Negative Regulation of T_H17 Differentiation

Mark S. Sundrud and Sergei Koralov

Abstract T_H17 cells and their associated cytokines act on resident parenchymal cells within tissues, thereby setting the stage for chronic inflammation. This realization, together with the finding that T_H17 cell development is reciprocally linked to that of T regulatory (Treg) cells, has revolutionized the way T cell-mediated immune pathology is viewed, and challenged the long-standing binary view of T cell differentiation (i.e., T_H1/T_H2), thereby opening exciting new opportunities to treat autoimmune inflammation. Much effort is now placed on understanding how T_H17 cells are restrained through endogenous mechanisms; the goal being to negatively regulate T_H17 development or function in clinical disease settings. The T_H1 and T_H2 cytokines, IFNγ and IL-4, as well as IL-27 and IL-10, all repress T_H17 cell differentiation. TGFβ signaling, which supports T_H17 differentiation in some contexts, can also strongly induce expression of the signature regulatory T cell transcription factor, Foxp3, which in turn cripples T_H17 differentiation through direct antagonism of the T_H17-specific orphan nuclear receptor RORγt. Emerging evidence also suggests that T_H17 cells are both inherently unstable and uniquely sensitive to metabolic stress. Here, we discuss some of the key molecular features of T_H17 cell development and highlight examples of cell-intrinsic and cell-extrinsic pathways that negatively influence T_H17 differentiation, the latter of which could be exploited for therapeutic application.

M.S. Sundrud (✉)
Department of Pathology, Harvard Medical School, Immune Disease Institute
and the Program in Cellular and Molecular Medicine at Children's Hospital
Boston, Boston, MA, USA
and
Tempero Pharmaceuticals, 200 Technology Square, Suite 602, Cambridge, MA 02139, USA
e-mail: msundrud@temperopharma.com

S. Jiang (ed.), *T_H17 Cells in Health and Disease*,
DOI 10.1007/978-1-4419-9371-7_7,

1 Introduction

T_H17 cells are uniquely equipped among lymphocytes to establish chronic tissue inflammation. As with other helper T cell lineages, their function is derived from the repertoire of pro-inflammatory cytokines they produce. A key feature of T_H17 cell biology is that, unlike T_H1 and T_H2 cells, T_H17 cells produce cytokines that lack classic immuno-regulatory (i.e., helper) function. IL-17A, IL-17F, and IL-22 made by T_H17 cells act primarily on tissue parenchyma, particularly at mucosal surfaces (Awane et al. 1999; Wolk et al. 2004; Zrioual et al. 2008). These cytokines have been implicated in wound healing and the maintenance of barrier immunity, but they also elicit antimicrobial peptide expression and production of pro-inflammatory cytokines and chemokines (Crome et al. 2010; Eyerich et al. 2009; Wolk et al. 2010). In contrast, IFNγ (produced by T_H1 cells) or IL-4, IL-5, and IL-13 (made by T_H2 cells), activate phagocytic and cytolytic immunity, or mobilize antibody responses, respectively (Amsen et al. 2009; Zhu and Paul 2008). The result is that T_H17-infiltrated tissues become a staging ground for chronic and progressive immune-mediated tissue damage, precipitating further recruitment of B and T cells, and well as innate immune cells, neutrophils in particular. T_H17 cells and their associated cytokines are found elevated in many common human auto-immune disorders, though their pioneering role in immune pathology is perhaps best exemplified in experimental auto-immune encephalomyelitis (EAE), a mouse model of multiple sclerosis. In EAE, myelin-reactive T_H17 cells are among the first cells to reach lesions within the central nervous system (Reboldi et al. 2009). Subsequent production of T_H17 cytokines activates resident parenchymal cells and damages integrity of the blood/brain barrier, fostering neutrophil and further lymphocyte recruitment (Reboldi et al. 2009). Thus, the propensity of T_H17 cells to drive propagate tissue inflammation is a distinguishing feature among lymphocytes and has been implicated in the pathogenesis of auto-immunity, fibrosis, and even cancer.

