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Review
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Th17 and Regulatory T Cells in Mediating and Restraining Inflammation

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The vertebrate immune system is poised in a state of equilibrium that permits accurate and rapid protective responses against pathogens but curtails potential for causing harm to the host through targeting of "self" and provoking overexuberant inflammatory processes. In this Review we discuss this balance achieved in large part by interactions of different classes of T lymphocytes that have potent pro- or anti-inflammatory activity in the context of genetic and environmental factors, particularly the commensal microbiota.

Introduction

Innate immune systems of lower organisms afford protection against infection through recruitment and activation of phagocytic effector cells and through elaboration of soluble mediators. The innate immune defenses are summoned upon recognition of microbial products by conserved, germline encoded sensors selected during evolution for their ability to recognize prototypical molecular features or patterns distinguishing microorganisms from their hosts (Janeway, 1989). These sensing mechanisms are exemplified by Toll-like receptors (TLR) and their ligands, including lipopolysaccharide (LPS), lipopeptide, and bacterial and viral nucleic acids (Medzhitov, 2009). Changes in the metabolic status of the host caused by the infectious agents or damage to the host tissues can also be detected by additional sets of sensors such as the inflammasome. Recent advances in understanding of molecular and cellular mechanisms of infection-associated inflammation are discussed in detail in Reviews by O. Takeuchi and S. Akira and K. Schroder and J. Tschopp (page 805 and page 821 of this issue, respectively). During the last several years it has become apparent that these sensing mechanisms are capable of distinguishing different classes of pathogens and are hard-wired to activate distinct effectors capable of protection against the corresponding type of pathogen.

The effectiveness of innate immune defenses is limited in higher organisms, likely due to the complexity of the body plan along with delayed onset and decreasing rate of reproduction, which result in steeper evolutionary penalties for failures of the immune system. Adaptive immune systems, which rely on the somatic generation of enormously diverse antigen-specific receptors, have thus evolved to anticipate encounters with invading pathogens and to effectively counter the rapid evolution of viruses and bacteria facilitated by their high reproduction and mutation rates. Two branches of adaptive immune recognition, mediated by T cell receptors (TCRs) and immunoglobulin (Ig) receptors utilized by T and B lymphocytes, are directed against pathogens hidden within the cells of the host or exposed for recognition outside the host cells or on their surfaces, respectively. Whereas Ig receptors displayed by B cells recognize intact antigens, T cell receptors recognize short peptides derived from foreign and self-antigens bound to products of highly polymorphic major histocompatibility complex (MHC) molecules. MHC molecules present peptide products of constitutive cellular protein breakdown and do not distinguish between self and foreign, i.e., pathogen-derived products, and hence their recognition by TCRs underlies the central roles that T cells have in both host defense and autoimmune inflammatory diseases. T cells must exist in a balanced state in which they are poised to pounce on offending microbes while remaining tolerant to host molecules and harmless commensal microorganisms. How T cells achieve a state of tolerance to self-antigens and how pathological conditions, environmental factors, and genetic predisposition result in breaking of tolerance and establishment of inflammatory processes have been areas of intense investigation during the past two decades. It is now known that, in addition to central tolerance established as a result of induced apoptosis of strongly self-reactive cells in the thymus, there are mechanisms of peripheral tolerance, achieved in large part through the activity of regulatory T (Treg) cells.

Distinct types of adaptive immune responses affording protection against different classes of pathogens are facilitated by the differentiation of $CD4^+$ T cells into the corresponding types of effector T cells, which currently include Th1, Th2, and Th17 subsets. Through elaboration of distinct sets of cytokines and other soluble and cell-bound products, these cells may act as immune effectors eliminating infected cells. More importantly, they act as principal amplifiers and inducers of the appropriate inflammatory and effector responses in cells of the innate immune system and "nonimmune" cells. The amplified blocks of adaptive and innate immune responses lead to efficient clearance or containment of the offending pathogen.

The downside of powerful mechanisms of protection against pathogen afforded by the immune system of higher organisms is inflammation associated with the "unwanted" immune

responses against ''self'' and environmental antigens and commensal microorganisms as well as ''collateral'' damage to the host as a side effect of immune responses against pathogens. These side effects are at times more devastating than infection itself. Thus, protective immune responses must ensure survival of the host in a trade-off between sterilizing immunity and its negative regulation limiting pathogen elimination. The restraint of overzealous immune responses involves numerous mechanisms operating in a cell-intrinsic and -extrinsic manner in relation to inflammation-inducing cells. Among these mechanisms, Treg cell-mediated suppression of inflammation associated with infection plays a prominent role.

In this Review, we discuss T cell-mediated inflammation and the mechanisms of its negative regulation by focusing on differentiation and function of Th17 effector cells, which elicit a highly inflammatory immune response, and of Treg cells that serve as critical gatekeepers in immune homeostasis. Th17 cells have been implicated in numerous autoimmune diseases and other inflammatory conditions, but they, as well as Treg cells, are most abundant at mucosal surfaces, particularly the intestinal lamina propria (LP). We will highlight recent studies linking signals from commensal microbes to the differentiation and function of these types of T cells.

Th17 Cells and Company

Following infection with diverse microbes, T cells undergo differentiation when their TCRs are triggered in the presence of particular combinations of cytokines produced by innate immune cells (Abbas et al., 1996; Mosmann and Coffman, 1989). Infection of myeloid cells with intracellular bacteria and viruses typically elicits production of IL-12, which induces differentiation of interferon-γ (IFN-γ)-producing Th1 cells and cytotoxic $CD8^+$ T cells that are best suited to clear such pathogens. Infection with parasitic worms, in contrast, induces production of IL-4 by cells of the innate immune system, and this, in turn, stimulates $CD4^+$ T cells to differentiate into Th2 cells that produce more IL-4, as well as IL-5 and IL-13, cytokines involved in controlling expulsion of the helminths.

A third subset of T helper cells, Th17 cells, are abundant at mucosal interfaces, where they contain infection with pathogenic bacteria and fungi (Weaver et al., 2007). These cells produce IL-17A (also referred to as IL-17), IL-17F, and IL-22, cytokines involved in neutrophilia, tissue remodeling and repair, and production of antimicrobial proteins. Since IL-17 cytokine family members are found in invertebrate organisms, where they appear to be involved in immune responses, Th17 cells may represent a relatively recent evolutionary adaptation of T cells to employ an effector program that predates the branching of chordates (Guo et al., 2009; Hibino et al., 2006; Roberts et al., 2008).

Th17 cells differentiate in response to the STAT3-activating cytokines IL-6, IL-21, and IL-23 along with TGF-β and IL-1β (Korn et al., 2009). Skin-homing T helper cells that produce IL-22, but not IL-17, have been described in humans, and it is possible that they represent a T cell subset with distinct effector functions (Duhen et al., 2009). The differentiation of $CD4^+$ T cells that produce IL-17 and IL-22 is influenced by the composition of the intestinal microbiota and by the presence of innate immune cells that amplify the Th17 cell response.

Th1 cells were long considered to be the major effectors in multiple autoimmune diseases, while Th2 cells have been known to be involved in atopy and asthma. More recently, Th17 cells have been implicated as culprits in a plethora of autoimmune and other inflammatory diseases in mice and humans. Many of the disease states previously associated with Th1 cells, e.g., experimental autoimmune encephalomyelitis (EAE, a model for multiple sclerosis), collagen-induced arthritis, and some forms of colitis, were shown to be caused by IL-23-dependent Th17 cells or other IL-17-producing lymphoid cell types (Ahern et al., 2008; Cua et al., 2003; McKenzie et al., 2006). An imbalance between Th17 and Treg cell function may be central in some of these diseases. Conversely, defects in the Th17 cell differentiation axis may predispose the host to bacterial and fungal infections at mucosal surfaces (Ouyang et al., 2008).

