Heterogeneity and Stability in Foxp3⁺ Regulatory T Cells

Booki Min

Foxp3⁺ regulatory T cells (Tregs) play an indispensable role in controlling tolerance and immunity against self- and foreign antigens. The failure of Tregs to properly function is the direct cause of systemic and chronic inflammation as well as immune suppression. It is now evident that Tregs are highly heterogeneous populations depending on the surface phenotypes, cytokine profiles, and anatomical locations. Yet, our understanding of the cellular and molecular pathways underlying such heterogeneity is very limited. Furthermore, some Tregs lose the phenotype (and suppressive functions) and instead acquire pathogenicity. Since utilizing Tregs as a tool for immunotherapy is being implemented in many clinical settings, it is of utmost importance to understand the precise mechanisms by which the loss of Treg phenotype (and function) is prevented. In this review, both cellular and molecular factors involved in Treg heterogeneity and stability are discussed.

Keywords: regulatory T cell, Foxp3, heterogeneity, stability

Introduction

FOXP3⁺ REGULATORY T cells (Tregs) are the central regulators of tolerance and immunity (Sakaguchi and others 2008). Mutations in the Foxp3 gene or defects in Foxp3 expression result in uncontrolled immune activation, resulting in systemic autoimmune inflammation and premature death (as shown in the scurfy mouse and immunodysregulation polyendocrinopathy enteropathy X-linked syndrome [IPEX] patients). Depending on the sites of generation, Tregs are largely divided into 2 types of cells: tTregs and pTregs (Shevach and Thornton 2014). tTregs generated in the thymus are thought to emerge through recognition of high-affinity self-antigens, which otherwise would be eliminated through negative selection process (Hsieh and others 2012). While tTregs are the dominant subset in the periphery, a sizable (yet unknown percentage) population of pTregs is generated from conventional CD4 T cells that are activated by antigens at the peripheral sites (Shevach and Thornton 2014). The uncertainty to estimate the size of pTregs is due to the lack of reliable surface markers that distinguish tTregs from pTregs. Surface neuropilin 1 (Nrp1) expression was reported to mark tTregs, but not pTregs, and is being used as the tTreg marker (Weiss and others 2012; Yadav and others 2012). However, Nrp1 expression is regulated by transforming growth factor beta (TGFβ) and, most importantly, it is also found at high levels on pTregs and *in vitro* generated iTregs (Shevach and Thornton 2014). With regard to their relative contribution to the Treg function, tTregs and pTregs may play overlapping roles in regulating immune responses, although distinct contribution of pTregs (and of

iTregs) has also been demonstrated (Haribhai and others 2011; Nguyen and others 2014).

Over the last several years, it has become clear that the population of Tregs in the periphery is highly heterogeneous not only by the site of generation but also by their properties to mediate regulatory functions (Sawant and Vignali 2014; Yuan and others 2014). Furthermore, some Tregs lose Foxp3 expression and become nonregulatory cells that often acquire inflammatory functions instead. Mechanisms underlying such heterogeneity and plasticity of Tregs especially in the context of inflammation remain unclear and these are the subject of extensive ongoing investigations. In this review, Treg heterogeneity in the context of location, surface phenotypes, and molecular markers will briefly be reviewed. In addition, factors contributing to Treg instability and the roles of Tregs that have lost “Treg-ness” in disease settings will be discussed.

Heterogeneity of Tregs

By the activation status and tissue trafficking molecule expression

In naïve mice raised in specific-pathogen free (SPF) conditions, roughly 10% of conventional CD4 T cells in the lymphoid tissues belong to effector/memory cells, expressing CD62^{low} CD44^{high} CCR7[−] phenotypes that enable them to readily traffic the nonlymphoid tissues. The proportion of effector/memory phenotype Tregs is substantially greater, 40%–50% (Min and others 2007). Similar to conventional memory T cells, they downregulate lymphoid homing

molecules, CD62L and CCR7, and upregulate tissue-infiltrating receptors such as ligands for E/P-selectin as well as adhesion molecules such as CD103 (α_E integrin) (Huehn and others 2004). In naïve animals, Tregs found in the skin or intestine, where its ligand, E-cadherin, is highly expressed, are almost CD103+ cells; however, ~30% of Tregs in the lymphoid tissues also express CD103 (Suffia and others 2005). CD103+ Tregs have been proposed as a specialized subset that preferentially homes to the inflamed tissues (Lehmann and others 2002; McHugh and others 2002; Stephens and others 2007). CD103 expression in Tregs also controls Treg retention at the sites of inflammation (Suffia and others 2005). They also display higher migratory capacity toward inflammatory chemokines such as CXCL9, CCL17, and CCL20 (Huehn and others 2004), further supporting their inflammatory site-seeking features. Higher levels of intracellular CTLA4 are noted in CD103+ subsets (Lehmann and others 2002), which may account for their potent suppressive functions (Huehn and others 2004). In the context of antitumor or antiparasite immunity, CD103-expressing Tregs accumulate in the parasite infection sites, conferring susceptibility to the infection (Suffia and others 2005), or tumor-infiltrating Tregs highly express CD103 in response to TGF β produced by tumor cells (Anz and others 2011). Compared with CD103- Tregs, CD103+ Tregs show significantly lower T cell receptor excision circle numbers, indicating that they have undergone extensive cell divisions (Huehn and others 2004). It remains unclear, though, what triggers the division and CD103 expression in Tregs. Besides integrin expression, Treg heterogeneity is also observed based on various chemokine receptor expression levels (Campbell and Koch 2011). For example, CCR5+ Tregs are specialized in trafficking into inflamed intestinal tissues (Kang and others 2007), while CXCR5-expressing Tregs are directed to regulate germinal center B cell responses (Lim and others 2004). As will be discussed below in more detail, the heterogeneity based on different trafficking molecules (chemokine receptors and/or adhesion molecules) is more closely associated with their anatomical location, which is critical not only during inflammatory responses but also as a homeostatic mechanism to position the specialized Treg subset in the right place (Fig. 1).

By localization

While effector/memory Tregs seek inflammatory tissue sites, certain Treg subsets are seeded early as tissue-resident Tregs. For example, it was reported that Tregs are highly enriched in adipose tissues [visceral adipose tissue (VAT)] of normal mice, representing 50%–80% of the total CD4 T cells found in the fat tissues (Feuerer and others 2009). Transcriptome analysis further uncovers that VAT Tregs are distinct from lymphoid tissue Tregs, in that they highly express certain chemokine receptors such as CCR1 and CCR2 and immunosuppressive cytokine interleukin (IL)-10 (Feuerer and others 2009). Another feature of VAT Tregs is that they express proteins involved in lipid metabolism (CD36 and Ldlr) and a transcription factor, peroxisome proliferator-activated receptor gamma (PPAR γ), which is important for adipocyte functions (Feuerer and others 2009). In fact, Treg-specific PPAR γ deletion greatly diminishes the proportion of VAT Tregs without affecting lymphoid Tregs (Cipolletta and others 2012). VAT Tregs seed the adipose tissues during the first weeks of life and may be maintained independently with minimal thymic contribution (Kolodin and others 2015). They are essential regulators of the inflammatory condition of adipose tissues. From the insulin-resistant model of obesity, the level of VAT Tregs is found to be specifically low (Feuerer and others 2009; Chen and others 2013a). Thiazolidinedione drug, pioglitazone, a synthetic agonist for PPAR γ , modulates the expression of genes involved in lipid metabolism, reducing insulin resistance (Cipolletta and others 2012). Importantly, pioglitazone treatment dramatically increases the proportion of VAT Tregs in obese mice; however, the PPAR γ deficiency in Tregs fails to respond to the treatment (Cipolletta and others 2012).

Another tissue site where Tregs are reported to play important regulatory roles is skeletal muscle. Utilizing a cardiotoxin-induced muscle injury model, Burzyn and others (2013) demonstrate that a specialized subset of Tregs is rapidly recruited into the injured muscle tissues. Similar to VAT Tregs that express a very restricted T cell receptor (TCR) repertoire distinct from lymphoid Tregs (Feuerer and others 2009), Tregs in the injured muscle include clonally expanding cells displaying drastic difference from that of

FIG. 1. Heterogeneity of lymphoid and tissue-resident Tregs. Treg, regulatory T cell.

splenic Tregs (Burzyn and others 2013). Treg depletion impairs muscle regeneration, suggesting that muscle Tregs are important regulator cells involved in muscle repair. Amphiregulin (Areg), a ligand for epidermal growth factor receptor (EGFR), is highly expressed in muscle Tregs (Zhou and others 2012; Burzyn and others 2013; Zaiss and others 2015). Areg has been proposed to play a critical role in regulating both inflammation and tissue repair process (Zaiss and others 2015). In fact, recombinant Areg administration is sufficient to ameliorate muscle injury even with the lack of muscle Tregs (Burzyn and others 2013). Moreover, Treg-specific Areg deletion substantially impairs tissue repair in the model of virus infection in the lung (Arpaia and others 2015).

