Published in final edited form as:

Cell Mol Life Sci. 2012 June ; 69(12): 1997–2008. doi:10.1007/s00018-011-0907-z.

The Battle Against Immunopathology: Infectious Tolerance Mediated by Regulatory T Cells

David M. Gravano and Dario A.A. Vignali

Department of Immunology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105-3678, USA.

Abstract

Infectious tolerance is a process whereby one regulatory lymphoid population confers suppressive capacity on another. Diverse immune responses are induced following infection or inflammatory insult that can protect the host, or potentially cause damage if not properly controlled. Thus, the process of infectious tolerance may be critical *in vivo* for exerting effective immune control and maintaining immune homeostasis by generating specialized regulatory subpopulations with distinct mechanistic capabilities. Foxp3⁺ regulatory T cells (T_{regs}) are a central mediator of infectious tolerance through their ability to convert conventional T cells into induced regulatory T cells (iT_{regs}) directly by secretion of the suppressive cytokines TGF- β , IL-10 or IL-35, or indirectly via dendritic cells. In this review we will discuss the mechanisms and cell populations that mediate and contribute to infectious tolerance, with a focus on the intestinal environment, where tolerance induction to foreign material is critical.

Keywords

Infectious tolerance; regulatory T cell; intestine; helminth; microbiota

Tolerance takes multiple forms

One of the most fundamental questions in immunology is how the immune system can target the diverse array of environmental pathogens we encounter on a daily basis while simultaneously maintaining non-reactivity, or tolerance, to self. A detailed understanding of tolerance mechanisms is beginning to emerge, presenting new therapeutic applications for the restoration of tolerance to self-antigens in autoimmune disease, promoting tolerance or ignorance to innocuous non-self-agents such as food antigens and commensal microbiota, or breaking tolerance to chronic pathogens or aberrant self (eg. cancer) [1–3]. Research over the last few decades has led to the realization that the immune system employs many strategies to exert tolerance, from the deletion of auto-reactive T cells in the thymus, termed 'central tolerance', to dominant mechanisms of 'peripheral tolerance' where suppressive populations, such as Foxp3⁺CD4⁺ regulatory T cells (T_{regs}), halt the activation of auto-reactive T cells. Given that T_{regs} are generally present at low numbers, a key process that appears to be required for the peripheral control of the immune system is a process termed 'infectious tolerance' whereby one suppressive lymphoid population can confer suppressive capabilities on another [4,5]. A complete understanding of the mechanism infectious tolerance will lead to advanced therapeutics that will impact the fields of transplantation biology, infectious disease, autoimmunity, and cancer research. The focus of this review will be to detail our current understanding of infectious tolerance with a particular focus on the

ability of T_{regs} to generate a regulatory microenvironment that nurtures the induction of infectious tolerance. This is especially relevant in mucosal sites where a barrage of foreign, mostly innocuous antigens are present and must be tolerated in order to avoid inflammatory disease [6].

The origins of infectious tolerance

Much of our understanding of peripheral and infectious tolerance has come from studies in transplantation immunology. Seminal studies by Billingham and Medawar on skin grafting in laboratory animals revealed that newborn mice could tolerate allogeneic skin grafts that adults would reject [7]. Later, it was realized that suppressive cells mediate this tolerance and that their suppressive capacity could be transferred from one population of cells to another. Gershon and Kondo were the first to describe this phenomenon in the 1970s coining the phrase 'infectious immunological tolerance' [4]. Subsequent studies by Gershon and colleagues, and later by North and colleagues, suggested that the suppressive population was a Ly1⁺ (CD5) Ly2⁻ (CD8) CD4⁺ T cell subset [8–11]. However, it was the work of Waldmann and colleagues firmly established the concept of infectious tolerance with the demonstration that tolerance to allogeneic skin grafts could be therapeutically induced by pre-treating recipients with nondepleting, blocking antibodies to T cell co-stimulatory pathways CD4, CD8 or CD154 [12–14]. T cell adoptive transfer at various points during the tolerance induction protocol revealed that tolerance took several weeks to initiate, but once established was long lasting and could be transferred [15]. Subsequent studies with congenically marked T cells demonstrated that tolerance could be transferred from one T cell population to another [16]. Amazingly, this infectious tolerance appeared to occur sequentially and seemingly indefinitely in this model.

Insight into the mechanism of infectious tolerance was provided by experiments which demonstrated the phenomenon of linked suppression (Fig 1). The observation was that a recipient mouse of strain A tolerized to a graft from donor strain B was also capable of accepting a second graft of an F1 cross of a strain B mouse with a strain C mouse, but a strain C graft alone was rejected [17]. After the tolerance inducing period, a new graft from a pure strain C mouse could then be tolerated in the absence of strain B antigens. This implied that infectious tolerance was expanded from strain B antigens to strain C antigens due to them being present on the same graft. Even placing grafts from strain B and strain C side by side in the same graft bed was not sufficient to induce infectious tolerance to graft C. This led to the notion that a highly localized regulatory microenvironment is critical for the spread of tolerance from one T cell population to another.

Foxp3⁺ regulatory T cells and tolerance

Suppressor T cell studies received early skepticism due to the tainted legacy of the suppressor cell era. The seminal discovery that the suppressive CD4⁺ T cell population could be uniquely identified by expression of the alpha chain of IL-2 receptor (CD25), led to the demonstration that the transplantable, suppressive T cell population was restricted to CD4⁺CD25⁺ T cells [18]. Adoptive transfer of CD25-depleted CD4⁺ T cells into T cell-deficient, nude mice resulted in systemic autoimmunity, which could be prevented by co-transfer with CD4⁺CD25⁺ T cells within a limited period of time [19]. The discovery of the forkhead transcription factor *Foxp3* as the defective gene in human immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome patients and scurfy mice, which both develop severe systemic autoimmunity, provided the first tangible marker for CD4⁺CD25⁺ T_{regs} [20–23]. Subsequent experiments proved that Foxp3 expression is restricted to the suppressive CD4⁺CD25⁺ T cell subset, is necessary for the development of suppressive cells, and when introduced via retroviral transduction can confer suppressive

capacity on naïve CD4⁺ T cells [24,25]. Collectively these seminal studies established Foxp3 as a master regulatory of T_{reg} development and function. Several studies have subsequently demonstrated that Foxp3⁺ T_{regs} are the primary suppressive population that mediates immunological tolerance to skin grafts. Female A1 *Rag1*^{-/-} TCR transgenic mice, specific for the male antigen DbY, fail to develop thymus-derived 'natural' Foxp3⁺ T_{regs} and readily reject male skin grafts. However, under tolerance induced through co-receptor blockade, Foxp3⁺ T_{reg} induction was observed that coincided with the establishment of tolerance [26]. These 'induced' T_{regs} (iT_{regs}) within the tolerated tissue were required to maintain tolerance to the allograft. This was demonstrated through transfer of the tolerated graft into *Rag1*^{-/-} recipient mice, which lack the capacity to reject the graft on their own [26,27]. Treatment with the CD25-depleting antibody, PC61, led to T_{reg} depletion within the transferred graft which was rapidly rejected due to the removal of suppression and restoration of alloreactivity by the graft resident T cells. These observations were recently supported by studies in transgenic mice expressing human CD2 under the control of the *Foxp3* promoter, which allowed targeted deletion of T_{regs} using ablative anti-human CD2 antibodies [28]. Thus Foxp3⁺ T_{regs} are required for the induction and maintenance of tolerance.