Differentiation of Th17 Cells

The existence of Th17 cells was only recently appreciated, following the discovery that the p40 subunit of IL-12 and its receptor subunit, IL-12Rβ1, are shared by IL-23 and its receptor, respectively. Selective inactivation of the p19 component of IL-23 or the IL-23 receptor resulted in loss of autoimmune disease manifestations, increased susceptibility to infections with mucosal pathogens, and marked reductions in the number of $CD4^+$ T cells expressing IL-17 and, especially, IL-22 (McKenzie et al., 2006; Ouyang et al., 2008). Th17 cells were shown to differentiate in vitro from naive $CD4^+$ T cells in response to TCR signaling in the presence of IL-6 and TGF-β, but not IL-23 (Bettelli et al., 2006; Veldhoen et al., 2006). The receptor for IL-23 is expressed on naive murine $CD4^+$ T cells only after stimulation in the presence of IL-6 or IL-21, and then these other cytokines can give way to the ability of IL-23 to stimulate continued differentiation of Th17 cells and, perhaps, their survival (Korn et al., 2007; Nurieva et al., 2007; Zhou et al., 2007). In human T cells, IL-23R may be constitutively expressed on $CD4^+$ T cells, and hence IL-17 expression can be induced by IL-23 in vitro (Manel et al., 2008). Mouse T cells bearing γδ TCRs, which are prominent in mucosal tissues, also express IL-23R constitutively and have been reported to differentiate into IL-17-producing cells early after exposure to IL-23 (see below) (Roark et al., 2008).

Whether Th17 cells represent a true terminally differentiated lineage versus a metastable state with distinct effector functions has been an area of much recent investigation. Many $IL\text{-}17^+$ cells present in inflammatory foci, for example in the central nervous system of mice with EAE, also express IFN-γ, and there is evidence that $IL\text{-}17^+$ T cells can differentiate into Th1 cells under inflammatory conditions in vivo (Bending et al., 2009). Th1 and Th2 cells were thought to be more stable, but recent studies found that Th2 cells can be induced to also express T-bet, the central transcriptional regulator for Th1 cells, and IFN-γ following infection with viruses (Hegazy et al., 2010). Thus, reprogramming of T helper cell effector functions may be critical for host defense to specific microbial challenge. In this context, studies of the epigenetic status of the genes that encode lineage-restricted transcription factors have been invoked as providing insight as to the reprogramming capacity of T cells. However, in vitro

polarized Th2 and Th17 cells displayed repressive histone modifications at the *Tbx21* locus encoding T-bet, rather than the bivalent configuration of positive and negative marks thought to represent a state poised for reprogramming. Thus, the chromatin state in these cells does not appear to reflect the differentiation potential observed in vivo. The epigenetic status of regulatory genes in T cells recovered directly from animals has yet to be determined. It may not be surprising if the gene expression state of multiple loci differs in T cells according to their site in the body and the type of inflammatory or microbial challenge confronted.

The Th17 program, whether stable or not, is defined both in vitro and in vivo by the expression of the signature cytokines, the chemokine receptor CCR6, and IL-23R. These same features are also found in other lymphoid cells, e.g., TCRγδ T cells, lymphoid tissue inducer (LTi) cells, and phenotypically related cells with NK cell markers, that secrete IL-17 and/or IL-22 (Colonna, 2009). These cells share with Th17 cells the expression of the orphan nuclear receptor RORγt, which is both necessary and sufficient for expression of the genes that currently define the Th17 program (Ivanov et al., 2006). In addition to RORγt, multiple other transcription factors (TFs) have been shown to be required for the expression of IL-17 in polarized T helper cells, and several of these are also required for upregulation of RORγt upon polarization. These include IRF4 and BATF, whose expression is induced upon TCR signaling, and STAT3 (Brustle et al., 2007; Schraml et al., 2009; Zhou and Littman, 2009). Additional transcription factors that contribute to the induction of IL-17 in polarized cells include Runx1/CBFβ, c-Maf, and the ligand-regulated aryl hydrocarbon receptor (Ahr) (Bauquet et al., 2009; Veldhoen et al., 2008; Zhang et al., 2008). RORα also contributes to some IL-17 expression in the absence of RORγt (Yang et al., 2008b). Except for Ahr, which is required for induction of IL-22 in response to xenobiotic ligands, the role of most of these factors in establishing a Th17 gene signature other than IL-17 itself has not been examined. STAT3, IRF4, and BATF are required for expression of RORγt in Th17-polarized T helper cells, yet each contributes additionally, in cooperation with RORγt, to expression of IL-17 and, likely, other key components of the Th17 program. The genetic underpinning of the program will likely be unraveled through a combination of high-throughput ChIP-sequence analysis and expression profiling of T cells with mutations in the different TF genes.

The expression of IL-17 by at least one lymphocyte subset, the γδ T cells, has been proposed to be important for Th17 cell differentiation. Unlike Th17 cells, which produce IL-17 in response to TCR and cytokine receptor stimulation, the γδ T cells do so independently of the TCR upon exposure to IL-23 and IL-1β (Sutton et al., 2009; Roark et al., 2008). The γδ cells produce IL-17 in vivo within hours following infection or other inflammatory stimuli, likely due to the presence of the inducing cytokines as well as stimulation of cell surface TLRs and dectin1 by microbial products (Martin et al., 2009). IL-17 then activates widely expressed receptors, on mesenchymal and epithelial cells, resulting in production of IL-6, IL-1β, and other inflammatory cytokines that act in a positive feedback loop on the γδ cells and on differentiating Th17 cells (Figure 1). Mice deficient for γδ cells have markedly attenuated Th17-mediated autoimmune disease, consistent with a need for this amplifying loop in the induction of pathogenic Th17 cells (Sutton et al., 2009).

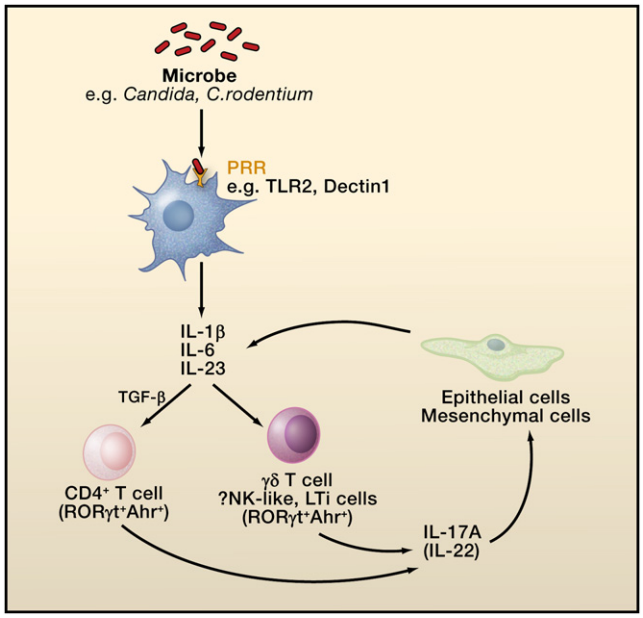

Figure 1. Role of Innate Lymphocytes in Initiation and Amplification of a Th17 Cell Response

Early activation of innate immune cells through their pattern recognition receptors (PRRs) results in production of IL-1β and IL-23 that, together, induce IL-17 production in γδ T cells (and potentially other RORγt+ lymphocytes) in the absence of TCR signaling. IL-17 induces production of inflammatory cytokines by epithelial and stromal cells, and these amplify responses by acting on TCR-activated CD4+ T cells and on other innate lymphocytes.