A subset of Tregs is also found in the skin of both mice and humans (Gratz and others 2013; Sanchez Rodriguez and others 2014). They also display properties associated with effector/memory cells (Gratz and others 2013). Utilizing α -1,3-fucosyltransferase VII (Fut7)-deficient cells in which expression of skin-homing molecules, E- and P-selectin ligands, is severely reduced, Campbell and colleagues (2008) reconstitute scurfy mice with Fut7^{-/-} Tregs and find that Treg migration into the skin is selectively blocked, resulting in rapid development of severe cutaneous inflammation. Skin-resident Tregs also facilitate wound healing (Nosbaum and others 2016). In particular, Tregs accumulating in the wounded skin reduce interferon gamma (IFN γ) production and macrophage accumulation. Skin Tregs express the EGFR and possibly respond to Areg produced by Tregs or mast cells (Zaiss and others 2013; Nosbaum and others 2016). In fact, Areg deficiency causes the development of dermatitis (Zaiss and others 2013). In support of this, Treg-specific EGFR deletion results in delayed wound healing and elevated inflammatory macrophage accumulation in the sites (Nosbaum and others 2016). Skin Tregs are also shown to maintain immune tolerance against commensal microbial antigens in the skin because blocking Treg accumulation in the skin disrupts immune tolerance and skin inflammation develops (Scharschmidt and others 2015).

The intestinal mucosal surfaces are separated from commensal microbes by a single layer of intestinal epithelial cell layer, demanding tight regulation to prevent unintended immune activation, yet preserving proper immune responses against invading pathogens. Tregs are highly enriched in the small and large intestine lamina propria (Tanoue and others 2016). One unique feature of intestinal Tregs is the dominance of pTregs (Weiss and others 2012). Commensal antigens and microbial products play a key role in the generation of pTregs as their numbers are drastically reduced in germ-free or antibiotic-treated mice. *Clostridium* species are particularly specialized in promoting pTreg generation (Atarashi and others 2011). Although unclear, potential mechanisms by which *Clostridium* sp. can induce pTregs include induction of TGF β and indoleamine 2,3-dioxygenase (IDO) by intestinal epithelial cells (Atarashi and others 2011; Ciorba 2013) and production of short-chain fatty acids (SCFAs) through fermentation of dietary fibers (Furusawa and others 2013; Narushima and others 2014). SCFAs, especially butyrate and propionate, greatly facilitate pTreg differentiation from naive T cells, in part, by inhibiting histone deacetylase (Arpaia and others 2013; Smith and others 2013). Polysaccharide A induces pTreg differentiation from naive CD4 T cells (Round and Mazmanian

2010). In addition to microbiota, dietary antigens are also capable of inducing pTregs, further limiting immune responses against food antigens (Kim and others 2016). Of note, provision of tTregs alone in Foxp3-deficient mice, while preventing lethal disease development, does not control inflammatory responses in the colon, which is markedly reduced only after cotransfer of pTregs (Haribhai and others 2011). Therefore, pTregs appear to play nonredundant roles in maintaining immune tolerance at the intestinal interface, and defects in pTregs may promote the development of inflammatory Th17/Th1-type responses in the intestine (Geuking and others 2011; Haribhai and others 2011).

T follicular helper (Tfh) cells are a CD4 T cell subset specialized in supporting germinal center B cell responses (Crotty 2011). It is now known that a subset of Tregs emerges to control Tfh cell activity (Linterman and others 2011). The phenotypes of T follicular regulatory (Tfr) cells mirror those of Tfh cells, namely they highly express CXCR5, a key chemokine receptor assisting their migration to the follicles, programmed cell death-1 (PD-1), and inducible T cell costimulator (ICOS) (Linterman and others 2011). Similar to Tfh cells, Tfr development depends on the transcription factors, Blimp-1 and Bcl-6 (Linterman and others 2011). Tfr cells, upon migration to the germinal center, inhibit Tfh-mediated B cell activation and the subsequent antibody responses (Sage and Sharpe 2016). Treg-specific Bcl6 deletion specifically eliminates Tfr cells. As expected, Tfh cells from these Tfr-free mice produce elevated levels of IL-21 and develop abnormal germinal center B cell responses (Wu and others 2016).

By transcription factor expression

T-bet, Gata3, and Ror γ t are the signature transcription factors responsible for the generation of Th1, Th2, and Th17 lineage effector cells. Analogously, Foxp3⁺ Tregs are induced to express key transcription factors to supposedly regulate each effector response. Naive Tregs, in response to IFN γ and IL-27, upregulate T-bet expression, which further promotes CXCR3 expression on Tregs (Koch and others 2009; Hall and others 2012). The T-bet/CXCR3 expression is critical for the accumulation at sites of Th1-mediated inflammatory responses (Koch and others 2009). Foxp3 regulates IRF4, a transcription factor involved in Th2 differentiation, by binding to the promoter region of the *Irf4* gene (Zheng and others 2009). Indeed, Foxp3 knockdown greatly diminishes *Irf4* mRNA expression (Zheng and others 2009). Treg-specific IRF4 deletion results in selective dysregulation of Th2-type inflammation, including immunoglobulin isotype switching, germinal center formation, and plasma cell infiltration (Zheng and others 2009). Gata3 is selectively expressed in Tregs residing in barrier tissues such as the gastrointestinal tract and skin (Wang and others 2011; Wohlfert and others 2011). Although Gata3 is not required to sustain Treg homeostasis and function at steady state, it plays a critical role during inflammation. Gata3-deficient Tregs fail to accumulate at inflamed sites and to maintain high levels of Foxp3 expression under inflammatory settings (Wohlfert and others 2011). Unlike canonical roles of Gata3 in generating effector cells producing the Th2-type cytokines, Gata3 expression in Tregs appears to be essential in limiting Tregs producing effector cytokines

within inflamed tissues (Wohlfert and others 2011). Utilizing triple-reporter mice where T-bet, Gata3, and Foxp3 expression is marked by different fluorescent proteins, Zhu and colleagues (2015) reported that 20%–30% and ~10% of Tregs in the lymph nodes and spleen express Gata3 and T-bet, respectively. Deletion of either T-bet or Gata3 did not result in loss of Treg functions. However, combined deficiency in Tregs results in reduced Foxp3 expression and severe autoimmune inflammation (Yu and others 2015). Interestingly, Th17-associated transcription factor ROR γ t expression is dramatically increased in the Tregs deficient in T-bet and Gata3 (Yu and others 2015). On the other hand, Tregs coexpressing ROR γ t represent stable effector Treg lineage cells with a highly suppressive function. ROR γ t+ Tregs exhibit enhanced suppressive function in the model of T cell-induced colitis, compared with that of ROR γ t– Tregs (Yang and others 2016). Of note, high ROR γ t expression of T-bet–/– Gata3–/– Tregs is particularly observed in Foxp3^{low} subsets of Tregs (Yu and others 2015). Since Gata3 expression is found to be universally high in all subsets of Tregs regardless of ROR γ t expression, ROR γ t expression in Tregs may be regulated, in part, by Gata3 (Yang and others 2016). STAT3 activation in response to IL-6 together with TGF β is the key pathway leading to the generation of Th17-type effector cells (Zhou and others 2007). Based on the finding that Foxp3 associates with active form of STAT3, Rudensky and colleagues (2009) reported that Treg expression of STAT3 is essential for controlling Treg functions.

In addition to the Th effector signature transcription factors, there are other transcription regulators that play a critical role in Treg functions. Bach2 is a transcriptional repressor highly expressed in B cells repressing Blimp1 expression (Ochiai and others 2006) and is critical for somatic hypermutation and class switch recombination (Muto and others 2004, 2010). Polymorphism in the Bach2 locus is associated with multiple inflammatory diseases in humans, and Bach2-deficient mice develop spontaneous lethal inflammation (Ferreira and others 2011; Roychoudhuri and others 2013). It was found that Bach2 is essential for efficient formation of Tregs in the thymus as well as iTreg generation (Roychoudhuri and others 2013). Even under *in vitro* Treg differentiation conditions, Bach2-deficient CD4 T cells express effector cell transcription factors, suggesting that Bach2 represses the differentiation programs of multiple effector lineages in CD4 to possibly stabilize Treg development while limiting full effector differentiation (Roychoudhuri and others 2013). Bcl11b is a zinc finger transcriptional regulator acting as transcriptional repressor as well as activator. Bcl11b-deficient mice develop lymphadenopathy and splenomegaly (Albu and others 2007). Treg-specific Bcl11b-deficient mice also develop massive colon inflammation, and wild type (WT) Treg transfer prevents the disease (Vanvalkenburgh and others 2011). Furthermore, Bcl11b-deficient Tregs express reduced suppressor function and Foxp3/IL-10 expression and elevated inflammatory cytokines (Vanvalkenburgh and others 2011). Ets-1 is a winged helix-turn-helix transcription factor originally identified as a fusion protein in the E26 avian erythroblastosis virus and subsequently as a key regulator in hematopoiesis, angiogenesis, and tumor progression (Dittmer 2003; Garrett-Sinha 2013). Mice deficient in Ets-1 display defects in B cell/plasma cell generation as well as in

thymocyte development and T cell activation (Bories and others 1995; Eyquem and others 2004; Grenningloh and others 2005; Moisan and others 2007). Moreover, these mice develop splenomegaly and systemic autoimmune inflammation that can be prevented by WT Treg transfer (Mouly and others 2010). Ets-1-deficient Tregs express impaired suppressive activity and fail to protect T cell-induced intestinal inflammation (Mouly and others 2010). Ets-1 directly binds to the Foxp3 enhancer element (CNS2), maintaining demethylation status of the region (Mouly and others 2010; Polansky and others 2010). Last, Kruppel-like factor 2 (KLF2) is a transcription factor that controls mature T cell migration from the thymus and the subsequent recirculation within secondary lymphoid tissues by inducing the sphingosine-1-phosphate receptor and CD62L expression (Carlson and others 2006; Bai and others 2007) and by repressing inflammatory chemokine receptors, including CCR3 and CCR5 (Sebzda and others 2008). It is shown that KLF2 can control Treg development by promoting Foxp3 transcription (Pabbisetty and others 2014). Interestingly, tTreg development is not affected by KLF2 deficiency. Instead, the generation of pTregs (including *in vitro* generated iTregs) is selectively impaired in the absence of KLF2 (Pabbisetty and others 2014).