A variety of CD4⁺ iT_{regs} populations have been shown to mediate dominant, infectious tolerance in a variety of model systems, and thus they will be the primary focus for the remainder of this review. However, other suppressive lymphoid populations have been described such as regulatory B cells and CD8⁺ regulatory T cells. However, their role in mediating infectious tolerance has not been extensively examined and they will not be discussed further. The diversity of iT_{reg} populations that can mediate infectious tolerance should not be surprising since the types of immune responses and environments in which regulatory populations must act are also diverse. However, evidence is emerging that there are several common characteristics of what constitute an environment capable of suppressing immune responses and inducing infectious tolerance.

Infectious tolerance mediated by T_{reg}-derived cytokines

Reductionist *in vitro* studies have provided considerable insight into the suppressive mechanisms used by T_{regs} and their ability to transfer their suppressive capacity to another T cell population (Fig 2). Considerable attention has focused on TGF-β, a pleiotropic cytokine that appears to play a central role in immune tolerance [29]. Genetic deletion of TGF-β1, which is predominantly expressed in the immune system, results in multi-organ immunopathology [30]. TGF-β induces Foxp3 expression by TCR-stimulated mouse and human T cells and confers suppressive capacity [31,32]. TGF-β has also been suggested regulate Foxp3⁺ T_{reg} development in the thymus [33]. Upon activation, the latent form of TGF-β is detected on a high percentage of mouse and human T_{regs} [34]. However, the role of TGF-β as a T_{reg} effector cytokine remains controversial [35]. Given the *in vitro* effects of TGF-β on T_{reg} conversion and the observation that latent TGF-β is expressed on activated T_{regs}, it was hypothesized that T_{reg}-derived TGF-β could be a key mediator of infectious tolerance, even in the absence of antigen presenting cells. Indeed, it was shown that Foxp3 is induced in effector T cells stimulated in the presence of pre-activated, latent TGF-β-expressing T_{regs} in a TGF-β- and cell contact-dependent manner, and that these iT_{regs} were suppressive *in vitro* and *in vivo* [36]. In these experiments, prior activation of T_{regs} and latent TGF-β expression were key as freshly activated T_{regs} were not strong mediators of infectious tolerance. Lastly, human T_{regs} have been shown to mediate infectious tolerance *in vitro* by induction of a regulatory population in a TGF-β-dependent manner [37].

The immunosuppressive cytokine IL-10 has been shown to induce a population of functionally suppressive CD4⁺ T cells, sometimes referred to as Tr1 cells [38,39]. They

share in common dependence on IL-10 and TGF- β to mediate their suppressive effects but differ from conventional T_{regs} by lacking expression of Foxp3 [39]. Human T_{regs} have been shown to induce Tr1 cells *in vitro* that are capable of suppressing third party T cells in an IL-10-dependent manner [40,41]. In the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis, CD4⁺CD25⁺ T_{regs} have also been observed to convert antigen-specific pathogenic effector T cells into IL-10 producing Tr1 cells [42,43]. These converted cells are capable of suppressing EAE in an IL-10-dependent manner. The development of IL-10 reporter mice has provided increased spatiotemporal resolution of IL-10 expression [44]. *In vivo*, both Foxp3⁺ and Foxp3⁻ IL-10-producing T_{regs} are not dependent on IL-10 expression to develop, as reporter expression can still be detected in IL-10 genetic knockouts. However, IL-10⁺ T_{reg} development is dependent on TGF- β signaling as TGF- β antibody neutralization can abrogate their induction. Recently, it has been shown that Foxp3⁺ T_{regs} respond to IL-10 by increasing production of IL-10 through a feed-forward mechanism that is critical for controlling T_H17 responses, particularly during intestinal inflammation [45–47]. It has also recently been observed that pathogenic T_H17 cells can be converted in the intestinal environment into suppressive T_H17 cells that express high levels of IL-10 and are partially dependent on IL-10 for their suppressive capacity [48]. However, it is unclear if T_{reg} derived IL-10 acts directly to confer suppressive capacity on this pathogenic T_H17 population through infectious tolerance or if other cellular and molecular mediators are required.

IL-35 is a heterodimeric cytokine composed of IL12a/p35 and Ebi3 that suppresses both mouse and human T cells. IL-35 secretion appears to be restricted to T_{regs} and is required for their maximal suppressive capacity *in vivo* [49,50]. Furthermore, T_{reg}-derived IL-35 can induce the conversion of CD4⁺ effector T cells into a regulatory population that mediates suppression via IL-35 production, termed iTr35 [49]. iTr35s do not express Foxp3, TGF- β and IL-10. Adoptive transfer studies with iTr35s has revealed their remarkably protective capacity and stability in five distinct autoimmune and inflammatory models *in vivo*. Importantly, T_{reg}-mediated iTr35 induction appears to contribute about half of the suppressive microenvironment in B16 melanoma, suggesting that iTr35 may contribute to infectious tolerance *in vivo* [49]. Human T_{regs} can also express IL-35 and mediate iTr35 induction in an IL-35-dependent fashion [51]. Further understanding of the role of T_{reg}-derived IL-35 *in vivo* in mediating infectious tolerance is clearly required.

Infectious tolerance mediated via dendritic cells

Experiments suggesting that infectious tolerance may be the result of linked suppression led to the hypothesis that antigen presenting cells in the tolerated tissue may be playing a critical role in this process. Dendritic cells (DCs) contribute to the generation of iT_{regs} in the periphery. DCs are the most potent cellular inducer of T_{regs} and DC immaturity is correlated with increased efficiency of T_{reg} generation [52,53]. CD8⁺ DCs in the spleen are more capable than CD8⁻ DCs at inducing T_{regs} in the presence of TGF- β [54]. This conversion process also requires signaling through the programmed death ligand 1 (PD-L1). It has also been observed that plasmacytoid DCs (pDCs) are particularly good inducers of T cell tolerance [55]. CCR9⁺ pDCs has been shown to induce Foxp3⁺ T_{regs} and suppress antigen-specific immune responses both *in vitro* and *in vivo* [56]. Bone marrow-derived DCs preconditioned with TGF- β , IL-10, or vitamin D3 have also have been reported to induce tolerance in a skin graft model [57,58]. In humans and mice a subset of IL-10-producing DCs have been characterized *in vivo*, and were found to be potent inducers of Tr1 cell *in vitro* [59,60]. Antibody mediated blockade of IL-10 signaling could inhibit the induction of Tr1 cells, indicating that DC production of IL-10 was critical. In addition to being found naturally, these IL-10 producing DCs can also be induced *in vitro* from monocytes in the

presence of IL-10 [59]. It is tempting to speculate that these IL-10-producing DCs may be a required intermediate *in vivo* for IL-10-mediated infectious tolerance via Tr1 cells.

Data also show that DCs are able to induce human iT_{reg} generation. Rhinovirus infected DCs cultured with T cells caused profound anergy and resulted in upregulation of IL-35 [61,62]. These Foxp3⁺ iT_{reg}s were able to suppress naïve T cell proliferation, comparable to results in the mouse. Furthermore, induction of human iT_{reg}s by infected DCs was dependent on PD-L1 and CD169 expression on DCs. It will be interesting to determine if iT_{reg}s generated via virus-exposed DCs or in the presence of Foxp3⁺ T_{regs} possess a similar phenotypic and functional profile.

A common observation in several studies of DC-induced tolerance is that an environment which strongly promotes T cell anergy or unresponsiveness is often potentiates T_{reg} induction (reviewed in [52,63]). This has led some to hypothesize that sub-optimal T cell activation by DCs under certain conditions is required for effective induction of infectious tolerance. However, the molecular details of this remain elusive. Tolerogenic DCs that preferentially induce iT_{reg} induction over effector T cell activation tend to exhibit low constitutive MHC expression, a low ratio of co-stimulatory to co-inhibitory molecules, have an impaired ability to synthesize T_H1-inducing cytokines (such as IL-12) [64]. It is not yet clear if anergy induction precedes iT_{reg} development in a linear and dependent fashion, or if these two events are independent outcomes of an anergy-inducing environment. Although questions and challenges remain, these findings have led some to attempt to utilize *in vitro* tolerized DCs in the clinic to drive immunosuppression [65].

