavages of active pulmonary tuberculosis patients. Clin. Immunol. 92, 224–234.

Gomez-Escobar, N., van den Biggelaar, A., and Maizels, R. (1997). A member of the TGF-beta receptor gene family in the parasitic nematode Brugia pahangi. Gene 199, 101–109.

Gorman, S., Kuritzky, L.A., Judge, M.A., Dixon, K.M., McGlade, J.P., Mason, R.S., Finlay-Jones, J.J., and Hart, P.H. (2007). Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. J. Immunol. 179, 6273–6283.

Goubier, A., Dubois, B., Gheit, H., Joubert, G., Villard-Truc, F., Asselin-Paturel, C., Trinchieri, G., and Kaiserlian, D. (2008). Plasmacytoid dendritic cells mediate oral tolerance. Immunity 29, this issue, 464–475.

Griffin, M.D., Lutz, W.H., Phan, V.A., Bachman, L.A., McKean, D.J., and Kumar, R. (2000). Potent inhibition of dendritic cell differentiation and maturation by vitamin D analogs. Biochem. Biophys. Res. Commun. 270, 701–708.

Hoffmann, K.F., Cheever, A.W., and Wynn, T.A. (2000). IL-10 and the dangers of immune polarization: Excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. J. Immunol. 164, 6406–6416.

Huang, F.P., Platt, N., Wykes, M., Major, J.R., Powell, T.J., Jenkins, C.D., and MacPherson, G.G. (2000). A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. J. Exp. Med. 191, 435–444.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor ROR-gammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121–1133.

Iwasaki, A., and Kelsall, B.L. (2001). Unique functions of CD11b+, CD8 alpha+, and double-negative Peyer's patch dendritic cells. J. Immunol. 166, 4884–4890.

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., and Song, S.Y. (2004). Retinoic acid imprints gut-homing specificity on T cells. Immunity 21, 527–538.

Jaensson, E., Uronen-Hansson, H., Pabst, O., Eksteen, B., Tian, J., Coombes, J.L., Berg, P.L., Davidsson, T., Powrie, F., Johansson-Lindbom, B., and Agace, W.W. (2008). Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans. J. Exp. Med. in press. Published online August 18, 2008. 10.1084/jem.20080414.

Jankovic, D., Kullberg, M.C., Feng, C.G., Goldszmid, R.S., Collazo, C.M., Wilson, M., Wynn, T.A., Kamanaka, M., Flavell, R.A., and Sher, A. (2007). Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. J. Exp. Med. 204, 273–283.

Johansson-Lindbom, B., Svensson, M., Wurbel, M.A., Malissen, B., Marquez, G., and Agace, W. (2003). Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): Requirement for GALT dendritic cells and adjuvant. J. Exp. Med. 198, 963–969.

Johansson-Lindbom, B., Svensson, M., Pabst, O., Palmqvist, C., Marquez, G., Forster, R., and Agace, W.W. (2005). Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. J. Exp. Med. 202, 1063–1073.

Jordan, M.S., Boesteanu, A., Reed, A.J., Petrone, A.L., Holenbeck, A.E., Lerman, M.A., Naji, A., and Caton, A.J. (2001). Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nat. Immunol. 2, 301–306.

Kang, S.G., Lim, H.W., Andrisani, O.M., Broxmeyer, H.E., and Kim, C.H. (2007). Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells. J. Immunol. 179, 3724–3733.

Kastelein, R.A., Hunter, C.A., and Cua, D.J. (2007). Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu. Rev. Immunol. 25, 221–242.

King, C.L., Medhat, A., Malhotra, I., Nafeh, M., Helmy, A., Khaudary, J., Ibrahim, S., El-Sherbiny, M., Zaky, S., Stupi, R.J., et al. (1996). Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. J. Immunol. 156, 4715–4721.

Knoechel, B., Lohr, J., Kahn, E., Bluestone, J.A., and Abbas, A.K. (2005). Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen. J. Exp. Med. 202, 1375–1386.

Korn, T., Bettelli, E., Gao, W., Awasthi, A., Jager, A., Strom, T.B., Oukka, M., and Kuchroo, V.K. (2007). IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 448, 484–487.

Kretschmer, K., Apostolou, I., Hawiger, D., Khazaie, K., Nussenzweig, M.C., and von Boehmer, H. (2005). Inducing and expanding regulatory T cell populations by foreign antigen. Nat. Immunol. 6, 1219–1227.

Lacy-Hulbert, A., Smith, A.M., Tissire, H., Barry, M., Crowley, D., Bronson, R.T., Roes, J.T., Savill, J.S., and Hynes, R.O. (2007). Ulcerative colitis and autoimmunity induced by loss of myeloid alphav integrins. Proc. Natl. Acad. Sci. USA 104, 15823–15828.