By cytokine expression

Whether IL-10 and TGF β represent nonreplaceable effector molecules produced by Tregs to mediate suppression has been a matter of debate (Asseman and others 1999; Shevach and others 2001; Fahlen and others 2005; Tran 2012). Rubtsov and others (2008) utilize a Treg-specific IL-10-deficient mouse model and report that Treg production of IL-10 is dispensable for the control of systemic autoimmune inflammation, but essential to control immune responses at the mucosal interface exposed to environmental antigens, such as the intestine, skin, and lungs. Treg-derived IL-10 is also critical to attenuate crescentic glomerulonephritis by regulating Th1/Th17-type cytokine responses as well as antiviral immune responses (Ostmann and others 2013; Jost and others 2014). In addition to producing it, IL-10 signaling in Tregs also plays a key role in Treg function to suppress Th17-type inflammation (Chaudhry and others 2011). Tregs are capable of inducing naive CD4 T cells to become Foxp3+ cells (pTregs *in vivo* or iTregs *ex vivo*), which acquire suppressive function (Jonuleit and others 2002; Zheng and others 2004; Shevach and others 2008). Treg-mediated iTreg generation occurs in a TGF β -dependent mechanism as TGF β -deficient Tregs are unable to generate iTregs (Andersson and others 2008). However, Treg-specific TGF β 1-deficient mice are healthy and do not show any signs of inflammation, which may be attributed to elevated expansion of Tregs in these mice, suggesting that TGF β 1 produced by Tregs controls Treg proliferation (Gutcher and others 2011).

Tregs can produce IFN γ under Th1 polarization conditions (Dominguez-Villar and others 2011), and Tregs can adopt a Th1 effector phenotype during infection, which often results in a reduced suppressive function (Oldenhove and others 2009). However, IFN γ produced by Tregs is beneficial for preventing experimental graft-versus-host disease (GvHD). During a major histocompatibility complex (MHC) mismatched acute GvHD, allogeneic Tregs produce

IFN γ after transplantation. Either neutralizing anti-IFN γ monoclonal antibody (mAb) treatment or Tregs from IFN γ -deficient donor mice fails to prevent development of lethal GVHD (Sawitzki and others 2005; Koenecke and others 2012). How does Treg-derived IFN γ exert the protective functions? IFN γ inhibits T cell proliferation and induces apoptosis (Kishimoto and others 2002; Refaelli and others 2002). IFN γ produced by Tregs can act on antigen presenting cells (APCs) to influence their function. For example, IFN γ induces nitric oxide synthase (NOS2) in APCs, and NOS2 metabolizes arginine to trigger production of reactive oxygen species, which then trigger apoptosis of neighboring cells (Atochina and others 2001). In fact, IFN γ neutralization reduces NOS2 expression. IFN γ produced by Tregs can stimulate the tryptophan catabolism enzyme, IDO (Fallarino and others 2003). IDO degrades tryptophan, thereby negatively influencing T cell function (Mellor and Munn 1999).

Voo and others (2009) showed that in human peripheral blood mononuclear cells (PBMCs) and lymphoid tissues, a significant number of Tregs that express ROR γ t are capable of producing IL-17 upon activation. Particularly, these cells strongly inhibit CD4 T cell proliferation through the cell-cell contact mechanism. Interestingly, these IL-17+ cells can emerge from Foxp3+CCR6- Tregs through activation in the presence of IL-1 β , IL-21, and IL-23. As CCR6+ Tregs are not found in the thymus, such IL-17+ Tregs appear to be generated in the periphery. Similarly, Bellemore and others (2015) reported that polarizing BDC2.5 Tg CD4 T cells with TGF β plus IL-6 generates nondiabetogenic Treg17 cells that express high levels of aryl hydrocarbon receptor (AHR) and IL-10. A separate study led by Hafler and colleagues (2009) reports that inflammatory condition can drive Tregs to secrete IL-17. CCR6+ Tregs produce IL-17 in the presence of IL-1 β and IL-6, while IL-17 production is inhibited by TGF β . Importantly, IL-17 production by Tregs inversely correlates with their suppressive activity.

From a genomic screen assay, Ebi3 was identified as a novel Treg-restricted molecule. Ebi3 pairs with IL-12p35 subunit to form IL-35 (Collison and others 2007). Tregs deficient in Ebi3 or IL-12p35 are functionally defective *in vitro* and *in vivo*. Non-Tregs transduced with retroviral vectors encoding IL-35 express regulatory phenotypes, suppressing T cell proliferation. Recombinant IL-35 itself is sufficient to suppress T cell proliferation (Collison and others 2007). IL-35 stimulation of naive T cells induces a regulatory population that does not express Foxp3 (iTr35). iTr35 cells are suppressive and stable *in vivo*. Interestingly, during parasite infection and tumor environments, iTr35 cells are generated, potentially contributing to the regulatory roles (Collison and others 2010). The stability of iTr35 cells (whether iTr35 cells lose regulatory phenotype such as Foxp3+ Tregs) remains to be determined.

Last, IL-9 produced by Tregs recruits mast cells that are essential for Treg-induced immune suppression (Eller and others 2011). MCs mobilize myeloid-derived suppressor cells (MDSCs) and Tregs through the IL-17 pathway (Yang and others 2010).

Stability of Tregs

Stable Foxp3 expression is required not only for the development of functional Tregs but also for proper functions

under homeostatic and inflammatory settings. Under inflammatory conditions, some Tregs lose Foxp3 expression, becoming so-called exTregs (Guo and Zhou 2015). Because exTregs are not only unable to exert suppressive functions but they also become pathogenic cells, identifying factors involved in the process is a subject of great interest.

One mechanism regulating a protein expression post-translationally is through protein ubiquitination, which marks the protein for degradation through proteasome. Protein ubiquitination is a tightly controlled process, involving series of ubiquitin ligases and deubiquitinases (Komander 2009). Ubiquitin ligase (called as E3 ubiquitin ligase) polyubiquitinates the protein substrate with Lys48-linked chains of ubiquitin, further targeting the substrate for proteasomal degradation (Komander 2009). Ubiquitin ligase known as Stub1 interacts with Foxp3 and causes polyubiquitination (Chen and others 2013b). Stub1 thus negatively regulates Foxp3 protein expression. Stub1 overexpression in Tregs abolishes Treg-suppressive function, while silencing Stub1 expression enhances Treg function (Chen and others 2013b). Another E3 ubiquitin ligase, Cbl-b, binds to Foxp3 following TCR stimulation and promotes degradation in the proteasome (Zhao and others 2015). Itch, another E3 ubiquitin ligase, plays a key role in TGF β -mediated Foxp3 expression and Treg generation (Venuprasad and others 2008). Unlike Stub1, Itch targets the transcription factor known as TGF β -inducible early gene 1 (TIEG1) for ubiquitination. Interestingly, TIEG1 is monoubiquitinated in a nonproteolytic pathway, which allows TIEG1 to induce Foxp3 transcription (Venuprasad and others 2008). Deubiquitinating enzyme removes ubiquitin from protein substrates, preventing degradation and increasing protein expression (Nijman and others 2005). One particular deubiquitinase known as USP7 is actively expressed in Tregs and associated with Foxp3 (van Loosdregt and others 2013). Ectopic USP7 expression decreases Foxp3 polyubiquitination and increases Foxp3 expression, whereas reducing USP7 activity decreases endogenous Foxp3 expression and Treg-mediated suppression (van Loosdregt and others 2013).