The factors that induce T cell anergy have been expanded from the classic two signal paradigm. Initially anergy was attributed to an antigen specific signal through the TCR (signal 1) in the absence of a costimulatory signal mediated by CD28 ligation (signal 2), which resulted in lack of IL-2 transcription (reviewed in [66]). Upon full T cell activation, signaling through the IL-2R activates the PI3K/AKT/mTOR pathway leading to cell cycle entry [67]. It is now recognized that several pathways can provide this second signal. Thus any signaling events that negatively affect the mTOR pathway and metabolism in stimulated T cells can induce anergy. For instance, inhibition of mTOR signaling with rapamycin is sufficient to induce anergy in the presence of antigen plus co-stimulation [68,69]. Since mTOR is downstream of several nutrient sensing pathways and the energy sensing AMP-activated protein kinase complex, which inhibits mTOR upon detection of a low ATP:ADP ratio (reviewed in [70,71]), the convergence of metabolic information is a determining factor in the induction of anergy versus activation. Conditional knockout of mTOR in CD4⁺ T cells has been shown to result in preferential induction of Foxp3, highlighting the importance of this signaling pathway in tolerance induction [72]. Likewise, anergy can result from mTOR independent signaling pathways such as the GCN2 amino acid sensing pathway [73]. GCN2 is a kinase that becomes activated upon binding to uncharged transfer RNAs, acting as a detector of amino acid deprivation. GCN2 activation results in S phase arrest and inhibition cell cycle entry [74]. Extracellular adenosine, which is generated by ATP hydrolysis, can act as a sensor of hypoxia. Adenosine signals through the A2A receptor and stimulates adenylyl cyclase to elevate intracellular levels of cAMP (reviewed in [75]). Adenosine-mediated reduction in Ras/MAPK signaling results in T cell anergy *in vitro* and *in vivo* and the generation of induced T_{regs} [76]. Thus, an anergy-inducing environment conducive to T_{reg} generation is one that lacks co-stimulatory molecules, nutrients and/or energy.

T_{regs} alter DC functionality

In order to confirm that T_{regs} drive the generation of new iT_{regs} through an APC intermediate, experiments needed to be performed which demonstrate that DC function is

altered by T_{regs} . Indeed, there is mounting evidence showing that $\text{Foxp3}^+ T_{\text{regs}}$ are capable of altering DC phenotype such that they promote tolerance induction of newly primed T cells (Fig 2). Two-photo laser scanning microscopy experiments have confirmed that strong interactions occur between T_{regs} and DCs in lymph nodes [77,78]. In the absence of T_{regs} , T cells were shown to have reduced locomotion and longer contact duration with DCs, which correlated with elevated cytokine production in the lymph nodes. Interactions between T_{regs} and naïve T cells were not observed in these studies, suggesting that the primary target of T_{regs} in these systems was the DC. However, these studies do not rule out the possibility that T_{reg} do directly alter T cell function and iT_{reg} conversion but that their interaction is very brief and/or cytokine driven and thus does not require direct contact.

T_{regs} may also mediate linked suppression and infectious tolerance by altering the ability of DCs to provide co-stimulation. Indeed, early *in vitro* studies using mouse and human DCs found that co-stimulatory molecules were down-regulated in the presence of T_{regs} [79,80]. T_{regs} have also been shown to physically outcompete naïve T cells for DC-presented antigen and costimulation *in vitro*. Antigen specific T_{regs} form stable clusters around DCs inhibiting their maturation and choking off productive interactions with naïve T cells [81]. These interactions resulted in decreased expression on CD80 and CD86 in the DC, which is partially dependent on T_{reg} expression of CTLA-4 [82]. Recently, CTLA-4 on T_{regs} was shown to interact with CD80 and CD86 DCs and mediate their trans-endocytosis into the T_{reg} where they were subsequently degraded [83]. This mechanism has been shown to occur both *in vivo* and *in vitro*, with a high proportion of T_{regs} utilizing this mechanism *in vivo*. The cell surface inhibitory molecule LAG-3 has been shown to be required for maximal T_{reg} function and is expressed by activated CD4^+ and CD8^+ T cells [84–86]. Interestingly, LAG-3 has been shown to inhibit DC maturation and their ability to present antigen by transducing negative signals in an MHC class II-, $\text{Fc}\gamma\text{R}\gamma$ - and SHP-1-dependent manner [87]. Via these mechanisms, the resulting paucity of TCR signal strength and costimulation can result in incomplete activation, anergy and potentially iT_{reg} conversion. One caveat is that many, but not all, of these proposed mechanisms have not been demonstrated *in vivo*, thus their physiological impact has yet to be assessed.

T_{regs} may also broaden their influence by transducing signals into DCs that alter nutrient availability. The ligation of CTLA-4 on T_{regs} with CD80/CD86 on DCs induces their expression of Indoleamine 2,3-dioxygenase (IDO), a tryptophan metabolizing enzyme [88,89]. *In vivo* IDO induction in response to CpG DNA is dependent upon PD1-expressing T cells, as $\text{Rag1}^{-/-}$ splenic DCs do not upregulate this enzyme [90]. Other catabolic enzymes may also be involved in T_{reg} -mediated infectious tolerance as it has been shown that T_{regs} specifically induce expression of several essential amino acid depleting enzymes both within skin grafts and in cultured DCs [91]. In response to the resulting amino acid depletion, T cells fail to proliferate due to reduced mTOR signaling and instead upregulate Foxp3 expression in a TGF- β -dependent manner. IDO may also have a non-enzymatic role in promoting long-term pDC-mediated tolerance [92]. TGF- β can signal independently of Smad to induce both IDO and TGF- β expression in pDCs. These tolerogenic pDCs can induce $\text{Foxp3}^+ T_{\text{regs}}$ *in vitro* in a TGF- β -dependent manner and retain their tolerogenic phenotype *in vivo* for at least 3 months, implying this may be a mechanism through which infectious tolerance occurs. Since TGF- β can be produced by many cell types and is present in a latent form bound to the extracellular matrix, it would be interesting to determine if TGF- β -derived from activated T_{regs} is sufficient to drive IDO and TGF- β production from pDCs and mediate infectious tolerance.

The intestinal mucosa as a specialized environment for inducing tolerance

The gut is recognized as being an anatomical location rich in immune suppressive factors such as TGF- β and IL-10, and possessing multiple regulatory lymphocytic and tolerogenic DC populations [93,94]. This bias towards a tolerogenic environment is critical for avoiding potentially disastrous inflammatory responses to beneficial bacterial symbionts and food antigens. Tolerogenic DCs in the Peyer's Patch and intestinal lamina propria express high levels of IL10 and thus may shape the intestinal microenvironment [95,96]. Furthermore, in the presence of exogenous TGF- β , DCs isolated from the lamina propria of the small intestine and from the mesenteric lymph node are superior to splenic DCs at inducing Foxp3 expression by activated T cells [97,98]. A high proportion of DCs isolated from mucosal tissues express the α E integrin CD103 and are potent inducers of iT_{regs} compared with other DC populations. Importantly, this conversion is mediated without the addition of exogenous factors due to their ability to release large amounts of bioactive TGF- β [97,98]. This feature is clearly critical as α _v β ₈ integrin-deficient DCs, which cannot release bioactive TGF- β , fail to generate T_{regs} in culture, and mice with a leukocyte-restricted deletion of α _v β ₈, which is predominantly expressed by DCs, lack colonic Foxp3⁺ T_{regs} and thus develop colitis [99]. The gut is also an environment rich in retinoic acid (RA), which enhances the capacity of TGF- β to induce T_{regs} [100,98,97,101]. The target population for RA during iT_{reg} induction has been contentious. RA can act directly on naïve CD4⁺CD25⁻CD44^{lo}CD62L^{hi} cells to promote their conversion *in vitro*, even in the absence of endogenous and exogenous IL-2 [102]. However, RA can also partially act through the inhibition of cytokine production by CD4⁺CD44^{hi} effector/memory cells, which functionally block iT_{reg} conversion [103]. Importantly, RA receptor antagonists can abrogate the T_{reg} induction seen in the presence of small intestine lamina propria DCs, and naïve OT-II T cells transferred into RA receptor α deficient mice exhibit limited iT_{reg} conversion, indicating that RA signaling plays a critical role in the peripheral T_{reg} induction process [103,97,98]. In addition, the mesenteric lymph node-resident DCs have constitutively elevated levels of the tolerance-inducing enzyme IDO relative to splenic DCs [88].