Latchman, Y.E., Liang, S.C., Wu, Y., Chernova, T., Sobel, R.A., Klemm, M., Kuchroo, V.K., Freeman, G.J., and Sharpe, A.H. (2004). PD-L1-deficient

mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. Proc. Natl. Acad. Sci. USA 101, 10691–10696.

Li, C., Corraliza, I., and Langhorne, J. (1999). A defect in interleukin-10 leads to enhanced malarial disease in Plasmodium chabaudi chabaudi infection in mice. Infect. Immun. 67, 4435–4442.

Liang, S., Alard, P., Zhao, Y., Parnell, S., Clark, S.L., and Kosiewicz, M.M. (2005). Conversion of CD4+ CD25- cells into CD4+ CD25+ regulatory T cells in vivo requires B7 costimulation, but not the thymus. J. Exp. Med. 201, 127–137.

Liu, Y., Teige, I., Birnir, B., and Issazadeh-Navikas, S. (2006). Neuron-mediated generation of regulatory T cells from encephalitogenic T cells suppresses EAE. Nat. Med. 12, 518–525.

López-Bravo, M., and Ardavín, C. (2008). In vivo induction of immune responses to pathogens by conventional dendritic cells. Immunity 29, this issue, 343–351.

MacDonald, A.J., Duffy, M., Brady, M.T., McKiernan, S., Hall, W., Hegarty, J., Curry, M., and Mills, K.H. (2002). CD4 T helper type 1 and regulatory T cells induced against the same epitopes on the core protein in hepatitis C virus-infected persons. J. Infect. Dis. 185, 720–727.

Mahanty, S., Ravichandran, M., Raman, U., Jayaraman, K., Kumaraswami, V., and Nutman, T.B. (1997). Regulation of parasite antigen-driven immune responses by interleukin-10 (IL-10) and IL-12 in lymphatic filariasis. Infect. Immun. 65, 1742–1747.

McGuirk, P., McCann, C., and Mills, K.H. (2002). Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: A novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. J. Exp. Med. 195, 221–231.

Meyaard, L., Hovenkamp, E., Otto, S.A., and Miedema, F. (1996). IL-12-induced IL-10 production by human T cells as a negative feedback for IL-12-induced immune responses. J. Immunol. 156, 2776–2782.

Mills, K.H., and McGuirk, P. (2004). Antigen-specific regulatory T cells–their induction and role in infection. Semin. Immunol. 16, 107–117.

Montagnoli, C., Fallarino, F., Gaziano, R., Bozza, S., Bellocchio, S., Zelante, T., Kurup, W.P., Pitzurra, L., Puccetti, P., and Romani, L. (2006). Immunity and tolerance to Aspergillus involve functionally distinct regulatory T cells and tryptophan catabolism. J. Immunol. 176, 1712–1723.

Moore, K.W., de Waal Malefyt, R., Coffman, R.L., and O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19, 683–765.

Mora, J.R., Bono, M.R., Manjunath, N., Weninger, W., Cavanagh, L.L., Rosemblatt, M., and Von Andrian, U.H. (2003). Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. Nature 424, 88–93.

Mora, J.R., Iwata, M., Eksteen, B., Song, S.Y., Junt, T., Senman, B., Otipoby, K.L., Yokota, A., Takeuchi, H., Ricciardi-Castagnoli, P., et al. (2006). Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Science 314, 1157–1160.

Mowat, A.M. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. Nat. Rev. Immunol. 3, 331–341.

Mucida, D., Kutchukhidze, N., Erazo, A., Russo, M., Lafaille, J.J., and Curotto de Lafaille, M.A. (2005). Oral tolerance in the absence of naturally occurring Tregs. J. Clin. Invest. 115, 1923–1933.

Mucida, D., Park, Y., Kim, G., Turovskaya, O., Scott, I., Kronenberg, M., and Cheroutre, H. (2007). Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. Science 317, 256–260.

Nurieva, R., Yang, X.O., Martinez, G., Zhang, Y., Panopoulos, A.D., Ma, L., Schluns, K., Tian, Q., Watowich, S.S., Jetten, A.M., and Dong, C. (2007). Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 448, 480–483.

O'Garra, A., Vieira, P.L., Vieira, P., and Goldfeld, A.E. (2004). IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. J. Clin. Invest. 114, 1372–1378.

O'Garra, A., Barrat, F.J., Castro, A.G., Vicari, A., and Hawrylowicz, C. (2008). Strategies for use of IL-10 or its antagonists in human disease. Immunol. Rev. 223, 114–131.

Ocana-Morgner, C., Wong, K.A., Lega, F., Dotor, J., Borras-Cuesta, F., and Rodriguez, A. (2007). Role of TGF-beta and PGE2 in T cell responses during Plasmodium yoelii infection. Eur. J. Immunol. 37, 1562–1574.