Lysine/histone acetyl transferases (KATs) mediate protein acetylation. Foxp3 is acetylated by the KAT and reciprocally deacetylated by the lysine/histone deacetylase (KDAC) (van Loosdregt and others 2010, 2011; Xiao and others 2010). Foxp3 acetylation prevents ubiquitination and subsequent proteasome degradation, stabilizing Foxp3 protein levels (van Loosdregt and others 2010). Ectopic expression of SIRT1 (KDAC) promotes Foxp3 deacetylation and degradation (van Loosdregt and others 2011). Treg-specific SIRT1 deletion enhances Foxp3 expression and Treg suppression (Beier and others 2011b). Foxp3 interacts with other KATs such as TIP60 and p300, thereby it is acetylated by these factors (Li and others 2007). It was recently demonstrated that the transcriptional factor activity of Foxp3 can be regulated by Foxp3 phosphorylation at Ser418 residue in the C-terminal DNA binding domain (Nie and others 2013). Nie and others (2013) reported that Tregs treated with a destabilizing cytokine, tumor necrosis factor alpha (TNF α), or with synovial fluid isolated from arthritis patients express decreased Foxp3 phosphorylation. Foxp3 also contains cyclin-dependent kinase motif within the N-terminal repressor domain and becomes phosphorylated by CDK2 (Morawski and others 2013). Therefore, while C-terminal phosphorylation is important for proper Treg

function (Nie and others 2013), N-terminal phosphorylation by CDK2 negatively regulates the stability and activity of Foxp3 (Morawski and others 2013).

Control of Foxp3 transcriptional activation is an important step for maintaining Treg stability (Fig. 2). Foxp3 gene activation is triggered by TCR stimulation both during the thymic development and during peripheral activation (Maruyama and others 2011). TCR activation results in the binding of transcription factors such as AP-1 and NFAT1 to the Foxp3 promoter (Mantel and others 2006). Cyclic-AMP-responsive element-binding protein (CREB) and activating transcription factor are induced to bind to the enhancer region of the Foxp3 gene upon TCR activation (Kim and Leonard 2007). TCR stimulation also promotes nuclear factor-kappaB (NFkB)-mediated Foxp3 transcription (Long and others 2009). C-Rel, the NFkB/Rel family member protein, plays an essential role during TGFβ-induced Foxp3 expression (Visekruna and others 2010).

Epigenetic modification is a process that controls gene activation (transcription) by allowing transcription factors and DNA-binding proteins to bind DNA regions. Histone can be a direct target of such modification process through acetylation or methylation, which eventually alters chromatin structure (Bannister and Kouzarides 2011). Alternatively, CpG motifs in the DNA are often clustered within promoters and can be methylated (Li 2002). Methylated CpG motifs result in the closed chromatin structure, whereas demethylation of CpG motifs results in the relaxed chromatin structure, allowing binding of transcription factors (Li 2002). CpG motifs in the Foxp3 promoter are completely demethylated in Tregs (Janson and others 2008). Likewise, acetylated histones are found in the Foxp3 promoter in Tregs (Beier and others 2011a). Treg-specific demethylated region known as TSDR is a highly conserved CpG-rich region of the Foxp3 gene (Polansky and others 2008). Fully demethylated TSDR is found in Tregs, while the region is methylated in conventional T cells (Polansky and others 2008; Schreiber and others 2014). Of note, TSDR methylation status is not always associated with Foxp3 expression.

TGFβ-induced iTregs highly express Foxp3, as high as that of tTregs. However, the TSDR of iTregs is partially demethylated, as opposed to tTregs in which the TSDR is fully demethylated (Floess and others 2007). The status of TSDR demethylation is a critical determinant of the stability of Foxp3 expression (Floess and others 2007). Unlike *in vitro* generated iTregs, pTregs generated *in vivo* exhibit demethylated TSDR (Polansky and others 2008; Miyao and others 2012; Yadav and others 2013).

Micro-RNA (miRNA) is a short-length RNA that plays a crucial role in regulating gene expression (Baumjohann and Ansel 2013). There is increasing evidence that miRNAs are critically involved in various T cell functions. In particular, miRNAs are important for Treg generation as well as suppressive functions, as deleting Dicer, the RNase III enzyme generating functional miRNA, impairs Treg development (Cobb and others 2006; Liston and others 2008). Specifically, miR-155 is highly expressed in Tregs, and miR-155-deficient mice harbor decreased Tregs (Lu and others 2009). The target for miR-155 is Socs1, a negative regulator of the IL-2 signaling pathway that is critical for Treg development (Lu and others 2009). miR-17 targets TGFβ receptor II and CREB 1, which are critical for Treg differentiation (Jiang and others 2011). miR-10a is selectively expressed in Tregs (Kuchen and others 2010). In particular, miR-10a supports Treg stability by maintaining Foxp3 expression (Jeker and others 2012).

Inflammatory conditions associated with Treg instability

Zhou and others (2009) reported the first evidence of Treg population that exhibits unstable Foxp3 expression. Utilizing lineage-tracing mice, Foxp3CreGFP crossed to a Rosa26-YFP in which the YFP controlled by the floxed stop codon permanently marks Foxp3+ cells even after they lose Foxp3 expression (Srinivas and others 2001); they found that a significant proportion of YFP+ Tregs lose Foxp3 expression (become GFP-) even in healthy animals and that these exTregs produce inflammatory cytokines upon activation (Zhou and others 2009). Especially, they noted that these exTregs tend to increase substantially in the setting of autoimmune diabetes and that they rapidly induce diabetes following adoptive transfer (Zhou and others 2009). exTreg generation disrupts the balance between Tregs and pathogenic effector T cells within the inflamed tissues, thus contributing to the development of inflammation (Tang and others 2008). A mechanism underlying exTreg generation in the context of diabetes, which remains to be determined, may include defective IL-2 production that causes CD25 downregulation and apoptotic cell death of Tregs (Tang and others 2008). IL-6 responsiveness of T cells is significantly elevated in type 1 diabetes patients (Hundhausen and others 2016). Given that IL-6 level is found to be higher in patients with diabetes (Bradshaw and others 2009; Talaat and others 2016) and that IL-6 triggers Foxp3 degradation (Gao and others 2015), it is alternatively possible that increased IL-6 level may promote exTreg generation, tip the balance, and cause disease development. In the model of experimental autoimmune encephalomyelitis (EAE), an animal model for human multiple sclerosis (MS), Bailey-Bucktrout and others (2013) reported that both polyclonal and antigen-specific exTregs peak in the inflamed target tissues during the peak

FIG. 2. Multiple factors affecting Foxp3 expression and Treg stability.

of the disease. However, the proportion of exTregs is higher in antigen-specific Tregs, suggesting that Foxp3 loss is triggered by antigen-induced activation (Bailey-Bucktrout and others 2013). Komatsu and others (2014) recently reported in the model of autoimmune arthritis that pathogenic Th17 cells arise from Tregs and that these Th17 cells play an essential role in pathogenesis. They also found that conversion of Tregs to pathogenic Th17 cells is mediated by IL-6 produced by synovial fibroblasts (Komatsu and others 2014). In addition to IL-6, TNF is another inflammatory cytokine capable of triggering Foxp3 degradation and downregulating Treg functions (Gao and others 2015). Particularly, Tregs isolated from active rheumatoid arthritis patients express reduced Foxp3 mRNA and protein and are functionally impaired, all of which are significantly restored by treatment with anti-TNF antibody (Valencia and others 2006). However, it was also noted that TNF alters Foxp3 phosphorylation without affecting Foxp3 protein expression (Nie and others 2013). Deleted in breast cancer 1 (DBC1) is a subunit of the Foxp3 complex in Tregs and physically interacts with Foxp3. DBC1 depletion maintains Foxp3 expression and Treg function during inflammation (Gao and others 2015). Mice deficient in DBC1 develop attenuated EAE and colitis and harbor increased Treg frequency (Gao and others 2015).

Is Treg instability the sole mechanism underlying Treg defects and favoring inflammatory responses? Not necessarily. The evidence supporting the instability—-independent mechanism—is plenty. In case of MS, Treg frequency is elevated in the cerebrospinal fluid (CSF) of MS patients, and the frequency of Tregs in the PBMC is similar between patients and healthy controls (Feger and others 2007). Instead, the suppressive function of Treg itself is significantly reduced in MS patients (Feger and others 2007). In a different study, it is noted that Treg frequencies are equally distributed in blood and CSF of MS patients, while suppressive function of patient-derived Tregs is severely impaired (Viglietta and others 2004; Haas and others 2005). Tregs from MS patients display impaired migratory capacity under noninflammatory conditions compared with healthy donors since proper migration may be a feature of functional Tregs that allows for an effective parenchymal immune surveillance (Schneider-Hohendorf and others 2010). Similar pattern of Treg dysregulation is also observed in other chronic inflammatory settings such as rheumatoid arthritis. Lawson and others (2006) reported that the proportion of circulating Tregs is reduced during early rheumatoid arthritis, while the frequency in synovial fluid is equally increased. They also noted no differences in suppressor function between patients and healthy controls (Lawson and others 2006), although others found that Tregs isolated from inflamed joint tissues of patients are unable to suppress inflammatory cytokine production by effector CD4 T cells (Ehrenstein and others 2004; van Amelsfort and others 2007).

Perspectives

The heterogeneity of Treg populations and the complexity through which Tregs execute the appropriate suppressive function in the right context continue to grow. With the help of various new tools that allow us to visualize population diversity, future investigations should focus on identifying

cellular mechanisms by which the diversity can emerge with both cellular and molecular factors that control the emergence. Probably, the biggest challenge of all will be how to translate the findings into human Tregs. There is no reliable marker that defines tTregs and pTregs in humans. Identifying markers distinguishing functionally competent Tregs from compromised exTregs is an important task to achieve. By better understanding the detailed mechanisms underlying Treg instability within inflammatory conditions, we may be able to devise novel therapeutic approaches to inhibit inflammatory responses, thereby treating the inflammatory diseases.