Several studies have highlighted the central role of IL-10 in maintaining a suppressive microenvironment in the gut. Using IL-10 reporter mice, high frequencies of Foxp3⁺IL10⁺ T_{regs} have been noted in the lamina propria, while a particularly large proportion of Foxp3⁺IL10⁺ Tr1-like cells were observed in the Peyer's Patches and within the intraepithelial lymphocyte population [44]. Anti-TGF- β treatment results in reduced conversion of CD4⁺Foxp3⁻ to CD4⁺Foxp3⁺IL10⁺ T_{regs} in the gut, implicating TGF- β as an important factor in this process. Recent evidence highlights an interesting new mechanism to eliminate inflammatory cells in the gut. *In vivo* CD3-specific antibody treatment, which leads to T_H17 induction, results in an exodus of T_H17 cells from the body via the lumen of the small intestine [48]. However, T_H17 cells remaining in the small intestine acquired a suppressive phenotype that could be abrogated by blocking the IL10, CTLA-4 and TGF- β pathways simultaneously. Taken together, these observations suggest that the microenvironment intestinal mucosa possess many unique cellular and molecular features which predispose towards the generation of tolerogenic responses.

The contribution of commensal bacteria in promoting tolerance

Many of the tolerogenic signals in the gut may be derived from interactions between the immune system and intestinal microbes. The hygiene hypothesis postulates that early encounter with microbial agents in life inhibits the later development of allergic and autoimmune responses [104]. There are substantial epidemiological data to support this notion. Several recent reports have highlighted the importance of commensal bacteria on the development of pro- and anti-inflammatory responses (reviewed in [105]). Germ free mice

have profoundly underdeveloped gut associated lymphoid tissues including Peyer's patches, mesenteric lymph nodes, and intestinal lamina propria [106]. Several recent studies have shown that specific microbes, such as *Clostridium spp.*, can mediate the induction of Foxp3⁺ T_{regs} [107–109]. This mechanism of Foxp3 induction is likely crucial for expanding the specificity of the T_{reg} repertoire to include commensal bacteria and dietary antigens to promote intestinal homeostasis.

Tolerance-inducing signaling pathways modulated by microbes can act by altering DC function or by directly acting on T cells. The bacterium *Bacteroides fragilis* has recently emerged as a model for characterizing microbiota-derived tolerogenic signals. The *B. fragilis* molecule polysaccharide A (PSA) signals through TLR2 on the surface of T cells, which results in immunoregulation rather than inflammation [107,110]. In the absence of PSA, *B. fragilis* was unable to colonize the mucosa and instead elicits a T_H17 response. PSA-mediated induction of IL-10 expression by CD4⁺ cells was observed, which appears to be critical as *Il10*^{-/-} mice cannot be protected from IL-17-mediated colitis. Likewise, resistance to T_H17-mediated pathology was dependent on T_{regs}, as their depletion abrogated PSA-mediated protection [110]. Recent evidence corroborates the important role of IL-10 production by T_{regs} in suppressing T_H17-mediated intestinal pathology [45]. Autocrine IL-10:IL10R signaling in T_{regs} has been shown to amplify IL-10 production via a Stat3 dependent pathway [45]. Thus, it is possible that microbial-derived signals propagate IL-10 signaling and thus promote a suppressive microenvironment, which is particularly effective in suppressing T_H17 cells. This finding has led to the hypothesis that signals from commensal microbes result in defined immunoregulatory populations and pathways that have co-evolved to perfect a mutualistic partnership between the host and symbiont [105].

Helminth parasites as immunoregulators

Helminth parasites have also been shown to induce T_{regs} and suppress allergic disease in several models [111]. The capacity of helminths to act as immune suppressors would argue against the notion that allergic disease reduction is simply due to a systemic deviation away from T_H2 responses, since many helminths that dampen allergy also induce strong T_H2 responses. Helminths have been reported to induce a variety of regulatory populations including Foxp3⁺ T_{regs} [112,113], CD8⁺T_{regs} [114], and regulatory B cells [115]. In addition to the induction of regulatory populations, helminths also appear to augment the regulatory capacity of the resident Foxp3⁺ T_{reg} population [112,113].

Unlike symbiotic microbes that are beneficial to the host, helminth parasites have likely evolved tolerance-inducing mechanisms as a mode of immune evasion. Only live parasites are capable of expanding T_{regs} suggesting that they are actively involved in inducing immunosuppression [116] and that helminth excretory/secretory (E/S) products mediate T_{reg} induction [117]. These findings argue against T_{reg} induction as merely a response to intestinal inflammatory signals in an effort to limit pathology. During helminth infection there is evidence that T_{reg} recruitment and induction occurs. T_{reg} depletion prior to infection with *Litomosoides sigmodontis* has shown that resident Foxp3⁺ T_{regs} contribute to filarial worm survival [118]. However, iT_{regs} are generated at a high rate during *Heligmosomoides polygyrus* infection [117], a model in which T_{regs} are known to protect against immune pathology [119]. Recent studies have shown that *H. polygyrus* modulates the immune system by secreting molecules that mimic TGF-β signaling [117]. Indeed, *H. polygyrus* E/S products plus TCR stimulation *in vitro* are sufficient to induce Foxp3⁺ T_{regs} development in the absence of antigen presenting cells. Interestingly, *H. polygyrus* infection during ovalbumin mediated oral tolerance induction resulted in greatly augmented frequencies of Foxp3⁺ iT_{regs} specific for ovalbumin [117]. This finding is further evidence that tolerance initiated by *H. polygyrus* is 'infectious' to non-parasite related antigens. *Schistosoma*

mansoni soluble egg antigen (SEA) can also act directly on CD4⁺ T cells via TLR2 to induce the secretion of bioactive TGF- β which is required for their induction of iT_{regs} [120].

In addition to direct induction of T_{regs} by helminths, there are examples where DCs provide a critical link in mediating suppression. For instance, *S. mansoni* SEA requires DCs to mediate TGF- β -dependent Foxp3⁺ T_{reg} conversion and also elevates DC IL-10 production, potentially augmenting T_{reg} development and function [121,45]. Thus the direct effects of SEA on both the DC and CD4⁺ compartments may act synergistically to maximize T_{reg} conversion. Likewise, *H. polygyrus* E/S product can mediate T_{reg} induction through a DC-dependent mechanism. Antigen-pulsed bone marrow-derived DCs treated with *H. polygyrus* E/S product were deficient in their ability to upregulate co-stimulatory molecules *in vitro* and initiate antigen specific immune responses *in vivo* [122]. These treated DCs induced IL-10 producing Tr1-like cells *in vitro*. *H. polygyrus* has recently been shown to expand a tolerogenic DC population characterized by low CD11c expression [123]. These DCs could induce T_{regs} *in vitro* in the presence of TGF- β in an RA-dependent manner. Furthermore, despite depletion of CD11c^{hi}DCs *in vivo*, *H. polygyrus* infection could still effectively expand T_{regs} implicating a CD11c^{lo} population in tolerance induction.