Th17 Cells in Inflammation

Although there is abundant evidence that Th17 cells have important roles in a variety of inflammatory conditions, there is considerable controversy as to whether the key cytokines produced by these cells are essential in those diseases. It also remains unclear what is the relative contribution to host protection and inflammation of the diverse cell types that have an effector cytokine profile similar to that of Th17 cells. IL-17, IL-17F, and IL-22 have been shown to be required to protect mice from pathology induced by air-borne *Klebsiella pneumoniae* and orally administered *Citrobacter rodentium* (Ouyang et al., 2008). However, the roles of these cytokines in several autoimmune disease models have been disputed. Thus, IL-17 and/or IL-17F deficiency does not prevent colitis mediated by transfer of Treg-depleted CD4+ T cells (Izcue et al., 2008; Leppkes et al., 2009), and colitis may even be exacerbated in the absence of IL-17 receptor signaling in pathogenic Th1 cells (O'Connor et al., 2009), presumably due to elevated IFN-γ. Although IL-17 was reported to have an important role in EAE (Komiyama et al., 2006), more recent reports have disputed that assertion and also showed no significant contribution of IL-22 (Haak et al., 2009; Kreymborg et al., 2007). However, multiple genetic studies have shown that IL-23 and its receptor as well as RORγt, which are essential for the differentiation of Th17 cells, are required for disease

manifestation in the colitis and EAE models (Cua et al., 2003; Ivanov et al., 2006; Kullberg et al., 2006; Uhlig et al., 2006). Moreover, polymorphisms in the *IL-23R* gene have been strongly associated with either protection from or susceptibility to Crohn's disease (Abraham and Cho, 2009). These apparently conflicting observations have two nonmutually exclusive explanations: there are effector functions other than production of the signature cytokines that contribute to inflammation mediated by Th17 cells; and other lymphoid cell types that are dependent on IL-23R signaling and RORγt may have pivotal roles in licensing T cell effector functions that result in inflammatory disease. The functions of such cells have not been studied in great detail, but IL-17 produced by γδ T cells, LTi cells, and NK-like cells may function to amplify Th17 effector functions. In this context, it remains difficult to assess relative contributions of Th17 cells versus other IL-23-dependent cells to inflammatory responses at mucosal sites. Selective inactivation of IL-23R and/or RORγt in different cell subsets will be required to clearly demarcate the functions of each cell type in mounting Th17 responses and in mediating both antimicrobial defense and inflammatory disease.

Th17-associated cytokines, if not Th17 cell per se, have been implicated as contributing to metabolic diseases and cancer. IL-23 and IL-17 have been shown to contribute to colon carcinogenesis in mice that had the multiple polyposis Apc^{min} mutation and that were colonized with enterotoxigenic *Bacteroides fragilis*, a bacterium present in the gut flora of a sizeable fraction of humans and associated with diarrhea (Wu et al., 2009). IL-23 has also been shown to promote the growth of multiple tumors in mice, particularly chemically induced papillary skin tumors (Langowski et al., 2006). A contribution of IL-17 to atherosclerotic disease has been proposed, but a direct link to Th17 cells or other inflammatory lymphocytes has not yet been established (Taleb et al., 2009). Diet-induced obesity in mice has been reported to result in an IL-6-dependent increase in Th17 cells, and the metabolic imbalance may thus contribute to increased severity or incidence of autoimmune disease (Winer et al., 2009). The positive amplifying loop in induction of Th17 cytokines suggests that, in the absence of countervailing regulation, e.g., production of IL-10 or Treg cells, Th17 cells may indeed contribute to the onset or persistence of metabolism-associated diseases.

Role of the Microbiota in T Cell Differentiation and in Maintenance of Barrier Function

Inflammatory responses are influenced by microbial products encountered during infections with pathogens and by host genetic polymorphisms that may predispose individuals to autoimmune disease. In addition, the commensal microbiota have a particularly important influence in regulating the immune system. Products of commensal microbes are likely to contribute to distribution of lymphoid cells in different mucosal tissues and to their effector functions, which protect against damage inflicted on the tissue by pathogens or toxins. Naive T lymphocytes receive innate immune cell-derived signals that guide them to specific tissues. Thus, DC-derived vitamin D and retinoic acid induce, respectively, CCR10 and CCR9, whose engagement by chemokines guides the T cells to skin and small intestine, respectively (Iwata et al., 2004; Sigmundsdottir et al., 2007). T cells that traffic to the intestine typically have effector or memory cell phenotypes, indicative of their previous exposure to antigenic stimuli. Such cells are present in large numbers in both large intestine and the terminal ileum, sites of extensive organized lymphoid tissue. These are also sites of abundant colonization with hundreds of different bacterial species that make up the commensal microbiota. The contribution of the microbiota to host immune responses has long been recognized, but it is only recently that specific bacterial species have been linked to the differentiation of specific types of T cells. The composition of the gut microbes has also been shown to correlate with proclivity for obesity or type 2 diabetes (Blaser and Falkow, 2009; Turnbaugh and Gordon, 2009), but it is not known whether microbial influence on T cells has a function in these metabolic disorders.

Th17 and Treg cells are most abundant in the intestinal LP. TCR transgenic, RAG-deficient mice have all T cells restricted to a single specificity and typically lack LP T cells. However, they accumulate such cells if they are fed antigen that is processed to be recognized by the transgenic TCR (Curotto de Lafaille and Lafaille, 2009). The specificity of the LP T cells present in mice with a full TCR repertoire is not currently known, although it is likely that many of the T cells are specific for products of the commensal microbiota. Nevertheless, the number of T cells in the LP is only modestly reduced in mice kept in germ-free conditions, which suggests that T cells accumulate due to activation in response to self and/or food antigens. In addition to antigenic stimuli, other commensal-derived signals influence the differentiation of T cells with distinct effector functions. Thus, colonization of mice with polysaccharide-A (PSA)-deficient *Bacteroides fragilis*, a commensal present in humans, promoted colitis in RAG-deficient mice into which Treg-depleted naive $CD4^+$ T cells had been transferred. However, administration of PSA protected the mice from transfer-mediated colitis, and this correlated with an increase in production of IL-10 and reduced levels of IFN-γ and IL-17 in colonic T cells (Mazmanian et al., 2008). PSA has therefore been proposed to be a commensal product that modulates the balance between inflammatory T cells and regulatory functions, e.g., IL-10, although it is not yet known whether discrete T cell subsets produce this regulatory cytokine. Indeed, it is now clear that all effector $CD4^+$ T cell subsets, including $Foxp3^+$ Treg cells, can produce IL-10 under appropriate conditions (Li and Flavell, 2008; Saraiva et al., 2009).