Oderup, C., Malm, H., Ekberg, H., Qi, Z., Veress, B., Ivars, F., and Corbascio, M. (2006). Costimulation blockade-induced cardiac allograft tolerance: inhibition of T cell expansion and accumulation of intragraft cD4(+)Foxp3(+) T cells. Transplantation 82, 1493–1500.

Onishi, Y., Fehervari, Z., Yamaguchi, T., and Sakaguchi, S. (2008). Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proc. Natl. Acad. Sci. USA 105, 10113–10118.

Penna, G., and Adorini, L. (2000). 1 Alpha,25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. J. Immunol. 164, 2405–2411.

Perruche, S., Zhang, P., Liu, Y., Saas, P., Bluestone, J.A., and Chen, W. (2008). CD3-specific antibody-induced immune tolerance involves transforming growth factor-beta from phagocytes digesting apoptotic T cells. Nat. Med. 14, 528–535.

Piemonti, L., Monti, P., Sironi, M., Fraticelli, P., Leone, B.E., Dal Cin, E., Allavena, P., and Di Carlo, V. (2000). Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. J. Immunol. 164, 4443–4451.

Plebanski, M., Flanagan, K.L., Lee, E.A., Reece, W.H., Hart, K., Gelder, C., Gillespie, G., Pinder, M., and Hill, A.V. (1999). Interleukin 10-mediated immunosuppression by a variant CD4 T cell epitope of Plasmodium falciparum. Immunity 10, 651–660.

Pohl-Koppe, A., Balashov, K.E., Steere, A.C., Logigian, E.L., and Hafler, D.A. (1998). Identification of a T cell subset capable of both IFN-gamma and IL-10 secretion in patients with chronic Borrelia burgdorferi infection. J. Immunol. 160, 1804–1810.

Poncini, C.V., Alba Soto, C.D., Batalla, E., Solana, M.E., and Gonzalez Cappa, S.M. (2008). Trypanosoma cruzi induces regulatory dendritic cells in vitro. Infect. Immun. 76, 2633–2641.

Rimoldi, M., Chieppa, M., Salucci, V., Avogadri, F., Sonzogni, A., Sampietro, G.M., Nespoli, A., Viale, G., Allavena, P., and Rescigno, M. (2005). Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. Nat. Immunol. 6, 507–514.

Roncarolo, M.G., Gregori, S., Battaglia, M., Bacchetta, R., Fleischhauer, K., and Levings, M.K. (2006). Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunol. Rev. 212, 28–50.

Saurer, L., McCullough, K.C., and Summerfield, A. (2007). In vitro induction of mucosa-type dendritic cells by all-trans retinoic acid. J. Immunol. 179, 3504–3514.

Schambach, F., Schupp, M., Lazar, M.A., and Reiner, S.L. (2007). Activation of retinoic acid receptor-alpha favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation. Eur. J. Immunol. 37, 2396–2399.

Siewert, C., Menning, A., Dudda, J., Siegmund, K., Lauer, U., Floess, S., Campbell, D.J., Hamann, A., and Huehn, J. (2007). Induction of organ-selective CD4(+) regulatory T cell homing. Eur. J. Immunol. 37, 978–989.

Sigmundsdottir, H., Pan, J., Debes, G.F., Alt, C., Habtezion, A., Soler, D., and Butcher, E.C. (2007). DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27. Nat. Immunol. 8, 285–293.

Spahn, T.W., Weiner, H.L., Rennert, P.D., Lugering, N., Fontana, A., Domschke, W., and Kucharzik, T. (2002). Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. Eur. J. Immunol. 32, 1109–1113.

Stagg, A.J., Kamm, M.A., and Knight, S.C. (2002). Intestinal dendritic cells increase T cell expression of alpha4beta7 integrin. Eur. J. Immunol. 32, 1445–1454.

Stumhofer, J.S., Laurence, A., Wilson, E.H., Huang, E., Tato, C.M., Johnson, L.M., Villarino, A.V., Huang, Q., Yoshimura, A., Sehy, D., et al. (2006). Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. Nat. Immunol. 7, 937–945.

Stumhofer, J.S., Silver, J.S., Laurence, A., Porrett, P.M., Harris, T.H., Turka, L.A., Ernst, M., Saris, C.J., O'Shea, J.J., and Hunter, C.A. (2007). Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. Nat. Immunol. *8*, 1363–1371.

Sun, C.M., Hall, J.A., Blank, R.B., Bouladoux, N., Oukka, M., Mora, J.R., and Belkaid, Y. (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.