Not only do tolerance-promoting microbes ensure their own survival by dampening immune responses, but they also promote tolerance to non-related antigens through the creation of a tolerogenic environment. Thus the intestinal microbiota appears to contribute substantially to systemic peripheral tolerance. For instance, PSA from *B. fragilis* limits the development of EAE [124]. Likewise, helminth parasites can protect against immunopathology in autoimmune diabetes and asthma mouse models [125,126]. Despite the clear expansion of tolerance by certain microbes, determining the contribution of infectious tolerance in these systems will be a challenge moving forward.

Conclusions

Dominant tolerance by regulatory T cell populations occurs through multiple mechanisms. This mechanistic diversity has likely evolved to match the plethora of distinct immune responses elicited to a multitude of different pathogens. Infectious tolerance represents an effective way for T_{regs} to establish a regulatory milieu to maintain immune balance. Immune tolerance may be maintained by strength in numbers, or through commissioning regulatory populations that can provide unique functional capabilities to ensure an effective immune response and prevent adverse immunopathology.

Future studies will be needed to fully understand the interrelationship between distinct T_{reg} populations. It will be a particular important to clarify these issues *in vivo* and define their mechanism of induction. For example, the relative contribution of direct cytokine-mediated conversion of suppressive populations, as seen *in vitro*, versus conversion that requires a cellular intermediate, such as a DC, will be challenging but important to ascertain. With further advances in our understanding of T_{reg} development and mechanisms of suppression, it may be possible to design targeted immunotherapies to modulate the immune response during cancer, allergy and autoimmunity.

Acknowledgments

Supported by the National Institutes of Health (AI39480, AI091977), St Jude NCI Comprehensive Cancer Center (CA-21765), and the American Lebanese Syrian Associated Charities.

Abbreviations

T_{regs}	regulatory T cells
IPEX	immunodysregulation polyendocrinopathy enteropathy X-linked
iT_{regs}	induced T _{regs}
EAE	experimental autoimmune encephalomyelitis
DC	Dendritic cells
IDO	Indoleamine 2,3-dioxygenase
RA	retinoic acid
PSA	polysaccharide A
E/S	excretory/secretory products
SEA	soluble egg antigen

References

1. Bluestone JA, Auchincloss H, Nepom GT, Rotrosen D, St Clair EW, Turka LA. The Immune Tolerance Network at 10 years: tolerance research at the bedside. *Nat Rev Immunol.* 2010; 10(11): 797–803. [PubMed: 20972473]
2. Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. *Immunol Rev.* 2011; 241(1):241–259. [PubMed: 21488901]
3. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol.* 2008; 26:677–704. [PubMed: 18173375]
4. Gershon RK, Kondo K. Infectious immunological tolerance. *Immunology.* 1971; 21(6):903–914. [PubMed: 4943147]
5. Waldmann H, Adams E, Fairchild P, Cobbold S. Infectious tolerance and the long-term acceptance of transplanted tissue. *Immunol Rev.* 2006; 212:301–313. [PubMed: 16903922]
6. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature.* 2011; 474(7351):298–306. [PubMed: 21677746]
7. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature.* 1953; 172(4379):603–606. [PubMed: 13099277]
8. Green DR, Flood PM, Gershon RK. Immunoregulatory T-cell pathways. *Annu Rev Immunol.* 1983; 1:439–463. [PubMed: 6152712]
9. Green DR, Gershon RK, Eardley DD. Functional deletion of different Ly-1 T-cell-inducer subset activities by Ly-2 suppressor T lymphocytes. *Proc Natl Acad Sci U S A.* 1981; 78(6):3819–3823. [PubMed: 6455665]
10. Bursuker I, North RJ. Generation and decay of the immune response to a progressive fibrosarcoma. II. Failure to demonstrate postexcision immunity after the onset of T cell-mediated suppression of immunity. *J Exp Med.* 1984; 159(5):1312–1321. [PubMed: 6232336]
11. North RJ, Bursuker I. T cell-mediated suppression of the concomitant antitumor immune response as an example of transplantation tolerance. *Transplant Proc.* 1984; 16(2):463–469. [PubMed: 6609455]
12. Benjamin RJ, Waldmann H. Induction of tolerance by monoclonal antibody therapy. *Nature.* 1986; 320(6061):449–451. [PubMed: 2938014]
13. Honey K, Cobbold SP, Waldmann H. CD40 ligand blockade induces CD4+ T cell tolerance and linked suppression. *J Immunol.* 1999; 163(9):4805–4810. [PubMed: 10528180]
14. Graca L, Honey K, Adams E, Cobbold SP, Waldmann H. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. *J Immunol.* 2000; 165(9):4783–4786. [PubMed: 11045999]

15. Scully R, Qin S, Cobbold S, Waldmann H. Mechanisms in CD4 antibody-mediated transplantation tolerance: kinetics of induction, antigen dependency and role of regulatory T cells. *Eur J Immunol.* 1994; 24(10):2383–2392. [PubMed: 7925565]
16. Qin S, Cobbold SP, Pope H, Elliott J, Kioussis D, Davies J, Waldmann H. "Infectious" transplantation tolerance. *Science.* 1993; 259:974–977.
17. Davies JD, Leong LY, Mellor A, Cobbold SP, Waldmann H. T cell suppression in transplantation tolerance through linked recognition. *J Immunol.* 1996; 156(10):3602–3607. [PubMed: 8621893]
18. Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, Kuniyasu Y, Nomura T, Toda M, Takahashi T. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev.* 2001; 182:18–32. [PubMed: 11722621]
19. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol.* 1995; 155(3):1151–1164. [PubMed: 7636184]
20. Brunkow ME, Jeffery EW, Hjerrild KA, Paepers B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet.* 2001; 27(1):68–73. [PubMed: 11138001]
21. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet.* 2001; 27(1):20–21. [PubMed: 11137993]
22. Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD, Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet.* 2001; 27(1):18–20. [PubMed: 11137992]
23. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J Clin Invest.* 2000; 106(12):R75–R81. [PubMed: 11120765]
24. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol.* 2003; 4(4):330–336. [PubMed: 12612578]
25. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003; 299(5609):1057–1061. [PubMed: 12522256]
26. Cobbold SP, Castejon R, Adams E, Zelenika D, Graca L, Humm S, Waldmann H. Induction of foxP3+ regulatory T cells in the periphery of T cell receptor transgenic mice tolerized to transplants. *J Immunol.* 2004; 172(10):6003–6010. [PubMed: 15128783]
27. Cobbold SP, Adams E, Graca L, Daley S, Yates S, Paterson A, Robertson NJ, Nolan KF, Fairchild PJ, Waldmann H. Immune privilege induced by regulatory T cells in transplantation tolerance. *Immunol Rev.* 2006; 213:239–255. [PubMed: 16972908]
28. Kendal AR, Chen Y, Regateiro FS, Ma J, Adams E, Cobbold SP, Hori S, Waldmann H. Sustained suppression by Foxp3+ regulatory T cells is vital for infectious transplantation tolerance. *J Exp Med.* 2011
29. Regateiro FS, Howie D, Cobbold SP, Waldmann H. TGF-beta in transplantation tolerance. *Curr Opin Immunol.* 2011
30. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature.* 1992; 359(6397):693–699. [PubMed: 1436033]
31. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. 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.* 2003; 198(12):1875–1886. [PubMed: 14676299]
32. Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. *J Immunol.* 2004; 172(9):5213–5221. [PubMed: 15100259]