Acknowledgments

This work is supported, in part, by grants from NIH (AI121524), American Asthma Foundation, and National Multiple Sclerosis Society (RG1411-02051).

Author Disclosure Statement

No competing financial interests exist.

References

- Albu DI, Feng D, Bhattacharya D, Jenkins NA, Copeland NG, Liu P, Avram D. 2007. BCL11B is required for positive selection and survival of double-positive thymocytes. *J Exp Med* 204(12):3003–3015.
- Andersson J, Tran DQ, Pesu M, Davidson TS, Ramsey H, O'Shea JJ, Shevach EM. 2008. CD4⁺ FoxP3⁺ regulatory T cells confer infectious tolerance in a TGF- β -dependent manner. *J Exp Med* 205(9):1975–1981.
- Anz D, Mueller W, Golic M, Kunz WG, Rapp M, Koelzer VH, Ellermeier J, Ellwart JW, Schnurr M, Bourquin C, Endres S. 2011. CD103 is a hallmark of tumor-infiltrating regulatory T cells. *Int J Cancer* 129(10):2417–2426.
- Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffey PJ, Rudensky AY. 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504(7480):451–455.
- Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, Yuan S, Treuting PM, Rudensky AY. 2015. A distinct function of regulatory T cells in tissue protection. *Cell* 162(5):1078–1089.
- Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 190(7):995–1004.
- 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. 2011. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* 331(6015):337–341.
- Atochina O, Daly-Engel T, Piskorska D, McGuire E, Harn DA. 2001. A schistosoma-expressed immunomodulatory glycoconjugate expands peritoneal Gr1(+) macrophages that suppress naive CD4(+) T cell proliferation via an IFN- γ and nitric oxide-dependent mechanism. *J Immunol* 167(8):4293–4302.
- Bai A, Hu H, Yeung M, Chen J. 2007. Kruppel-like factor 2 controls T cell trafficking by activating L-selectin (CD62L) and sphingosine-1-phosphate receptor 1 transcription. *J Immunol* 178(12):7632–7639.
- Bailey-Bucktrout SL, Martinez-Llordella M, Zhou X, Anthony B, Rosenthal W, Luche H, Fehling HJ, Bluestone JA. 2013.

- Self-antigen-driven activation induces instability of regulatory T cells during an inflammatory autoimmune response. *Immunity* 39(5):949–962.
- Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. *Cell Res* 21(3):381–395.
- Baumjohann D, Ansel KM. 2013. MicroRNA-mediated regulation of T helper cell differentiation and plasticity. *Nat Rev Immunol* 13(9):666–678.
- Beier UH, Akimova T, Liu Y, Wang L, Hancock WW. 2011a. Histone/protein deacetylases control Foxp3 expression and the heat shock response of T-regulatory cells. *Curr Opin Immunol* 23(5):670–678.
- Beier UH, Wang L, Bhatti TR, Liu Y, Han R, Ge G, Hancock WW. 2011b. Sirtuin-1 targeting promotes Foxp3⁺ T-regulatory cell function and prolongs allograft survival. *Mol Cell Biol* 31(5):1022–1029.
- Bellemore SM, Nikoobpour E, Schwartz JA, Krougly O, Lee-Chan E, Singh B. 2015. Preventative role of interleukin-17 producing regulatory T helper type 17 (Treg 17) cells in type 1 diabetes in non-obese diabetic mice. *Clin Exp Immunol* 182(3):261–269.
- Beriu G, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C, Hafler DA. 2009. IL-17-producing human peripheral regulatory T cells retain suppressive function. *Blood* 113(18):4240–4249.
- Bories JC, Willerford DM, Grevin D, Davidson L, Camus A, Martin P, Stehelin D, Alt FW. 1995. Increased T-cell apoptosis and terminal B-cell differentiation induced by inactivation of the Ets-1 proto-oncogene. *Nature* 377(6550):635–638.
- Bradshaw EM, Raddassi K, Elyaman W, Orban T, Gottlieb PA, Kent SC, Hafler DA. 2009. Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells. *J Immunol* 183(7):4432–4439.
- Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y, Sefik E, Tan TG, Wagers AJ, Benoist C, Mathis D. 2013. A special population of regulatory T cells potentiates muscle repair. *Cell* 155(6):1282–1295.
- Campbell DJ, Koch MA. 2011. Phenotypical and functional specialization of FOXP3⁺ regulatory T cells. *Nat Rev Immunol* 11(2):119–130.
- Carlson CM, Endrizzi BT, Wu J, Ding X, Weinreich MA, Walsh ER, Wani MA, Lingrel JB, Hogquist KA, Jameson SC. 2006. Kruppel-like factor 2 regulates thymocyte and T-cell migration. *Nature* 442(7100):299–302.
- Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, Rudensky AY. 2009. CD4⁺ regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* 326(5955):986–991.
- Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, Jack RS, Wunderlich FT, Bruning JC, Muller W, Rudensky AY. 2011. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* 34(4):566–578.
- Chen X, Wu Y, Wang L. 2013a. Fat-resident Tregs: an emerging guard protecting from obesity-associated metabolic disorders. *Obes Rev* 14(7):568–578.
- Chen Z, Barbi J, Bu S, Yang HY, Li Z, Gao Y, Jinasena D, Fu J, Lin F, Chen C, Zhang J, Yu N, Li X, Shan Z, Nie J, Gao Z, Tian H, Li Y, Yao Z, Zheng Y, Park BV, Pan Z, Zhang J, Dang E, Li Z, Wang H, Luo W, Li L, Semenza GL, Zheng SG, Loser K, Tsun A, Greene MI, Pardoll DM, Pan F, Li B. 2013b. The ubiquitin ligase Stub1 negatively modulates regulatory T cell suppressive activity by promoting degradation of the transcription factor Foxp3. *Immunity* 39(2):272–285.
- Ciorba MA. 2013. Indoleamine 2,3 dioxygenase in intestinal disease. *Curr Opin Gastroenterol* 29(2):146–152.
- Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelson SE, Benoist C, Mathis D. 2012. PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* 486(7404):549–553.
- Cobb BS, Hertweck A, Smith J, O'Connor E, Graf D, Cook T, Smale ST, Sakaguchi S, Livesey FJ, Fisher AG, Merkenchlagher M. 2006. A role for Dicer in immune regulation. *J Exp Med* 203(11):2519–2527.
- Collison LW, Chaturvedi V, Henderson AL, Giacomini PR, Guy C, Bankoti J, Finkelstein D, Forbes K, Workman CJ, Brown SA, Rehag JE, Jones ML, Ni HT, Artis D, Turk MJ, Vignali DA. 2010. IL-35-mediated induction of a potent regulatory T cell population. *Nat Immunol* 11(12):1093–1101.
- Collison LW, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450(7169):566–569.
- Crotty S. 2011. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* 29:621–663.
- Dittmer J. 2003. The biology of the Ets1 proto-oncogene. *Mol Cancer* 2:29.
- Dominguez-Villar M, Baecher-Allan CM, Hafler DA. 2011. Identification of T helper type 1-like, Foxp3⁺ regulatory T cells in human autoimmune disease. *Nat Med* 17(6):673–675.
- Dudda JC, Perdue N, Bachtanian E, Campbell DJ. 2008. Foxp3⁺ regulatory T cells maintain immune homeostasis in the skin. *J Exp Med* 205(7):1559–1565.
- Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C. 2004. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J Exp Med* 200(3):277–285.
- Eller K, Wolf D, Huber JM, Metz M, Mayer G, McKenzie AN, Maurer M, Rosenkranz AR, Wolf AM. 2011. IL-9 production by regulatory T cells recruits mast cells that are essential for regulatory T cell-induced immune suppression. *J Immunol* 186(1):83–91.
- Eyquem S, Chemin K, Fasseu M, Chopin M, Sigaux F, Cumano A, Bories JC. 2004. The development of early and mature B cells is impaired in mice deficient for the Ets-1 transcription factor. *Eur J Immunol* 34(11):3187–3196.
- Fahlen L, Read S, Gorelik L, Hurst SD, Coffman RL, Flavell RA, Powrie F. 2005. T cells that cannot respond to TGF-beta escape control by CD4(+)CD25(+) regulatory T cells. *J Exp Med* 201(5):737–746.
- Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P. 2003. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 4(12):1206–1212.
- Feger U, Luther C, Poeschel S, Melms A, Tolosa E, Wiendl H. 2007. Increased frequency of CD4⁺ CD25⁺ regulatory T cells in the cerebrospinal fluid but not in the blood of multiple sclerosis patients. *Clin Exp Immunol* 147(3):412–418.
- Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, Le Souef P, Danoy P, Baltic S, Nyholt DR, Jenkins M, Hayden C, Willemsen G, Ang W, Kuokkanen M, Beilby J, Cheah F, de Geus EJ, Ramasamy A, Vedantam S, Salomaa V, Madden PA, Heath AC, Hopper JL, Visscher PM, Musk B, Leeder SR, Jarvelin MR, Pennell C, Boomsma DI, Hirschhorn JN, Walters H, Martin NG, James A, Jones G, Abramson MJ, Robertson CF, Dharmage SC, Brown MA, Montgomery GW, Thompson PJ, Australian Asthma Genetics Consortium. 2011. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet* 378(9795):1006–1014.

- Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D. 2009. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* 15(8):930–939.
- Floess S, Freyer J, Siewert C, Baron U, Olek S, Polansky J, Schlawe K, Chang HD, Bopp T, Schmitt E, Klein-Hessling S, Serfling E, Hamann A, Huehn J. 2007. Epigenetic control of the *foxp3* locus in regulatory T cells. *PLoS Biol* 5(2):e38.
- Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. 2013. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504(7480):446–450.
- Gao Y, Tang J, Chen W, Li Q, Nie J, Lin F, Wu Q, Chen Z, Gao Z, Fan H, Tsun A, Shen J, Chen G, Liu Z, Lou Z, Olsen NJ, Zheng SG, Li B. 2015. Inflammation negatively regulates FOXP3 and regulatory T-cell function via DBC1. *Proc Natl Acad Sci U S A* 112(25):E3246–E3254.
- Garrett-Sinha LA. 2013. Review of Ets1 structure, function, and roles in immunity. *Cell Mol Life Sci* 70(18):3375–3390.
- Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ. 2011. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* 34(5):794–806.
- Gratz IK, Truong HA, Yang SH, Maurano MM, Lee K, Abbas AK, Rosenblum MD. 2013. Cutting edge: memory regulatory T cells require IL-7 and not IL-2 for their maintenance in peripheral tissues. *J Immunol* 190(9):4483–4487.
- Greeningloh R, Kang BY, Ho IC. 2005. Ets-1, a functional cofactor of T-bet, is essential for Th1 inflammatory responses. *J Exp Med* 201(4):615–626.
- Guo J, Zhou X. 2015. Regulatory T cells turn pathogenic. *Cell Mol Immunol* 12(5):525–532.
- Gutcher I, Donkor MK, Ma Q, Rudensky AY, Flavell RA, Li MO. 2011. Autocrine transforming growth factor-beta1 promotes in vivo Th17 cell differentiation. *Immunity* 34(3):396–408.
- Haas J, Hug A, Viehover A, Fritzsche B, Falk CS, Filser A, Vetter T, Milkova L, Korporal M, Fritz B, Storch-Hagenlocher B, Krammer PH, Suri-Payer E, Wildemann B. 2005. Reduced suppressive effect of CD4⁺CD25^{high} regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. *Eur J Immunol* 35(11):3343–3352.
- Hall AO, Beiting DP, Tato C, John B, Oldenhove G, Lombana CG, Pritchard GH, Silver JS, Bouladoux N, Stumhofer JS, Harris TH, Grainger J, Wojno ED, Wagage S, Roos DS, Scott P, Turka LA, Cherry S, Reiner SL, Cua D, Belkaid Y, Elloso MM, Hunter CA. 2012. The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. *Immunity* 37(3):511–523.
- Haribhai D, Williams JB, Jia S, Nickerson D, Schmitt EG, Edwards B, Ziegelbauer J, Yassai M, Li SH, Relland LM, Wise PM, Chen A, Zheng YQ, Simpson PM, Gorski J, Salzman NH, Hessner MJ, Chatila TA, Williams CB. 2011. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. *Immunity* 35(1):109–122.
- Hsieh CS, Lee HM, Lio CW. 2012. Selection of regulatory T cells in the thymus. *Nat Rev Immunol* 12(3):157–167.
- Huehn J, Siegmund K, Lehmann JC, Siewert C, Haubold U, Feuerer M, Debes GF, Lauber J, Frey O, Przybylski GK, Niesner U, de la Rosa M, Schmidt CA, Brauer R, Buer J, Scheffold A, Hamann A. 2004. Developmental stage, phenotype, and migration distinguish naive- and effector/memory-like CD4⁺ regulatory T cells. *J Exp Med* 199(3):303–313.
- Hundhausen C, Roth A, Whalen E, Chen J, Schneider A, Long SA, Wei S, Rawlings R, Kinsman M, Evanko SP, Wight TN, Greenbaum CJ, Cerosaletti K, Buckner JH. 2016. Enhanced T cell responses to IL-6 in type 1 diabetes are associated with early clinical disease and increased IL-6 receptor expression. *Sci Transl Med* 8(356):356ra119.
- Janson PC, Winerdal ME, Marits P, Thorn M, Ohlsson R, Winqvist O. 2008. FOXP3 promoter demethylation reveals the committed Treg population in humans. *PLoS One* 3(2):e1612.
- Jeker LT, Zhou X, Gershberg K, de Kouchkovsky D, Morar MM, Stadthagen G, Lund AH, Bluestone JA. 2012. MicroRNA 10a marks regulatory T cells. *PLoS One* 7(5):e36684.
- Jiang S, Li C, Olive V, Lykken E, Feng F, Sevilla J, Wan Y, He L, Li QJ. 2011. Molecular dissection of the miR-17–92 cluster's critical dual roles in promoting Th1 responses and preventing inducible Treg differentiation. *Blood* 118(20):5487–5497.
- Jonuleit H, Schmitt E, Kakirman H, Stassen M, Knop J, Enk AH. 2002. Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. *J Exp Med* 196(2):255–260.
- Jost NH, Abel S, Hutzler M, Sparwasser T, Zimmermann A, Roers A, Muller W, Klopfeisch R, Hengel H, Westendorf AM, Buer J, Hansen W. 2014. Regulatory T cells and T-cell-derived IL-10 interfere with effective anti-cytomegalovirus immune response. *Immunol Cell Biol* 92(10):860–871.
- Kang SG, Piniecki RJ, Hogenesch H, Lim HW, Wiebke E, Braun SE, Matsumoto S, Kim CH. 2007. Identification of a chemokine network that recruits FoxP3(+) regulatory T cells into chronically inflamed intestine. *Gastroenterology* 132(3):966–981.
- Kim HP, Leonard WJ. 2007. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. *J Exp Med* 204(7):1543–1551.
- Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG, Lee JY, Lee M, Surh CD. 2016. Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine. *Science* 351(6275):858–863.
- Kishimoto K, Sandner S, Imitola J, Sho M, Li Y, Langmuir PB, Rothstein DM, Strom TB, Turka LA, Sayegh MH. 2002. Th1 cytokines, programmed cell death, and alloreactive T cell clone size in transplant tolerance. *J Clin Invest* 109(11):1471–1479.
- Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. 2009. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat Immunol* 10(6):595–602.
- Koenecke C, Lee CW, Thamm K, Fohse L, Schafferus M, Mittrucker HW, Floess S, Huehn J, Ganser A, Forster R, Prinz I. 2012. IFN-gamma production by allogeneic Foxp3⁺ regulatory T cells is essential for preventing experimental graft-versus-host disease. *J Immunol* 189(6):2890–2896.
- Kolodin D, van Panhuys N, Li C, Magnuson AM, Cipolletta D, Miller CM, Wagers A, Germain RN, Benoist C, Mathis D. 2015. Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. *Cell Metab* 21(4):543–557.