Segmented filamentous bacterium (SFB) is another gut commensal that can have a profound influence on the balance of T cells in the intestine and, potentially, systemically as well. Colonization with SFB, which adhere tightly to intestinal epithelial cells in the terminal ileum, was found to correlate with the presence of Th17 cells in the intestinal LP. Whereas LP Th17 cells were absent in germ-free mice, they were abundant upon colonization with SFB, and such mice were more resistant to growth of *C. rodentium* (Ivanov et al., 2009). Colonization of mice with SFB from a different source was reported to induce accumulation of more diverse LP $CD4^+$ T cells, including Th1, Th17, and Treg cells (Gaboriau-Routhiau et al., 2009). The difference in the influence of SFB between the two studies may be due to

differences in the bacterial strains employed or to genetic differences in the host. Mice engineered to express a human α-defensin in intestinal epithelial Paneth cells displayed reduced numbers of SFB and LP Th17 cells, but there was no effect on Th1 cells, consistent with a specific influence of SFB on Th17 cell differentiation or accumulation in the LP (Salzman et al., 2009) Together, these studies suggest that SFB contribute to the balance of effector T cells (and potentially Treg cells) in the LP and, hence, to the ability of the host immune system to maintain an effective barrier against potentially pathogenic microbes. Although our understanding of how this barrier is regulated is still rudimentary, there has been substantial recent progress. Antimicrobial peptides (AMPs) produced by intestinal epithelial cells in response to TLR signals have been shown to have critical roles in protecting the host from damage mediated by pathogenic bacteria such as vancomycin-resistant enterococcus and *Listeria monocytogenes* (Brandl et al., 2007, 2008). AMPs like the lectin RegIIIγ are induced in Paneth cells upon colonization of germ-free mice with commensal bacteria (Cash et al., 2006) and hence serve to protect the mucosal barrier from pathogens and resident commensals alike, reducing penetration into the intestinal tissues and draining lymph nodes. Although AMP expression can be induced directly by TLR signaling in epithelial cells (Vaishnava et al., 2008), there is evidence that it is also regulated indirectly, by IL-22 produced by Th17 cells and other lymphoid cells in the LP. Mice deficient for IL-22 or its upstream regulator, IL-23, were highly susceptible to infection with *C. rodentium*, and this correlated with the loss of epithelial-derived AMPs (Zheng et al., 2008). Colonization with SFB correlated with elevated levels of RegIIIγ and other AMPs and with high-level production of the acute phase serum amyloid A (SAA) proteins (Ivanov et al., 2009). However, there was no apparent difference with or without SFB in IL-17 and IL-22 production by cells other than Th17 cells, which may explain why SFB-deficient mice survive infection with *C. rodentium*. This is consistent with the finding that protection from *C. rodentium*-induced lethality was observed even in the absence of adaptive immunity (Zheng et al., 2008). Whether there are specific commensal microbes that influence production of cytokines by lymphoid cells other than Th17 cells is an important issue that needs to be addressed.

The signaling pathways involved in commensal microbe-induced accumulation of LP Th17 cells have not yet been elucidated. Lumenal ATP can induce accumulation of Th17 cells in germ-free mice, and it has been suggested that bacteria-derived ATP activates a subset of intestinal dendritic cells (DCs) that, in turn, produce Th17-inducing cytokines (Atarashi et al., 2008). However, SFB induction of Th17 cells was independent of ATP, and no single innate signaling pathway has yet been shown to be required for accumulation of gut Th17 cells. DNA from commensal bacteria has also been proposed to regulate the Th17/Treg balance, presumably through activation of TLR9 (Hall et al., 2008). Paradoxically, although TLR9-deficient mice had reduced proportions of both Th1 and Th17 cells and increased Treg cells in the small intestine, there was no effect in the absence of Myd88, which is required for signals downstream of most TLRs. Thus, individual TLRs may convey divergent signals that influence the balance of T cells within the LP.

The role of commensal microbes in regulating the balance of pro- and anti-inflammatory cells is an important topic that has only recently become amenable to investigation as a consequence of advances in genomic sequencing and taxonomic approaches for classifying bacterial species. The recent discoveries outlined above suggest that growth of pathogenic and commensal bacteria is regulated by members of the commensal community both directly, through mechanisms like quorum sensing, and indirectly, through signals in the host mucosal microenvironment. For example, potentially pathogenic *Clostridium difficile* spores germinate and undergo vegetative growth following treatment of humans and experimental animals with antibiotics, presumably due to the loss of bacterial inhibitory factors (Rupnik et al., 2009). It is likely that a balance between Th17 and other effector T cell types and Treg cells will impact the outcome, i.e., colitis versus containment of bacterial growth.

The commensal microbiota have also been implicated in having a key role in multiple tissue-specific autoimmune and inflammatory diseases, although the evidence in human disease is largely anecdotal. Treatment with antibiotics has been reported to ameliorate symptoms in a variety of autoimmune diseases, from rheumatoid arthritis to Crohn's disease and multiple sclerosis (Guarner, 2007; Toivanen, 2003). Asthma is a notable example in which there is a clear association of protection from the disease in individuals raised in rural environments, where there may be increased exposure to microbes from farm animals (von Mutius, 2009). Treatment of experimental animals with antibiotics or transfer into a germ-free environment can result in either exacerbation or reduction in autoimmune disease manifestation. In this regard, mice reared in a colony with high levels of SFB may be predisposed to Th17-mediated autoimmune diseases but may also be protected from other inflammatory diseases through the beneficial activity of IL-22. A more detailed understanding of how individual bacteria and their products induce signaling pathways that influence host immune system composition and responses may result in new therapeutic approaches for strengthening mucosal immunity and for attenuating inflammatory conditions.

In addition to commensal microorganisms, other environmental factors can contribute to the differentiation of lymphoid cells that mediate either proinflammatory or anti-inflammatory functions. For example, xenobiotic agents, by inducing the activity of Ahr, promote the differentiation of lymphoid cells that produce inflammatory cytokines, particularly IL-22 (Veldhoen et al., 2008). Ahr ligands have also been reported to induce expression of Foxp3 in T cells and to also increase transplantation tolerance by both direct and indirect influence on Treg cells (Hauben et al., 2008; Quintana et al., 2008). However, in the absence of Ahr, there were reduced levels of IL-17 and IL-22 in intestinal Th17 and LTi cells, and mice were highly susceptible to *C. rodentium*-mediated lethality (L. Zhou and D.R.L., unpublished data). An attractive possibility is that microbial products or metabolites generated in response to microbes act as ligands to modulate Ahr activity and, hence, immune homeostasis.

Regulatory T Cells

Multiple immune cell subsets, including tolerogenic DCs, myeloid suppressor cells, NK-like T cells, IL-10-producing $CD4^+$

T cells, and B cells, and their products have been implicated in inhibition of immune response-associated inflammation. It appears that many of these cell types cause immunomodulation, i.e., oppose the predominant pathogenic inflammatory response, leading to mutual restraint. Over the last decade and a half, Treg cells expressing the transcription factor Foxp3 have emerged as dedicated suppressors of diverse immune responses and inflammation and vitally important keepers of immune homeostasis. Mechanisms of peripheral tolerance achieved through the activity of Treg cells complement deletional mechanisms of tolerance and anergy induction operating both in the thymus and in the periphery.