33. Liu Y, Zhang P, Li J, Kulkarni AB, Perruche S, Chen W. A critical function for TGF- β signaling in the development of natural CD4⁺CD25⁺Foxp3⁺ regulatory T cells. *Nat Immunol.* 2008; 9(6):632–640. [PubMed: 18438410]
34. Tran DQ, Andersson J, Hardwick D, Bebris L, Illei GG, Shevach EM. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3⁺ regulatory T cells allows for their purification from expansion cultures. *Blood.* 2009; 113(21):5125–5133. [PubMed: 19299332]
35. Shevach EM. Mechanisms of foxp3⁺ T regulatory cell-mediated suppression. *Immunity.* 2009; 30(5):636–645. [PubMed: 19464986]
36. Andersson J, Tran DQ, Pesu M, Davidson TS, Ramsey H, O'Shea JJ, Shevach EM. CD4⁺ FoxP3⁺ regulatory T cells confer infectious tolerance in a TGF- β -dependent manner. *J Exp Med.* 2008; 205(9):1975–1981. [PubMed: 18710931]
37. Jonuleit H, Schmitt E, Kakirman H, Stassen M, Knop J, Enk AH. Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. *J Exp Med.* 2002; 196(2):255–260. [PubMed: 12119350]
38. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature.* 1997; 389(6652):737–742. [PubMed: 9338786]
39. Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. *Immunol Rev.* 2006; 212:28–50. [PubMed: 16903904]
40. Stassen M, Fondel S, Bopp T, Richter C, Muller C, Kubach J, Becker C, Knop J, Enk AH, Schmitt S, Schmitt E, Jonuleit H. Human CD25⁺ regulatory T cells: two subsets defined by the integrins α 4 β 7 or α 4 β 1 confer distinct suppressive properties upon CD4⁺ T helper cells. *Eur J Immunol.* 2004; 34(5):1303–1311. [PubMed: 15114663]
41. Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells [corrected]. *J Exp Med.* 2002; 196(2):247–253. [PubMed: 12119349]
42. Mekala DJ, Alli RS, Geiger TL. IL-10-dependent infectious tolerance after the treatment of experimental allergic encephalomyelitis with redirected CD4⁺CD25⁺ T lymphocytes. *Proc Natl Acad Sci U S A.* 2005; 102(33):11817–11822. [PubMed: 16087867]
43. Selvaraj RK, Geiger TL. Mitigation of experimental allergic encephalomyelitis by TGF- β induced Foxp3⁺ regulatory T lymphocytes through the induction of anergy and infectious tolerance. *J Immunol.* 2008; 180(5):2830–2838. [PubMed: 18292504]
44. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, Weaver CT. Regulatory T cells expressing interleukin 10 develop from Foxp3⁺ and Foxp3[–] precursor cells in the absence of interleukin 10. *Nat Immunol.* 2007; 8(9):931–941. [PubMed: 17694059]
45. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, Jack RS, Wunderlich FT, Bruning JC, Muller W, Rudensky AY. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity.* 2011; 34(4):566–578. [PubMed: 21511185]
46. Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, Chaudhry A, Kamanaka M, Kobayashi Y, Booth CJ, Rudensky AY, Roncarolo MG, Battaglia M, Flavell RA. Th17 20 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3⁺ regulatory CD4⁺ T cells in an interleukin-10-dependent manner. *Immunity.* 2011; 34(4):554–565. [PubMed: 21511184]
47. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, Treuting P, Siewe L, Roers A, Henderson WR Jr, Muller W, Rudensky AY. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity.* 2008; 28(4):546–558. [PubMed: 18387831]
48. Esplugues E, Huber S, Gagliani N, Hauser AE, Town T, Wan YY, O'Connor W Jr, Rongvaux A, Van Rooijen N, Haberman AM, Iwakura Y, Kuchroo VK, Kolls JK, Bluestone JA, Herold KC, Flavell RA. Control of TH17 cells occurs in the small intestine. *Nature.* 2011; 475(7357):514–518. [PubMed: 21765430]
49. Collison LW, Chaturvedi V, Henderson AL, Giacomini PR, Guy C, Bankoti J, Finkelstein D, Forbes K, Workman CJ, Brown SA, Rehgle JE, Jones ML, Ni HT, Artis D, Turk MJ, Vignali DA.

- IL-35-mediated induction of a potent regulatory T cell population. *Nat Immunol.* 2010; 11(12): 1093–1101. [PubMed: 20953201]
50. Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature.* 2007; 450(7169):566–569. [PubMed: 18033300]
 51. Chaturvedi V, Collison LW, Guy CS, Workman CJ, Vignali DA. Cutting edge: Human regulatory T cells require IL-35 to mediate suppression and infectious tolerance. *J Immunol.* 2011; 186(12): 6661–6666. [PubMed: 21576509]
 52. Cobbold SP, Adams E, Nolan KF, Regateiro FS, Waldmann H. Connecting the mechanisms of T-cell regulation: dendritic cells as the missing link. *Immunol Rev.* 2010; 236:203–218. [PubMed: 20636819]
 53. Yamazaki S, Bonito AJ, Spisek R, Dhodapkar M, Inaba K, Steinman RM. Dendritic cells are specialized accessory cells along with TGF- for the differentiation of Foxp3+ CD4+ regulatory T cells from peripheral Foxp3 precursors. *Blood.* 2007; 110(13):4293–4302. [PubMed: 17699744]
 54. Wang L, Pino-Lagos K, de Vries VC, Guleria I, Sayegh MH, Noelle RJ. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. *Proc Natl Acad Sci U S A.* 2008; 105(27):9331–9336. [PubMed: 18599457]
 55. Martin P, Del Hoyo GM, Anjuere F, Arias CF, Vargas HH, Fernandez LA, Parrillas V, Ardavin C. Characterization of a new subpopulation of mouse CD8alpha+ B220+ dendritic cells endowed with type 1 interferon production capacity and tolerogenic potential. *Blood.* 2002; 100(2):383–390. [PubMed: 12091326]
 56. Hadeiba H, Sato T, Habtezion A, Oderup C, Pan J, Butcher EC. CCR9 expression defines tolerogenic plasmacytoid dendritic cells able to suppress acute graft-versus-host disease. *Nat Immunol.* 2008; 9(11):1253–1260. [PubMed: 18836452]
 57. Farquhar CA, Paterson AM, Cobbold SP, Garcia Rueda H, Fairchild PJ, Yates SF, Adams E, Saunders NJ, Waldmann H, Nolan KF. Tolerogenicity is not an absolute property of a dendritic cell. *Eur J Immunol.* 2010; 40(6):1728–1737. [PubMed: 20373289]
 58. Yates SF, Paterson AM, Nolan KF, Cobbold SP, Saunders NJ, Waldmann H, Fairchild PJ. Induction of regulatory T cells and dominant tolerance by dendritic cells incapable of full activation. *J Immunol.* 2007; 179(2):967–976. [PubMed: 17617588]
 59. Gregori S, Tomasoni D, Pacciani V, Scirpoli M, Battaglia M, Magnani CF, Hauben E, Roncarolo MG. Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway. *Blood.* 2010; 116(6):935–944. [PubMed: 20448110]
 60. Levings MK, Gregori S, Tresoldi E, Cazzaniga S, Bonini C, Roncarolo MG. Differentiation of Tr1 cells by immature dendritic cells requires IL-10 but not CD25+CD4+ Tr cells. *Blood.* 2005; 105(3):1162–1169. [PubMed: 15479730]
 61. Kirchberger S, Majdic O, Steinberger P, Bluml S, Pfistershammer K, Zlabinger G, Deszcz L, Kuechler E, Knapp W, Stockl J. Human rhinoviruses inhibit the accessory function of dendritic cells by inducing sialoadhesin and B7-H1 expression. *J Immunol.* 2005; 175(2):1145–1152. [PubMed: 16002716]
 62. Seyerl M, Kirchberger S, Majdic O, Seipelt J, Jindra C, Schrauf C, Stockl J. Human rhinoviruses induce IL-35-producing Treg via induction of B7-H1 (CD274) and sialoadhesin (CD169) on DC. *Eur J Immunol.* 2010; 40(2):321–329. [PubMed: 19950173]
 63. Chappert P, Schwartz RH. Induction of T cell anergy: integration of environmental cues and infectious tolerance. *Curr Opin Immunol.* 2010; 22(5):552–559. [PubMed: 20869863]
 64. Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. *Nat Rev Immunol.* 2007; 7(8):610–621. [PubMed: 17627284]
 65. Hilkens CM, Isaacs JD, Thomson AW. Development of dendritic cell-based immunotherapy for autoimmunity. *Int Rev Immunol.* 2010; 29(2):156–183. [PubMed: 20199240]
 66. Schwartz RH. T cell anergy. *Annu Rev Immunol.* 2003; 21:305–334. [PubMed: 12471050]
 67. Wells AD. New insights into the molecular basis of T cell anergy: anergy factors, avoidance sensors, and epigenetic imprinting. *J Immunol.* 2009; 182(12):7331–7341. [PubMed: 19494254]