- Komander D. 2009. The emerging complexity of protein ubiquitination. *Biochem Soc Trans* 37 (Pt 5):937–953.
- Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, Tanaka S, Bluestone JA, Takayanagi H. 2014. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. *Nat Med* 20(1):62–68.
- Kuchen S, Resch W, Yamane A, Kuo N, Li Z, Chakraborty T, Wei L, Laurence A, Yasuda T, Peng S, Hu-Li J, Lu K, Dubois W, Kitamura Y, Charles N, Sun HW, Muljo S, Schwartzberg PL, Paul WE, O'Shea J, Rajewsky K, Casellas R. 2010. Regulation of microRNA expression and abundance during lymphopoiesis. *Immunity* 32(6):828–839.
- Lawson CA, Brown AK, Bejarano V, Douglas SH, Burgoyne CH, Greenstein AS, Boylston AW, Emery P, Ponchel F, Isaacs JD. 2006. Early rheumatoid arthritis is associated with a deficit in the CD4+CD25high regulatory T cell population in peripheral blood. *Rheumatology (Oxford)* 45(10):1210–1217.
- Lehmann J, Huehn J, de la Rosa M, Maszyra F, Kretschmer U, Krenn V, Brunner M, Scheffold A, Hamann A. 2002. Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25- regulatory T cells. *Proc Natl Acad Sci U S A* 99(20):13031–13036.
- Li B, Samanta A, Song X, Iacono KT, Bembas K, Tao R, Basu S, Riley JL, Hancock WW, Shen Y, Saouaf SJ, Greene MI. 2007. FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. *Proc Natl Acad Sci U S A* 104(11):4571–4576.
- Li E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 3(9):662–673.
- Lim HW, Hillsamer P, Kim CH. 2004. Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. *J Clin Invest* 114(11):1640–1649.
- Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, Srivastava M, Divekar DP, Beaton L, Hogan JJ, Fagarasan S, Liston A, Smith KG, Vinuesa CG. 2011. Foxp3+ follicular regulatory T cells control the germinal center response. *Nat Med* 17(8):975–982.
- Liston A, Lu LF, O'Carroll D, Tarakhovsky A, Rudensky AY. 2008. Dicer-dependent microRNA pathway safeguards regulatory T cell function. *J Exp Med* 205(9):1993–2004.
- Long M, Park SG, Strickland I, Hayden MS, Ghosh S. 2009. Nuclear factor-kappaB modulates regulatory T cell development by directly regulating expression of Foxp3 transcription factor. *Immunity* 31(6):921–931.
- Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, Loeb GB, Lee H, Yoshimura A, Rajewsky K, Rudensky AY. 2009. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity* 30(1):80–91.
- Mantel PY, Ouaked N, Ruckert B, Karagiannidis C, Welz R, Blaser K, Schmidt-Weber CB. 2006. Molecular mechanisms underlying FOXP3 induction in human T cells. *J Immunol* 176(6):3593–3602.
- Maruyama T, Konkel JE, Zamarron BF, Chen W. 2011. The molecular mechanisms of Foxp3 gene regulation. *Semin Immunol* 23(6):418–423.
- McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC. 2002. CD4(+)/CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 16(2):311–323.
- Mellor AL, Munn DH. 1999. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? *Immunol Today* 20(10):469–473.
- Min B, Thornton A, Caucheteux SM, Younes SA, Oh K, Hu-Li J, Paul WE. 2007. Gut flora antigens are not important in the maintenance of regulatory T cell heterogeneity and homeostasis. *Eur J Immunol* 37(7):1916–1923.
- Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, Huehn J, Hori S. 2012. Plasticity of Foxp3(+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. *Immunity* 36(2):262–275.
- Moisan J, Grenningloh R, Bettelli E, Oukka M, Ho IC. 2007. Ets-1 is a negative regulator of Th17 differentiation. *J Exp Med* 204(12):2825–2835.
- Morawski PA, Mehra P, Chen C, Bhatti T, Wells AD. 2013. Foxp3 protein stability is regulated by cyclin-dependent kinase 2. *J Biol Chem* 288(34):24494–24502.
- Mouly E, Chemin K, Nguyen HV, Chopin M, Mesnard L, Leite-de-Moraes M, Burlen-defranoux O, Bandeira A, Bories JC. 2010. The Ets-1 transcription factor controls the development and function of natural regulatory T cells. *J Exp Med* 207(10):2113–2125.
- Muto A, Ochiai K, Kimura Y, Itoh-Nakadai A, Calame KL, Ikebe D, Tashiro S, Igarashi K. 2010. Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch. *EMBO J* 29(23):4048–4061.
- Muto A, Tashiro S, Nakajima O, Hoshino H, Takahashi S, Sakoda E, Ikebe D, Yamamoto M, Igarashi K. 2004. The transcriptional programme of antibody class switching involves the repressor Bach2. *Nature* 429(6991):566–571.
- Narushima S, Sugiura Y, Oshima K, Atarashi K, Hattori M, Suematsu M, Honda K. 2014. Characterization of the 17 strains of regulatory T cell-inducing human-derived *Clostridia*. *Gut Microbes* 5(3):333–339.
- Nguyen TL, Makhlof NT, Anthony BA, Teague RM, DiPaolo RJ. 2014. In vitro induced regulatory T cells are unique from endogenous regulatory T cells and effective at suppressing late stages of ongoing autoimmunity. *PLoS One* 9(8):e104698.
- Nie H, Zheng Y, Li R, Guo TB, He D, Fang L, Liu X, Xiao L, Chen X, Wan B, Chin YE, Zhang JZ. 2013. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-alpha in rheumatoid arthritis. *Nat Med* 19(3):322–328.
- Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R. 2005. A genomic and functional inventory of deubiquitinating enzymes. *Cell* 123(5):773–786.
- Nosbaum A, Prevel N, Truong HA, Mehta P, Ettinger M, Scharschmidt TC, Ali NH, Pauli ML, Abbas AK, Rosenblum MD. 2016. Cutting edge: regulatory T cells facilitate cutaneous wound healing. *J Immunol* 196(5):2010–2014.
- Ochiai K, Katoh Y, Ikura T, Hoshikawa Y, Noda T, Karasuyama H, Tashiro S, Muto A, Igarashi K. 2006. Plasmacytic transcription factor Blimp-1 is repressed by Bach2 in B cells. *J Biol Chem* 281(50):38226–38234.
- Oldenhove G, Bouladoux N, Wohlfert EA, Hall JA, Chou D, Dos Santos L, O'Brien S, Blank R, Lamb E, Natarajan S, Kastanmayer R, Hunter C, Grigg ME, Belkaid Y. 2009. Decrease of Foxp3+ Treg cell number and acquisition of effector cell phenotype during lethal infection. *Immunity* 31(5):772–786.
- Ostmann A, Paust HJ, Panzer U, Wegscheid C, Kapffer S, Huber S, Flavell RA, Erhardt A, Tiegs G. 2013. Regulatory T cell-derived IL-10 ameliorates crescentic GN. *J Am Soc Nephrol* 24(6):930–942.
- Pabbisetty SK, Rabacal W, Maseda D, Cendron D, Collins PL, Hoek KL, Parekh VV, Aune TM, Sebzda E. 2014. KLF2 is a

- rate-limiting transcription factor that can be targeted to enhance regulatory T-cell production. *Proc Natl Acad Sci U S A* 111(26):9579–9584.
- Polansky JK, Kretschmer K, Freyer J, Floess S, Garbe A, Baron U, Olek S, Hamann A, von Boehmer H, Huehn J. 2008. DNA methylation controls Foxp3 gene expression. *Eur J Immunol* 38(6):1654–1663.
- Polansky JK, Schreiber L, Thelemann C, Ludwig L, Kruger M, Baumgras R, Cording S, Floess S, Hamann A, Huehn J. 2010. Methylation matters: binding of Ets-1 to the demethylated Foxp3 gene contributes to the stabilization of Foxp3 expression in regulatory T cells. *J Mol Med (Berl)* 88(10):1029–1040.
- Rafaeli Y, Van Parijs L, Alexander SI, Abbas AK. 2002. Interferon gamma is required for activation-induced death of T lymphocytes. *J Exp Med* 196(7):999–1005.
- Round JL, Mazmanian SK. 2010. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A* 107(27):12204–12209.
- Roychoudhuri R, Hirahara K, Mousavi K, Clever D, Klebanoff CA, Bonelli M, Sciume G, Zare H, Vahedi G, Dema B, Yu Z, Liu H, Takahashi H, Rao M, Muranski P, Crompton JG, Punkosdy G, Bedognetti D, Wang E, Hoffmann V, Rivera J, Marincola FM, Nakamura A, Sartorelli V, Kanno Y, Gattinoni L, Muto A, Igarashi K, O'Shea JJ, Restifo NP. 2013. BACH2 represses effector programs to stabilize T(reg)-mediated immune homeostasis. *Nature* 498(7455):506–510.
- 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. 2008. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28(4):546–558.
- Sage PT, Sharpe AH. 2016. T follicular regulatory cells. *Immunol Rev* 271(1):246–259.
- Sakaguchi S, Yamaguchi T, Nomura T, Ono M. 2008. Regulatory T cells and immune tolerance. *Cell* 133(5):775–787.
- Sanchez Rodriguez R, Pauli ML, Neuhaus IM, Yu SS, Arron ST, Harris HW, Yang SH, Anthony BA, Sverdrup FM, Krow-Lucal E, MacKenzie TC, Johnson DS, Meyer EH, Lohr A, Hsu A, Koo J, Liao W, Gupta R, Debbaneh MG, Butler D, Huynh M, Levin EC, Leon A, Hoffman WY, McGrath MH, Alvarado MD, Ludwig CH, Truong HA, Maurano MM, Gratz IK, Abbas AK, Rosenblum MD. 2014. Memory regulatory T cells reside in human skin. *J Clin Invest* 124(3):1027–1036.
- Sawant DV, Vignali DA. 2014. Once a Treg, always a Treg? *Immunol Rev* 259(1):173–191.
- Sawitzki B, Kingsley CI, Oliveira V, Karim M, Herber M, Wood KJ. 2005. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function in vivo. *J Exp Med* 201(12):1925–1935.
- Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, Pauli ML, Nosbaum A, Gratz IK, Otto M, Moon JJ, Liese J, Abbas AK, Fischbach MA, Rosenblum MD. 2015. A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity* 43(5):1011–1021.
- Schneider-Hohendorf T, Stenner MP, Weidenfeller C, Zozulya AL, Simon OJ, Schwab N, Wiendl H. 2010. Regulatory T cells exhibit enhanced migratory characteristics, a feature impaired in patients with multiple sclerosis. *Eur J Immunol* 40(12):3581–3590.
- Schreiber L, Pietzsch B, Floess S, Farah C, Jansch L, Schmitz I, Huehn J. 2014. The Treg-specific demethylated region stabilizes Foxp3 expression independently of NF-kappaB signaling. *PLoS One* 9(2):e88318.
- Sebzda E, Zou Z, Lee JS, Wang T, Kahn ML. 2008. Transcription factor KLF2 regulates the migration of naive T cells by restricting chemokine receptor expression patterns. *Nat Immunol* 9(3):292–300.
- Shevach EM, McHugh RS, Piccirillo CA, Thornton AM. 2001. Control of T-cell activation by CD4+ CD25+ suppressor T cells. *Immunol Rev* 182:58–67.
- Shevach EM, Thornton AM. 2014. tTregs, pTregs, and iTregs: similarities and differences. *Immunol Rev* 259(1):88–102.
- Shevach EM, Tran DQ, Davidson TS, Andersson J. 2008. The critical contribution of TGF-beta to the induction of Foxp3 expression and regulatory T cell function. *Eur J Immunol* 38(4):915–917.
- Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS. 2013. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* 341(6145):569–573.
- Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F. 2001. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1:4.
- Stephens GL, Andersson J, Shevach EM. 2007. Distinct subsets of FoxP3+ regulatory T cells participate in the control of immune responses. *J Immunol* 178(11):6901–6911.
- Suffia I, Reckling SK, Salay G, Belkaid Y. 2005. A role for CD103 in the retention of CD4+CD25+ Treg and control of Leishmania major infection. *J Immunol* 174(9):5444–5455.
- Talaat IM, Nasr A, Alsulaimani AA, Alghamdi H, Alswat KA, Almalki DM, Abushouk A, Saleh AM, Allam G. 2016. Association between type 1, type 2 cytokines, diabetic auto-antibodies and 25-hydroxyvitamin D in children with type 1 diabetes. *J Endocrinol Invest* 39(12):1425–1434.
- Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, Piccirillo CA, Salomon BL, Bluestone JA. 2008. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity* 28(5):687–697.
- Tanoue T, Atarashi K, Honda K. 2016. Development and maintenance of intestinal regulatory T cells. *Nat Rev Immunol* 16(5):295–309.
- Tran DQ. 2012. TGF-beta: the sword, the wand, and the shield of FOXP3(+) regulatory T cells. *J Mol Cell Biol* 4(1):29–37.
- Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. 2006. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. *Blood* 108(1):253–261.
- van Amelsfort JM, van Roon JA, Noordegraaf M, Jacobs KM, Bijlsma JW, Lafeber FP, Taams LS. 2007. Proinflammatory mediator-induced reversal of CD4+, CD25+ regulatory T cell-mediated suppression in rheumatoid arthritis. *Arthritis Rheum* 56(3):732–742.
- van Loosdregt J, Brunen D, Fleskens V, Pals CE, Lam EW, Coffey PJ. 2011. Rapid temporal control of Foxp3 protein degradation by sirtuin-1. *PLoS One* 6(4):e19047.
- van Loosdregt J, Fleskens V, Fu J, Brenkman AB, Bekker CP, Pals CE, Meerding J, Berkens CR, Barbi J, Grone A, Sijts AJ, Maurice MM, Kalkhoven E, Prakken BJ, Ovaa H, Pan F, Zaiss DM, Coffey PJ. 2013. Stabilization of the transcription factor Foxp3 by the deubiquitinase USP7 increases Treg-cell-suppressive capacity. *Immunity* 39(2):259–271.
- van Loosdregt J, Vercoulen Y, Guichelaar T, Gent YY, Beekman JM, van Beekum O, Brenkman AB, Hijnen DJ, Mutis T, Kalkhoven E, Prakken BJ, Coffey PJ. 2010. Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization. *Blood* 115(5):965–974.