The existence of a subset of T cells capable of limiting autoimmunity and associated inflammation was unveiled by neonatal thymectomy studies in mice, where chronic inflammation was unexpectedly observed in various tissues. Subsequent work revealed that autoimmunity accounted for the wide-spectrum inflammatory lesions that included oophoritis, prostatitis, diabetes, thyroiditis, gastritis, myositis, and hepatitis. The autoimmunity commencing in these mice was T cell dependent and was averted by provision of T cells from euthymic mice (reviewed in Sakaguchi and Sakaguchi, 2005). Furthermore, adoptive transfers of naive $CD4^+$ T cells into lymphopenic rodents implicated this subset of T cells as culprits of immune mediated lesions, most prominently colitis (Mason and Powrie, 1998). Finally, a genetic study showed that, in mice expressing a transgenic TCR specific for myelin basic protein, a few T cells expressing endogenously rearranged TCR prevented spontaneous EAE (Lafaille et al., 1994). This groundbreaking series of experiments demonstrated that elimination or inactivation of differentiating self-reactive thymocytes is incomplete and that the thymus is a source of both pathogenic T cells that cause autoimmunity and protective T cells. A subset of $CD4^+$ T cells expressing large amounts of high-affinity IL-2R α chain (also known as CD25) was shown to provide potent suppression of immune inflammation in neonatally thymectomized mice and in lymphopenia-associated experimental models.

Identification of CD25 as a marker of Treg cells enabled their isolation and facilitated characterization of their functional properties in vitro and in vivo using adoptive cell transfers. Unlike effector T cells, Treg cells failed to proliferate on their own in response to TCR engagement but instead were able to suppress proliferation of effector T cells in a contact-dependent manner. Importantly, TCR-stimulated Treg cells also failed to produce IL-2, but their survival and expansion was dependent upon provision of IL-2 by activated effector T cells (Fontenot et al., 2005a; Setoguchi et al., 2005). These observations explained another old finding, that mice with targeted disruption of the *Il2* gene exhibited a fatal lymphoproliferative autoimmune syndrome. Mice lacking IL-2Rα or IL-2Rβ chains also developed a similar syndrome that was rescued by transferring Treg cells or IL-2R-sufficient bone marrow (Malek, 2008). These findings established an essential role for IL-2 in homeostasis of Treg cells. They also suggested that activated effector T cells and Treg cells are present at a certain equilibrium maintained by a simple negative regulatory loop—under physiologic conditions activation of effector T cells associated with heightened IL-2 production is expected to increase the numbers of suppressive Treg cells, which in turn limit effector T cell response.

Since CD25 is upregulated upon activation of all effector T cells, it was initially thought that, instead of being a distinct T cell lineage, $CD25^+$ Treg cells represent a metastable state of T cell activation and that Treg cells function exclusively by competing for IL-2 with recently activated effector T cells. However, the discovery of Foxp3 and its role in Treg cells has dispelled this view.

Role of Foxp3 in Treg Cells

Positional cloning of the X chromosome gene encoding the transcription factor Foxp3 in studies aimed at elucidation of the genetic basis of the rare human autoimmune disorder IPEX (Immune-dysregulation Polyendocrinopathy Enteropathy X-linked) (Brunkow et al., 2001; Chatila et al., 2000; Fontenot et al., 2003; Wildin et al., 2001) led to the discovery that Foxp3 is required for Treg cell differentiation and that its high expression is a characteristic feature of Treg cells (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). Loss-of-function mutations in the *Foxp3* gene result in fatal autoimmune lesions affecting multiple tissues, reminiscent of the less severe inflammation observed in neonatally thymectomized mice. IPEX patients and *Foxp3* mutant mice present with a variety of immune and inflammatory lesions with a massive cytokine storm including both Th1 and Th2 cytokines. In both mice and humans, only mutant males are affected, whereas heterozygous female carriers are spared because the latter harbor a normal population of Treg cells expressing the wild-type *Foxp3* allele due to random X chromosome inactivation.

The possibility that the absence of Treg cells leads to a systemic breakdown of immunological tolerance and uncontrolled inflammatory responses in *Foxp3* mutant individuals has been experimentally confirmed in a series of genetic studies. First, analyses of the Foxp3 reporter mice have revealed that Foxp3 expression is surprisingly restricted to a subset of T cells with suppressor function (Fontenot et al., 2005b; Wan and Flavell, 2005). High amounts of Foxp3 protein have not been detected in other hematopoietic and nonhematopoietic lineage cells (Fontenot et al., 2005b; Kim et al., 2009; Liston et al., 2007). Evidence that the paucity of Treg cells accounts for fatal autoimmune and inflammatory lesions associated with the *Foxp3* deficiency was provided by studies in which the adoptive transfer of Treg cells rescued disease in neonatal Foxp3-deficient mice (Fontenot et al., 2003). Furthermore, mice with T cell-specific and germline ablation of *Foxp3* were indistinguishable in the progression and severity of the autoimmune lesions (Fontenot et al., 2005b).

The question of whether anti-inflammatory Treg suppressor function is critical during the development of the immune system or after the immune system is fully developed was addressed through the analysis of $Foxp3^{DTR}$ knockin and Foxp3-DTR BAC transgenic mice harboring an ''ablatable'' Treg population expressing human diphtheria toxin receptor (Kim et al., 2007; Lahl et al., 2007). Chronic ablation of Treg cells in adult $Foxp3^{DTR}$ mice showed that Treg-mediated suppression is indispensable for preventing fatal lympho- and myelo-proliferative

inflammatory syndrome throughout the life spans of normal mice (Kim et al., 2007).

Thymic and Peripheral Origin of Treg Cells

Neonatal thymectomy studies indirectly implicated the thymus as a site of generation of Treg cells. Indeed, Foxp3$^+$ cells with suppressive function are found in the thymus. However, peripheral T cells can also acquire Foxp3 expression upon suboptimal activation in a TGF-β-dependent manner (Chen et al., 2003). Considering that intestinal commensal microbiota and food represent the largest source of microbial inflammatory stimuli, gut-associated lymphoid tissues are thought to be a main site for generation of peripheral Treg (iTreg) cells. The generation of iTreg cells is likely promoted by the environment rich in TGF-β and retinoic acid, a combination that efficiently induces Foxp3 expression in antigen-stimulated T cells at the expense of effector T cells, Th17 cells in particular (Mucida et al., 2007; Sun et al., 2007; Coombes et al., 2007). Gut-resident DCs, distinguished by the expression of CD103, produce retinoic acid and facilitate iTreg generation (reviewed in Coombes and Powrie, 2008). A delicate balance between inflammatory Th17 cells and suppressive Treg cells in the gut microenvironment is illustrated by a number of mouse studies demonstrating that a change in composition of the commensal flora leading to an increase in Th17 cells in the gut is frequently associated with a reciprocal decrease in Treg cells and vice versa (Ivanov et al., 2008).These observations are in agreement with the in vitro studies, which implicate a combination of high amounts of IL-6 and intermediate amounts of TGF-β in Th17 induction, whereas high amounts of TGF-β alone facilitate iTreg differentiation (Veldhoen et al., 2006; Zhou et al., 2008). Furthermore, many Foxp3$^+$ T cells in the gut express high amounts of RORγ and produce IL-17 (Zhou et al., 2008). Finally, genetic tagging of cells using Cre recombinase expressed under the control of *Foxp3* regulatory elements and a recombination reporter allele R26Y (R26Y: loxP-flanked STOP cassette followed by a YFP-coding sequence inserted into the ubiquitously expressed *Rosa26* locus) showed that at least a quarter of IL-17-producing cells expressed Foxp3 at some point in their history (Zhou et al., 2008). These results speak strongly of a close relationship between iTreg and Th17 cells and suggest that they might represent alternative cell fates.