Tadokoro, C.E., Shakhar, G., Shen, S., Ding, Y., Lino, A.C., Maraver, A., Lafaille, J.J., and Dustin, M.L. (2006). Regulatory T cells inhibit stable contacts between CD4+ T cells and dendritic cells in vivo. J. Exp. Med. *203*, 505–511.

Tai, P., Wang, J., Jin, H., Song, X., Yan, J., Kang, Y., Zhao, L., An, X., Du, X., Chen, X., et al. (2008). Induction of regulatory T cells by physiological level estrogen. J. Cell. Physiol. *214*, 456–464.

Tang, Q., Adams, J.Y., Tooley, A.J., Bi, M., Fife, B.T., Serra, P., Santamaria, P., Locksley, R.M., Krummel, M.F., and Bluestone, J.A. (2006). Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. Nat. Immunol. *7*, 83–92.

Tarbell, K.V., Yamazaki, S., Olson, K., Toy, P., and Steinman, R.M. (2004). CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J. Exp. Med. *199*, 1467–1477.

Travis, M.A., Reizis, B., Melton, A.C., Masteller, E., Tang, Q., Proctor, J.M., Wang, Y., Bernstein, X., Huang, X., Reichardt, L.F., et al. (2007). Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice. Nature *449*, 361–365.

Trinchieri, G. (2001). Regulatory role of T cells producing both interferon gamma and interleukin 10 in persistent infection. J. Exp. Med. *194*, F53–F57.

Van der Kleij, D., Van Remoortere, A., Schuitemaker, J.H., Kapsenberg, M.L., Deelder, A.M., Tielens, A.G., Hokke, C.H., and Yazdanbakhsh, M. (2002). Triggering of innate immune responses by schistosome egg glycolipids and their carbohydrate epitope GalNAc beta 1–4(Fuc alpha 1–2Fuc alpha 1–3)GlcNAc. J. Infect. Dis. *185*, 531–539.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179–189.

Vignali, D. (2008). How many mechanisms do regulatory T cells need? Eur. J. Immunol. *38*, 908–911.

Vijay-Kumar, M., Aitken, J.D., Kumar, A., Neish, A.S., Uematsu, S., Akira, S., and Gewirtz, A.T. (2008). Toll-like receptor 5-deficient mice have dysregulated intestinal gene expression and nonspecific resistance to Salmonella-induced typhoid-like disease. Infect. Immun. *76*, 1276–1281.

Walther, M., Tongren, J.E., Andrews, L., Korbel, D., King, E., Fletcher, H., Andersen, R.F., Bejon, P., Thompson, F., Dunachie, S.J., et al. (2005). Upregulation of TGF-beta, FOXP3, and CD4+CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. Immunity *23*, 287–296.

Wang, L., Pino-Lagos, K., de Vries, V.C., Guleria, I., Sayegh, M.H., and Noelle, R.J. (2008). Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. Proc. Natl. Acad. Sci. USA *105*, 9331–9336.

Wei, J., Duramad, O., Perng, O.A., Reiner, S.L., Liu, Y.J., and Qin, F.X. (2007). Antagonistic nature of T helper 1/2 developmental programs in opposing peripheral induction of Foxp3+ regulatory T cells. Proc. Natl. Acad. Sci. USA *104*, 18169–18174.

Wong, K.A., and Rodriguez, A. (2008). Plasmodium infection and endotoxic shock induce the expansion of regulatory dendritic cells. J. Immunol. *180*, 716–726.

Yamazaki, S., Bonito, A.J., Spisek, R., Dhodapkar, M., Inaba, K., and Steinman, R.M. (2007). Dendritic cells are specialized accessory cells along with TGF- for the differentiation of Foxp3+ CD4+ regulatory T cells from peripheral Foxp3 precursors. Blood *110*, 4293–4302.

Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K., and Steinman, R.M. (2003). Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J. Exp. Med. *198*, 235–247.

Zaccone, P., Fehervari, Z., Jones, F.M., Sidobre, S., Kronenberg, M., Dunne, D.W., and Cooke, A. (2003). Schistosoma mansoni antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. Eur. J. Immunol. *33*, 1439–1449.

Zheng, S.G., Wang, J.H., Stohl, W., Kim, K.S., Gray, J.D., and Horwitz, D.A. (2006). TGF-beta requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive CD4+CD25+ regulatory cells. J. Immunol. *176*, 3321–3329.

Zhou, L., Ivanov, I.I., Spolski, R., Min, R., Shenderov, K., Egawa, T., Levy, D.E., Leonard, W.J., and Littman, D.R. (2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat. Immunol. *8*, 967–974.

Ziegler, T.R., Evans, M.E., Fernandez-Estivariz, C., and Jones, D.P. (2003). Trophic and cytoprotective nutrition for intestinal adaptation, mucosal repair, and barrier function. Annu. Rev. Nutr. *23*, 229–261.