- Vanvalkenburgh J, Albu DI, Bapanpally C, Casanova S, Califano D, Jones DM, Ignatowicz L, Kawamoto S, Fagarasan S, Jenkins NA, Copeland NG, Liu P, Avram D. 2011. Critical role of Bcl11b in suppressor function of T regulatory cells and prevention of inflammatory bowel disease. *J Exp Med* 208(10):2069–2081.
- Venuprasad K, Huang H, Harada Y, Elly C, Subramaniam M, Spelsberg T, Su J, Liu YC. 2008. The E3 ubiquitin ligase Itch regulates expression of transcription factor Foxp3 and airway inflammation by enhancing the function of transcription factor TIEG1. *Nat Immunol* 9(3):245–253.
- Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. 2004. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J Exp Med* 199(7):971–979.
- Visekruna A, Huber M, Hellhund A, Bothur E, Reinhard K, Bollig N, Schmidt N, Joeris T, Lohoff M, Steinhoff U. 2010. c-Rel is crucial for the induction of Foxp3(+) regulatory CD4(+) T cells but not T(H)17 cells. *Eur J Immunol* 40(3):671–676.
- Voo KS, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Littman DR, Liu YJ. 2009. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. *Proc Natl Acad Sci U S A* 106(12):4793–4798.
- Wang Y, Su MA, Wan YY. 2011. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity* 35(3):337–348.
- Weiss JM, Bilate AM, Gobert M, Ding Y, Curotto de Lafaille MA, Parkhurst CN, Xiong H, Dolpady J, Frey AB, Ruocco MG, Yang Y, Floess S, Huehn J, Oh S, Li MO, Niec RE, Rudensky AY, Dustin ML, Littman DR, Lafaille JJ. 2012. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. *J Exp Med* 209(10):1723–1742, S1.
- Wohlfert EA, Grainger JR, Bouladoux N, Konkel JE, Oldenhove G, Ribeiro CH, Hall JA, Yagi R, Naik S, Bhairavabhotla R, Paul WE, Bosselut R, Wei G, Zhao K, Oukka M, Zhu J, Belkaid Y. 2011. GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. *J Clin Invest* 121(11):4503–4515.
- Wu H, Chen Y, Liu H, Xu LL, Teuscher P, Wang S, Lu S, Dent AL. 2016. Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice. *Eur J Immunol* 46(5):1152–1161.
- Xiao Y, Li B, Zhou Z, Hancock WW, Zhang H, Greene MI. 2010. Histone acetyltransferase mediated regulation of FOXP3 acetylation and Treg function. *Curr Opin Immunol* 22(5):583–591.
- Yadav M, Louvet C, Davini D, Gardner JM, Martinez-Llordella M, Bailey-Bucktrout S, Anthony BA, Sverdrup FM, Head R, Kuster DJ, Ruminski P, Weiss D, Von Schack D, Bluestone JA. 2012. Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. *J Exp Med* 209(10):1713–1722, S1–S19.
- Yadav M, Stephan S, Bluestone JA. 2013. Peripherally induced tregs—role in immune homeostasis and autoimmunity. *Front Immunol* 4:232.
- Yang BH, Hagemann S, Mamarelli P, Lauer U, Hoffmann U, Beckstette M, Fohse L, Prinz I, Pezoldt J, Suerbaum S, Sparwasser T, Hamann A, Floess S, Huehn J, Lochner M. 2016. Foxp3(+) T cells expressing RORgammat represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. *Mucosal Immunol* 9(2):444–457.
- Yang Z, Zhang B, Li D, Lv M, Huang C, Shen GX, Huang B. 2010. Mast cells mobilize myeloid-derived suppressor cells and Treg cells in tumor microenvironment via IL-17 pathway in murine hepatocarcinoma model. *PLoS One* 5(1):e8922.
- Yu F, Sharma S, Edwards J, Feigenbaum L, Zhu J. 2015. Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance. *Nat Immunol* 16(2):197–206.
- Yuan X, Cheng G, Malek TR. 2014. The importance of regulatory T-cell heterogeneity in maintaining self-tolerance. *Immunol Rev* 259(1):103–114.
- Zaiss DM, Gause WC, Osborne LC, Artis D. 2015. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. *Immunity* 42(2):216–226.
- Zaiss DM, van Loosdregt J, Gorlani A, Bekker CP, Grone A, Sibilia M, van Bergen en Henegouwen PM, Roovers RC, Coffey PJ, Sijts AJ. 2013. Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity* 38(2):275–284.
- Zhao Y, Guo H, Qiao G, Zucker M, Langdon WY, Zhang J. 2015. E3 Ubiquitin ligase Cbl-b regulates thymic-derived CD4+CD25+ regulatory T cell development by targeting Foxp3 for ubiquitination. *J Immunol* 194(4):1639–1645.
- Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. 2004. 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* 172(9):5213–5221.
- Zheng Y, Chaudhry A, Kas A, deRoos P, Kim JM, Chu TT, Corcoran L, Treuting P, Klein U, Rudensky AY. 2009. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. *Nature* 458(7236):351–356.
- Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. 2007. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 8(9):967–974.
- Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martinez-Llordella M, Ashby M, Nakayama M, Rosenthal W, Bluestone JA. 2009. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat Immunol* 10(9):1000–1007.
- Zhou Y, Lee JY, Lee CM, Cho WK, Kang MJ, Koff JL, Yoon PO, Chae J, Park HO, Elias JA, Lee CG. 2012. Amphiregulin, an epidermal growth factor receptor ligand, plays an essential role in the pathogenesis of transforming growth factor-beta-induced pulmonary fibrosis. *J Biol Chem* 287(50):41991–42000.

Address correspondence to:

Dr. Booki Min

Department of Immunology/NB30

Lerner Research Institute

Cleveland Clinic Foundation

9500 Euclid Avenue

Cleveland, OH 44195

E-mail: minb@ccf.org

Received 13 March 2017/Accepted 7 June 2017