Differentiation of Treg Cells

Thymic induction of Foxp3 requires TCR signaling of increased strength (reviewed in Hsieh and Rudensky, 2005). Besides TCR, engagement of common γ-chain (γc) cytokine receptors, foremost IL-2R, is required for both thymic and peripheral generation of Treg cells (Vang et al., 2008; Fontenot et al., 2005a). According to a two-step model of Treg differentiation, TCR engagement first leads to CD25 upregulation making precursor cells receptive to receiving IL-2 signals leading to Stat5 activation (Lio and Hsieh, 2008; Burchill et al., 2008). Finally, CD28 signaling induced upon binding of its ligands CD80 and CD86 has also a cell-intrinsic role in Foxp3 induction in the thymus (Salomon et al., 2000; Tai et al., 2005). Activation of these signaling pathways leads to recruitment of a number of transcription factors including NFAT, cRel, Creb, and Stat5 to the promoter and several conserved noncoding regulatory elements of the *Foxp3* gene and its expression (Kim and Leonard, 2007; Long et al., 2009; Zheng et al., 2010). Unexpectedly, activation of the PI3K-Akt pathway opposes Foxp3 induction in the thymus (Haxhinasto et al., 2008; Josefowicz et al., 2009; Sauer et al., 2008). Since Akt-mediated phosphorylation is known to inactivate Foxo, it is possible that Foxo family members also contribute to Foxp3 induction or survival of Foxp3-expressing thymocytes.

As mentioned above a distinguishing feature of iTreg generation is its dependence upon TGF-βR signaling. The latter might facilitate Foxp3 expression in more than one way. First, TGF-β restrains recruitment of Dnmt1, the key maintenance DNA methyltransferase whose activity likely leads to silencing of the newly induced *Foxp3* gene (Josefowicz et al., 2009). Second, TGF-βR signaling leads to Smad recruitment to a conserved noncoding element within the *Foxp3* locus (Tone et al., 2008). The ablation of this element in mice impairs iTreg induction, whereas thymic Treg differentiation is unaffected (Zheng et al., 2010). Unexpectedly, Th17 cell numbers do not change in the gut-draining mesenteric lymph nodes or gut-associated lymphoid tissues in these mice, which remain free of unprovoked inflammation in the intestines or elsewhere. Increased numbers of Foxp3$^-$CD4$^+$ T cells producing the immunomodulatory cytokine IL-10, known as Tr1 cells, might compensate for a paucity of iTreg cells and maintain immune homeostasis in the gut (Zheng et al., 2010). Thus, suppressive Tr1 and iTreg cells might also represent alternative fates of T cell differentiation in the gut (Figure 2). Single-cell analysis of the precursor-product relationship between iTreg, Th17, and Tr1 cells is needed to inform the mechanisms regulating the delicate balance between inflammatory and suppressive T cell lineages in the gut tissues.

Foxp3-Dependent Transcriptional Program

Once expressed, Foxp3 orchestrates distinct gene expression and functional programs by amplifying and making permanent the features of precursor cells that are beneficial to Treg function and homeostasis, while obviating those that are detrimental (Gavin et al., 2007). The "opportunistic" character of Foxp3-dependent Treg cell differentiation has been revealed by comparison of cells isolated from healthy heterozygous female mice that express *Foxp3* reporter null and functional alleles (Gavin et al., 2007; Lin et al., 2007). The former cells are exposed to signals inducing Foxp3 yet lack Foxp3 protein. Although the expression pattern of a sizable gene cluster in these "Foxp3-less" "wannabes" and some of their functional properties resemble those of Foxp3$^+$ Treg cells, they fail to suppress. In addition, Foxp3 confers proliferative potential and metabolic fitness to Treg precursors (Gavin et al., 2007). Finally, Foxp3 enforces repression of immune response effector cytokines. For example, cells expressing a *Foxp3* reporter null allele produce high amounts of IL-17, whereas Foxp3$^+$ Treg cells do not (Gavin et al., 2007). In agreement with these results, expression of a hypomorphic *Foxp3* allele results in a marked impairment of suppressor function of Treg cells and in their production of IL-4 (Wan and Flavell, 2005). Genome-wide analyses of Foxp3-binding genes have suggested that Foxp3 acts as a transcriptional repressor and activator and its binding is accompanied

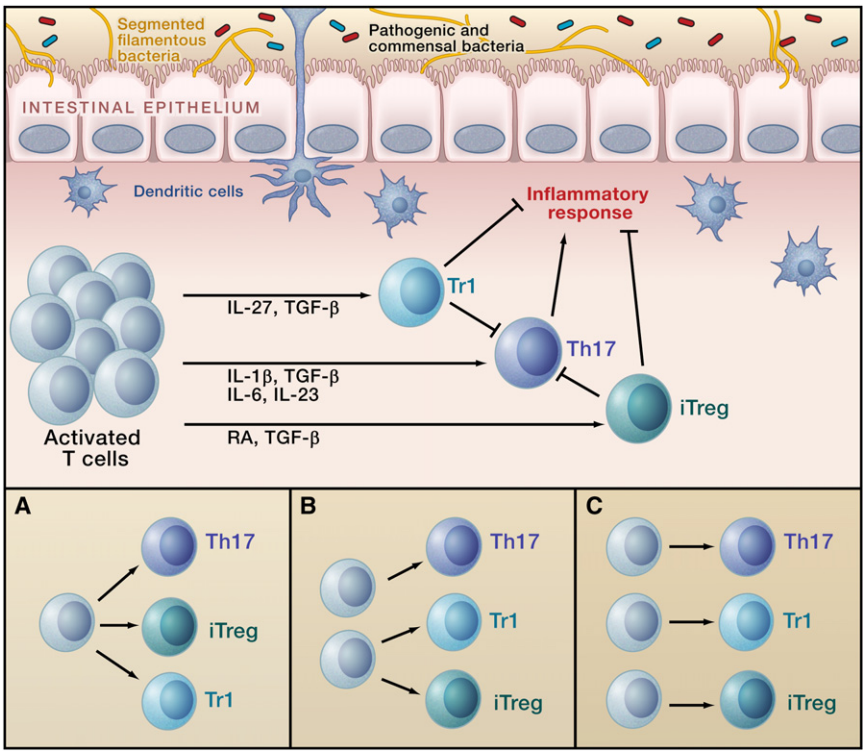

Figure 2. Possible Relationship between Precursors Differentiating into Proinflammatory and Protective T Cells in the Gut Microenvironment from Activated Precursor T Cell Populations

Signals initiated by interaction of microbiota and, potentially, pathogenic bacteria with epithelium and dendritic cells result in production of diverse cytokines that influence T cell differentiation. The relative amounts of IL-6, TGF-β, retinoic acid, and additional cytokines skew differentiation of highly inflammatory Th17 or anti-inflammatory Foxp3$^+$ iTreg cells or IL-10-producing Foxp3$^-$ Tr1 cells in several possible ways: (A) Single precursor can commit to any one of the three cell fates under the influence of a particular cytokine combination. (B) Suppressive iTreg and Tr1 cells represent alternative cell fates, whereas proinflammatory Th17 cells originate from a distinct pool of cells. (C) The three T cell fates originate from separate activated precursor T cells. Additional studies are needed to clarify the relationship between the three cell lineages.

by corresponding inhibitory or permissive histone modifications in the target genes (Marson et al., 2007; Zheng et al., 2007).