68. Powell JD, Lerner CG, Schwartz RH. Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. *J Immunol.* 1999; 162(5):2775–2784. [PubMed: 10072524]
69. Zheng Y, Collins SL, Lutz MA, Allen AN, Kole TP, Zarek PE, Powell JD. A role for mammalian target of rapamycin in regulating T cell activation versus anergy. *J Immunol.* 2007; 178(4):2163–2170. [PubMed: 17277121]
70. Thomson AW, Turnquist HR, Raimondi G. Immunoregulatory functions of mTOR inhibition. *Nat Rev Immunol.* 2009; 9(5):324–337. [PubMed: 19390566]
71. Hardie DG. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat Rev Mol Cell Biol.* 2007; 8(10):774–785. [PubMed: 17712357]
72. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, Worley PF, Kozma SC, Powell JD. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity.* 2009; 30(6):832–844. [PubMed: 19538929]
73. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, Mellor AL. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity.* 2005; 22(5):633–642. [PubMed: 15894280]
74. Grallert B, Boye E. The Gcn2 kinase as a cell cycle regulator. *Cell Cycle.* 2007; 6(22):2768–2772. [PubMed: 17986863]
75. Zarek PE, Powell JD. Adenosine and anergy. *Autoimmunity.* 2007; 40(6):425–432. [PubMed: 17729036]
76. Zarek PE, Huang CT, Lutz ER, Kowalski J, Horton MR, Linden J, Drake CG, Powell JD. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. *Blood.* 2008; 111(1):251–259. [PubMed: 17909080]
77. Tadokoro CE, Shakhar G, Shen S, Ding Y, Lino AC, Maraver A, Lafaille JJ, Dustin ML. Regulatory T cells inhibit stable contacts between CD4+ T cells and dendritic cells in vivo. *J Exp Med.* 2006; 203(3):505–511. [PubMed: 16533880]
78. Tang Q, Adams JY, Tooley AJ, Bi M, Fife BT, Serra P, Santamaria P, Locksley RM, Krummel MF, Bluestone JA. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat Immunol.* 2006; 7(1):83–92. [PubMed: 16311599]
79. Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. *J Immunol.* 2004; 172(8):4676–4680. [PubMed: 15067041]
80. Serra P, Amrani A, Yamanouchi J, Han B, Thiessen S, Utsugi T, Verdaguer J, Santamaria P. CD40 ligation releases immature dendritic cells from the control of regulatory CD4+CD25+ T cells. *Immunity.* 2003; 19(6):877–889. [PubMed: 14670304]
81. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc Natl Acad Sci U S A.* 2008; 105(29):10113–10118. [PubMed: 18635688]
82. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science.* 2008; 322(5899):271–275. [PubMed: 18845758]
83. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, Hou TZ, Futter CE, Anderson G, Walker LS, Sansom DM. Transendocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science.* 2011; 332(6029):600–603. [PubMed: 21474713]
84. Workman CJ, Cauley LS, Kim IJ, Blackman MA, Woodland DL, Vignali DA. Lymphocyte activation gene-3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo. *J Immunol.* 2004; 172(9):5450–5455. [PubMed: 15100286]
85. Workman CJ, Vignali DA. Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223). *J Immunol.* 2005; 174(2):688–695. [PubMed: 15634887]
86. Huang CT, Workman CJ, Flies D, Pan X, Marson AL, Zhou G, Hipkiss EL, Ravi S, Kowalski J, Levitsky HI, Powell JD, Pardoll DM, Drake CG, Vignali DA. Role of LAG-3 in regulatory T cells. *Immunity.* 2004; 21(4):503–513. [PubMed: 15485628]

87. Liang B, Workman C, Lee J, Chew C, Dale BM, Colonna L, Flores M, Li N, Schweighoffer E, Greenberg S, Tybulewicz V, Vignali D, Clynes R. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J Immunol.* 2008; 180(9):5916–5926. [PubMed: 18424711]
88. Onodera T, Jang MH, Guo Z, Yamasaki M, Hirata T, Bai Z, Tsuji NM, Nagakubo D, Yoshie O, Sakaguchi S, Takikawa O, Miyasaka M. Constitutive expression of IDO by dendritic cells of mesenteric lymph nodes: functional involvement of the CTLA-4/B7 and CCL22/CCR4 interactions. *J Immunol.* 2009; 183(9):5608–5614. [PubMed: 19843945]
89. Mellor AL, Chandler P, Baban B, Hansen AM, Marshall B, Pihkala J, Waldmann H, Cobbold S, Adams E, Munn DH. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int Immunol.* 2004; 16(10):1391–1401. [PubMed: 15351783]
90. Baban B, Chandler PR, Johnson BA 3rd, Huang L, Li M, Sharpe ML, Francisco LM, Sharpe AH, Blazar BR, Munn DH, Mellor AL. Physiologic Control of IDO Competence in Splenic Dendritic Cells. *J Immunol.* 2011; 187(5):2329–2335. [PubMed: 21813777]
91. Cobbold SP, Adams E, Farquhar CA, Nolan KF, Howie D, Lui KO, Fairchild PJ, Mellor AL, Ron D, Waldmann H. Infectious tolerance via the consumption of essential amino acids and mTOR signaling. *Proc Natl Acad Sci U S A.* 2009; 106(29):12055–12060. [PubMed: 19567830]
92. Pallotta MT, Orabona C, Volpi C, Vacca C, Belladonna ML, Bianchi R, Servillo G, Brunacci C, Calvitti M, Biccato S, Mazza EM, Boon L, Grassi F, Fioretti MC, Fallarino F, Puccetti P, Grohmann U. Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells. *Nat Immunol.* 2011; 12(9):870–878. [PubMed: 21804557]
93. Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. *Annu Rev Immunol.* 2009; 27:313–338. [PubMed: 19302043]
94. Grainger JR, Hall JA, Bouladoux N, Oldenhove G, Belkaid Y. Microbe-dendritic cell dialog controls regulatory T-cell fate. *Immunol Rev.* 2010; 234(1):305–316. [PubMed: 20193027]
95. Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med.* 1999; 190(2):229–239. [PubMed: 10432286]
96. Chirido FG, Millington OR, Beacock-Sharp H, Mowat AM. Immunomodulatory dendritic cells in intestinal lamina propria. *Eur J Immunol.* 2005; 35(6):1831–1840. [PubMed: 16010704]
97. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, Belkaid Y. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med.* 2007; 204(8):1775–1785. [PubMed: 17620362]
98. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. 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.* 2007; 204(8):1757–1764. [PubMed: 17620361]
99. Travis MA, Reizis B, Melton AC, Masteller E, Tang Q, Proctor JM, Wang Y, Bernstein X, Huang X, Reichardt LF, Bluestone JA, Sheppard D. Loss of integrin alpha(v)beta8 on dendritic cells causes autoimmunity and colitis in mice. *Nature.* 2007; 449(7160):361–365. [PubMed: 17694047]
100. Benson MJ, Pino-Lagos K, Roseblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of costimulation. *J Exp Med.* 2007; 204(8):1765–1774. [PubMed: 17620363]
101. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, Cheroutre H. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science.* 2007; 317(5835):256–260. [PubMed: 17569825]
102. Mucida D, Pino-Lagos K, Kim G, Nowak E, Benson MJ, Kronenberg M, Noelle RJ, Cheroutre H. Retinoic acid can directly promote TGF-beta-mediated Foxp3(+) Treg cell conversion of naive T cells. *Immunity.* 2009; 30(4):471–472. author reply 472. [PubMed: 19371709]
103. Hill JA, Hall JA, Sun CM, Cai Q, Ghyselinck N, Chambon P, Belkaid Y, Mathis D, Benoist C. Retinoic acid enhances Foxp3 induction indirectly by relieving inhibition from CD4+CD44hi Cells. *Immunity.* 2008; 29(5):758–770. [PubMed: 19006694]

104. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. 2010; 160(Clin Exp Immunol)(1):1–9.
105. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science. 2010; 330(6012):1768–1773. [PubMed: 21205662]
106. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol. 2004; 4(6):478–485. [PubMed: 15173836]
107. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010; 107(27):12204–12209. [PubMed: 20566854]
108. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011; 331(6015):337–341. [PubMed: 21205640]
109. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity. 2011; 34(5):794–806. [PubMed: 21596591]
110. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011; 332(6032):974–977. [PubMed: 21512004]
111. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol Biochem Parasitol. 2009; 167(1):1–11. [PubMed: 19406170]
112. Finney CA, Taylor MD, Wilson MS, Maizels RM. Expansion and activation of CD4(+)CD25(+) regulatory T cells in Heligmosomoides polygyrus infection. Eur J Immunol. 2007; 37(7):1874–1886. [PubMed: 17563918]
113. Rausch S, Huehn J, Kirchhoff D, Rzepecka J, Schnoeller C, Pillai S, Loddenkemper C, Scheffold A, Hamann A, Lucius R, Hartmann S. Functional analysis of effector and regulatory T cells in a parasitic nematode infection. Infect Immun. 2008; 76(5):1908–1919. [PubMed: 18316386]
114. Metwali A, Setiawan T, Blum AM, Urban J, Elliott DE, Hang L, Weinstock JV. Induction of CD8+ regulatory T cells in the intestine by Heligmosomoides polygyrus infection. Am J Physiol Gastrointest Liver Physiol. 2006; 291(2):G253–G259. [PubMed: 16825660]
115. Husaarts L, van der Vlugt LE, Yazdanbakhsh M, Smits HH. Regulatory B-cell induction by helminths: Implications for allergic disease. J Allergy Clin Immunol. 2011
116. McSorley HJ, Hargus YM, Murray J, Taylor MD, Maizels RM. Expansion of Foxp3+ regulatory T cells in mice infected with the filarial parasite Brugia malayi. J Immunol. 2008; 181(9):6456–6466. [PubMed: 18941236]
117. Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Hargus Y, Filbey KJ, Finney CA, Greenwood EJ, Knox DP, Wilson MS, Belkaid Y, Rudensky AY, Maizels RM. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-beta pathway. J Exp Med. 2010; 207(11):2331–2341. [PubMed: 20876311]
118. Taylor MD, van der Werf N, Harris A, Graham AL, Bain O, Allen JE, Maizels RM. Early recruitment of natural CD4+ Foxp3+ Treg cells by infective larvae determines the outcome of filarial infection. Eur J Immunol. 2009; 39(1):192–206. [PubMed: 19089814]
119. Rausch S, Huehn J, Loddenkemper C, Hepworth MR, Klotz C, Sparwasser T, Hamann A, Lucius R, Hartmann S. Establishment of nematode infection despite increased Th2 responses and immunopathology after selective depletion of Foxp3+ cells. Eur J Immunol. 2009; 39(11):3066–3077. [PubMed: 19750483]
120. Burton OT, Gibbs S, Miller N, Jones FM, Wen L, Dunne DW, Cooke A, Zaccane P. Importance of TLR2 in the direct response of T lymphocytes to Schistosoma mansoni antigens. Eur J Immunol. 2010; 40(8):2221–2229. [PubMed: 20480503]
121. Zaccane P, Burton O, Miller N, Jones FM, Dunne DW, Cooke A. Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice. Eur J Immunol. 2009; 39(4):1098–1107. [PubMed: 19291704]

122. Segura M, Su Z, Piccirillo C, Stevenson MM. Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. *Eur J Immunol.* 2007; 37(7):1887–1904. [PubMed: 17563917]
123. Smith KA, Hochweller K, Hammerling GJ, Boon L, MacDonald AS, Maizels RM. Chronic helminth infection promotes immune regulation in vivo through dominance of CD11c⁺CD103⁺ dendritic cells. *J Immunol.* 2011; 186(12):7098–7109. [PubMed: 21576507]
124. Ochoa-Reparaz J, Mielcarz DW, Ditrio LE, Burroughs AR, Begum-Haque S, Dasgupta S, Kasper DL, Kasper LH. Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *J Immunol.* 2010; 185(7):4101–4108. [PubMed: 20817872]
125. Saunders KA, Raine T, Cooke A, Lawrence CE. Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. *Infect Immun.* 2007; 75(1):397–407. [PubMed: 17043101]
126. Dittrich AM, Erbacher A, Specht S, Diesner F, Krokowski M, Avagyan A, Stock P, Ahrens B, Hoffmann WH, Hoerauf A, Hamelmann E. Helminth infection with *Litomosoides sigmodontis* induces regulatory T cells and inhibits allergic sensitization, airway inflammation, and hyperreactivity in a murine asthma model. *J Immunol.* 2008; 180(3):1792–1799. [PubMed: 18209076]

Fig. 1. Infectious tolerance through linked suppression

Allogeneic transplantation models have revealed that tolerance to a particular graft induced through co-stimulatory molecule blockade can be transferred to unrelated antigens. This is possible if a second graft from an F1 cross of the tolerated strain and an unrelated strain is given, highlighting the importance of a localized regulatory environment [17]. Linked suppression may be the result of tolerated antigens and foreign antigens being presented by the same DCs or in a highly localized cytokine environment conducive to T_{reg} induction. After tolerance induction to the F1 graft is established, a graft from the unrelated strain can be tolerated in the absence of the initially tolerated antigens.

Fig. 2. Cytokine-mediated infectious tolerance by T_{regs} in the presence or absence of DCs
 Direct infectious tolerance via the cytokines IL-10, TGF- β , and IL-35 have been described *in vitro* in the absence of a requirement for antigen presenting cells. Foxp3⁺ T_{regs} have also been described to interact with DCs to promote a tolerogenic phenotype. This occurs through modulation of co-stimulatory molecule expression and promotion of tolerance-inducing factors like IDO and immunosuppressive cytokines, leading to an environment that promotes the induction of T_{regs}.