Stability of Foxp3 Expression and Treg Lineage

Despite epigenetic marking of target genes repressed or induced by Foxp3, its ablation in mature, fully differentiated Treg cells results in eventual loss of suppression and reversal of the Foxp3-dependent transcriptional signature to that of activated T cells or Treg "wannabes" (Williams and Rudensky, 2007). Thus, continuous expression of Foxp3 is required for the maintenance of the developmentally established Treg functional program. This feature is likely not unique to Treg cells as ablation of Pax5, a key transcription factor in the B cell lineage, results in a loss of B cell identity and dedifferentiation of B cells into T cells (Cobaleda et al., 2007).

Importantly, "former" Treg cells devoid of Foxp3 differentiate into effector T cells and cause pronounced tissue inflammation upon transfer into lymphopenic recipients (Williams and Rudensky, 2007). The observed pathology is likely due to activation of "former" Treg cells driven by self-reactive TCRs. These results raise a question as to the stability of Foxp3 expression in Treg cells and, therefore, of the Treg lineage. The possibility that Treg cells represent an intrinsically unstable lineage and may serve as a "fifth column" aiding, rather than opposing, inflammation has also a significant practical interest as Treg cell therapies and drugs enhancing production of Treg cells are being considered for treatment of autoimmune diseases and for preventing transplant rejection.

Under what circumstances might Treg cells lose their identity? One obvious possibility is that the inflammatory environment might favor "conversion" of Treg cells into effector T cells. Specifically, in the presence of high amounts of IL-6, the Foxp3$^+$ Treg cell population might become Th17 cells (Xu et al., 2007; Yang et al., 2008a). A striking example of acquisition of characteristic Th1 effector cell features by Treg cells has been recently reported in a study of lethal Toxoplasma infection in mice (Oldenhove et al., 2009). Furthermore, adoptive transfer of Foxp3$^+$ Treg cells into lymphopenic hosts also leads to a loss of Foxp3 expression and their differentiation into so-called follicular T helper cells (T_{FH}) in Peyer's patches (Tsuji et al., 2009). T_{FH} cells have recently emerged as principal assistants of B cell responses in the germinal centers. These observations were in line with the results of recent experiments utilizing a R26Y recombination reporter allele combined with a *Foxp3* BAC transgene-expressing Cre recombinase. This study suggested that a considerable proportion of YFP-tagged cells might have lost Foxp3 expression and are able to produce proinflammatory cytokines, most prominently IFN-γ, and become pathogenic under certain circumstances (Zhou et al., 2009).

On the other hand, adoptive transfer of CD25$^+$CD4$^+$ Treg cells into recipients with a full lymphoid compartment did not lead to a loss of Foxp3 expression (Floess et al., 2007; Komatsu et al., 2009). In agreement with these results, another study has shown that the majority of Treg cells expressing high amounts of CD25, a direct transcriptional target of Foxp3, are stable and do not lose Foxp3 upon adoptive transfer into lymphopenic hosts, whereas a relatively minor fraction of CD25$^-$ or CD25^{lo}Foxp3$^+$ T cells can lose Foxp3 expression and divert into effector T cell lineages (Komatsu et al., 2009).

Thus, the issue of Treg lineage stability versus plasticity remains controversial and awaits further experimentation. In this regard, we would like to posit that the instability of Foxp3 expression in "transitional" cells, some of which are on

their way to becoming Treg cells or to losing Foxp3 expression, is hardly surprising. However, if fully differentiated Treg cells exhibit intrinsically unstable Foxp3 expression, does this represent a failure of mechanisms enforcing Foxp3 stability or is there a purposeful process for destabilizing Foxp3 expression?

Integration of Environmental Cues by Treg Cells

The diversity of inflammatory lesions and increased production of Th1, Th2, and Th17 cytokines in Treg-deficient mice suggest that under physiologic conditions Treg cells control different types of immune responses and associated inflammation. These observations also raise the question as to whether Treg cells employ a single mechanism of suppression or their function is tailored to suppress a particular type of inflammation.

Indirect support for the latter possibility comes from reports that acquisition of tissue-specific homing capability, including cytokine-driven upregulation of appropriate chemokine receptors, is important for the ability of Treg cells to suppress inflammation in a given tissue (Siegmund et al., 2005; Siewert et al., 2007). If Treg cells acquire the capacity to suppress a particular type of inflammation in response to environmental cues that elicit it (e.g., to upregulate a chemokine receptor shared between effector and Treg cells), one may expect that some of the transcription factors driving differentiation of inflammatory cells would also be required for their restraint by Treg cells. In agreement with this concept, IRF4, a transcription factor essential for Th2 and Th17 cell differentiation, is expressed in Treg cells in a Foxp3-dependent manner and is required for Treg-mediated suppression of fatal Th2-mediated inflammation and hyper-IgE syndrome (Zheng et al., 2009). Likewise, Treg cells devoid of Stat3, whose activation is required for Th17 cell differentiation, fail to restrain fatal colitis instigated by a dysregulated Th17 response. However, Stat3-deficient Treg cells exercise full control over Th1 and Th2 responses, IL-2 production, and lymphoproliferation (Chaudhry et al., 2009) (Figure 2). Finally, both effector T cells and Treg cells upregulate the signature Th1 transcription factor, T-bet, in response to IFN-γR/Stat1 activation. In Treg cells, T-bet induction is required for efficient homing to the Th1 response site and for Treg survival and expansion and, thus, for efficient suppression of Th1 responses (Koch et al., 2009). These data imply functional heterogeneity within peripheral Treg populations in terms of Treg cell states or subsets able to suppress different types of inflammation (Figure 3). How do these Treg cell subsets mediate suppression of a given type of inflammation and how stable are they? Although the answer to the second question is lacking, a few clues in response to the first one have started to emerge. At least some effector T cell-specific transcription factors modulate Foxp3-dependent transcriptional programs in part by being recruited into Foxp3 complexes (Zheng et al., 2009; Chaudhry et al., 2009). As a result, the "tuned" Treg cells upregulate distinct sets of chemokine and cytokine receptors in a pattern matching that of the immune effector cells. While chemokine receptors such as CCR6 or CXCR3 likely facilitate spatial proximity of suppressive Treg and inflammatory effector cells, cytokine receptors (e.g., ST2, IL-1R, IL-6R) may act as a "sink" competing for important factors and, thereby, limiting activation or differentiation of effector cells (Koch et al., 2009; Chaudhry et al., 2009; Zheng et al., 2009). As discussed above, activation of effector T cell-specific transcription factors in Treg cells may also confer the ability to survive and expand and to elaborate a mechanism(s) of suppression corresponding to a particular type of inflammation.

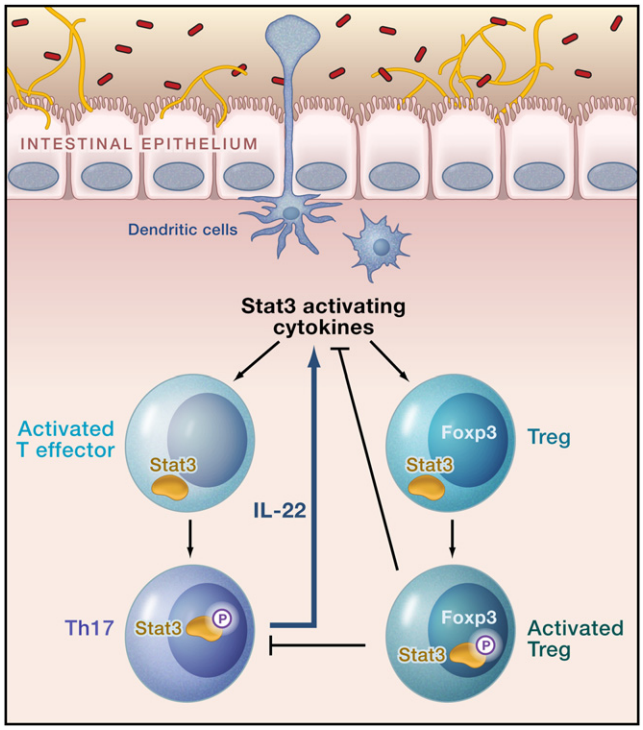

Figure 3. Integration of Environmental Cues by Proinflammatory Effector T Cells and Anti-inflammatory Treg Cells in the Gut

Cytokine microenvironment impacted by the gut microbiota promotes Stat3 phosphorylation, which in turn induces differentiation of activated T cells into Th17 effector cells. At the same time, Stat3 phosphorylation endows Foxp3$^+$ Treg cells with the ability to suppress Th17 responses and associated inflammation.

The observed "symmetry" in requirements for certain transcription factors for eliciting a particular inflammatory response and Treg-mediated suppression of this response may represent a general principle of coordinated regulation of gene expression in different cell types in response to extracellular stimuli. We suggest that formation of transcriptional complexes between cell type-specific, function-defining transcription factors (e.g., Foxp3) and activation-dependent transcription factors acting as environmental sensors (e.g., Stat3), or their cooperative binding to DNA, form the basis for such regulation. This concept is further propped by the observation that Treg cells found in the fat pads exhibit a distinct gene expression signature that includes a subset of genes characteristic of adipocytes and are able to keep sterile inflammation and associated glucose intolerance in check. The distinct features of "fat" Treg cells are likely elicited by the unique environmental factors, which include adipokines and other products of adipocytes (Feuerer et al., 2009a). We suggest that similar "tuning" of Treg and other immune cell types by the environment is not limited to fat pads but extends to other types of tissues.

Uncovering modules of transcriptional regulation in Treg cells and their associated functions in different inflammatory and tissue settings will facilitate understanding of mechanisms of suppression and likely inform novel means of selective therapeutic intervention for inflammatory disorders of different origins.

Suppression Mechanisms and Their Cellular Targets

Despite intensive investigation, mechanisms of Treg-mediated suppression remain relatively obscure. Until recently, it was unclear whether a single or multiple mechanisms are employed by Treg cells to curb inflammatory responses and how redundant such mechanisms might be. Another important question has been the identity of cell types serving as targets of Treg-mediated suppression. Ablation of Treg cells in adult healthy mice results in proliferation and activation of multiple immune cell types including NK cells, T and B cells, DCs, granulocytes, macrophages, and monocytes (Kim et al., 2007). Furthermore, activation of T cells, DCs, and NK cells occurs very early after Treg ablation suggesting that Treg cells might directly suppress these cells (Feuerer et al., 2009b; Kim et al., 2007). Although simultaneous elimination of Foxp3$^+$ Treg and effector CD4$^+$ T cells prevents both lympho- and myelo-proliferative syndromes, DC activation is still observed. This finding implies that activation of self-reactive CD4$^+$ T cells, normally restrained by Treg cells, fuels expansion of activated myeloid and lymphoid cells and the resulting inflammation (Kim et al., 2007; Kim and A.Y.R., unpublished). In vitro Treg cells efficiently suppress activation of T and B cells, NK cells, and DCs (Zhao et al., 2006; Shevach, 2009). However, intravital imaging studies have thus far failed to reveal selective (i.e., nonrandom) Treg and effector T cell interactions, whereas stable Treg-DC contacts were readily observed (Tadokoro et al., 2006; Tang et al., 2006). Together, existing data suggest that DCs and potentially NK cells serve as direct targets of Treg cells in vivo. It seems likely that a number of other cell types of hematopoietic and nonhematopoietic origin can also be kept in check by Treg cells. Consistent with this idea are reported inhibitory effects of Treg cells on inflammatory and metabolic properties of adipocytes, suggesting that they may limit metabolic causes and consequences of inflammation and associated pathologies like type II diabetes (Feuerer et al., 2009a).

Considering that multiple cell types serve as targets of Treg-mediated suppression and distinct transcription factors partake in Treg mediated suppression of different types of inflammation, it seems likely that Treg cells utilize multiple means of restraining inflammation. In fact, Treg-restricted inactivation of the immunosuppressive cytokine IL-10 resulted in increased inflammation at major environmental interfaces, with late-onset colitis, but not in the systemic autoimmunity characteristic of mice lacking Treg cells (Rubtsov et al., 2008). In addition to IL-10, TGF-β and the newly described cytokine IL-35 have been implicated in restraining inflammation in the colon (Collison et al., 2007).

In addition to cytokine-mediated modulation of inflammatory responses, Treg cells display high amounts of CTLA4 and TIGIT, two Ig family members that are able to inhibit the immunostimulatory potential of DCs by reducing expression of the costimulatory molecules CD80 and CD86 and by inducing IL-10 production in DCs, respectively (Wing et al., 2008; Wing and Sakaguchi, 2009; Yu et al., 2009). Furthermore, a yet unknown mechanism enables Treg-mediated suppression of extracellular thiol release by DCs that supports effector T cell proliferation (Yan et al., 2009).

Small molecules elaborated by Treg cells have also been implicated in restraining inflammation. Specifically, Foxp3-dependent expression of the ectonucleotidases CD39 and CD73 endows Treg cells with the ability to sequentially convert highly inflammatory extracellular ATP, which accumulates under hypoxic conditions, into adenosine via an AMP intermediate (Deaglio et al., 2007). Adenosine binding to A2A and A2B receptors on lymphocytes and myeloid cells leads to their inactivation, at least in part due to an increase in intracellular cAMP (Sitkovsky, 2009; Sitkovsky et al., 2004). It has been also suggested that Treg cells directly extrude cAMP, which is transported through the gap junctions into the effector T cells (Bopp et al., 2007). However, formation of gap junction requires stable cell-cell contacts between Treg and effector T cells, which have not been demonstrated so far.

Besides immunomodulation or inactivation, perforin- and granzyme-mediated cytolysis of immune effector and accessory cells by Treg cells has been considered as a means of suppression (Cao et al., 2007; Gondek et al., 2005; Grossman et al., 2004). In addition to direct killing, an indirect way of elimination of effector T cells is through induction of apoptosis due to a growth factor withdrawal. In this regard, Treg cells are able to deprive effector T cells of IL-2 due to a much higher level of CD25 expression (Pandiyan et al., 2007).

Concluding Remarks

Although we briefly overviewed many means of suppression employed by Treg cells, the list of putative suppressor mechanisms will undoubtedly continue to grow. We would like to emphasize that no single mechanism of suppression can account for expansive Treg functions in regulation of autoimmune and autoinflammatory states, allergic and metabolic inflammation, tissue injury, immunity to infection, and tumorigenesis. The diversity of effector mechanisms affords versatility to a Treg-mediated suppression program capable of restraining diverse types of inflammatory responses in different tissues. Likewise, functions of Th17 cells, a prototypic inflammatory T cell type, are most likely not limited to production of IL-17 or IL-22. Both Treg and Th17 cells can exert both beneficial and pathogenic effects depending on a particular biological setting. The intricate mechanisms governing differentiation and functions of these pro- and anti-inflammatory cells speak to major challenges ahead—the need to dissect the fine balance between innate and adaptive pro- and anti-inflammatory cells in the context of their complex relationships with infectious agents and commensal microbiota.

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