By virtue of their effects on resident epithelial cells, T_H17 cells are also important for mucosal immunity, most notably against fungal and bacterial insults. This role has been elucidated using numerous mouse models of infection (Chung et al. 2003; Huang et al. 2004; Mangan et al. 2006; Zelante et al. 2007; Zheng et al. 2008) and it is further illustrated when considering hyper IgE syndrome (HIES) in humans. HIES patients suffer from a primary immuno-deficiency due to somatic loss-of-function mutations within *Stat3* (Holland et al. 2007; Minegishi et al. 2007). HIES patients have a paucity of circulating T_H17 cells and afflicted individuals present clinically with recurrent fungal and bacterial infections (Holland et al. 2007; Milner et al. 2008; Minegishi et al. 2007). In mice, T_H17 cells have been shown to be key in warding off certain species of bacteria that colonize in the gut, such as *Citrobacter rodentium* (Chung et al. 2003; Dubin and Kolls 2008; Mangan et al. 2006). These results support previous work demonstrating that the vast majority of T_H17 cells in mice at steady-state are present within mucosal layers of the gut, such as the lamina propria of the small intestine and colon (Ivanov et al. 2006, 2009). Development of T_H17 cells in the gut, however, does not require pathogenic infection, but rather

appears to be driven by commensal flora (Ivanov et al. 2008, 2009; Wu et al. 2010). Accordingly, gut-resident T_H17 cells can be abrogated if mice are housed in a germ free environment or treated with antibiotics, such as vancomycin, which target filamentous bacteria (Ivanov et al. 2008).

While there is still some debate as to whether T_H17 cells are inherently "good" or "bad", there is little doubt that the identification of T_H17 cells dramatically expands our understanding of potentially pathogenic T cell subsets. The T_H17 paradigm also opens up new therapeutic opportunities to more specifically treat chronic and auto-immune inflammation whilst leaving intact other aspects of protective immunity (vis-à-vis general immuno-suppressants). To fully exploit such opportunities, we must first understand the endogenous mechanisms that normally keep T_H17 responses in check, and also appreciate how these checkpoints are subverted in context of immune pathology.

Like all T cell responses generated in the periphery, T_H17 cell differentiation is a multi-step process consisting of lineage-commitment, amplification, and stabilization. Underlying each of these steps is a highly coordinated and tightly regulated program of gene expression (Miller and Weinmann 2009; Wilson et al. 2009). T_H17 differentiation begins with cognate interactions between naïve T cells and antigen-presenting cells (APC). Depending on the type of pathogen encountered (and therefore the combination of pathogen-associated molecular pattern receptors engaged), APC can express a variety of co-stimulatory or inhibitory cell surface receptors and secrete inflammatory or regulatory cytokines (Adema 2009; Vance et al. 2009). The engagement of peptide-MHC complexes with cognate T cell antigen receptors (TCR) triggers a number of outside-in signal transduction pathways that mirror antigen dose. This information is integrated, together with the type and quality of co-stimulation and cytokine gradients, in the form of post-translational activation or *de novo* expression of lineage-defining transcription factors (Miller and Weinmann 2009; Sharpe 2009; Sundrud and Nolan 2010). As a general rule, lineage-defining transcription factors such as T-bet (for T_H1 cells), GATA-3 (for T_H2 cells), Foxp3 (for inducible Treg cells), and RORγt (for T_H17 cells) interact with ubiquitously expressed transcription factors to form a dense regulatory network that specifies T cell lineage commitment (Miller and Weinmann 2009; Sundrud and Nolan 2010). Still other soluble factors, including cytokines, hormones, and growth factors, are produced by local bystander cells; they are sensed by activated T cells via an equally dense network of metabolic signaling pathways and can play a profound role in tuning peripheral T cell responses (discussed below).

2 Control of T_H17 Responses by Cytokines and STAT Proteins

Although many factors can influence the outcome of naïve T cell differentiation, cytokines are arguably the most dominant force. Hematopoietic cytokines bind to multimeric receptors comprised of both unique and shared sub-units. Cytokine binding induces rapid clustering and tyrosine phosphorylation of receptors mediated

by receptor-associated Janus kinases (JAKs). Phosphorylated receptors then serve as docking surfaces for the SH2 domains of signal transducer activator of transcription (STAT) proteins, which exist in the cytoplasm of resting cells as latent monomers. Newly recruited STAT proteins are promptly phosphorylated by JAKs, leading to their dimerization and subsequent nuclear translocation. Activated STAT dimers subsequently direct gene transcription through interactions with cognate promoter elements (Fig. 1a). A number of excellent reviews are available discussing the modes and mechanics of JAK/STAT signal transduction (Adamson et al. 2009; Hu and Ivashkiv 2009; O'Shea and Murray 2008). In addition to their initial activation, several mechanisms exist to control the amplitude and duration of STAT signaling following cytokine stimulation. These mechanisms include receptor downregulation, active dephosphorylation or ubiquitination by Protein Inhibitor of Activated Stat (PIAS) proteins, and feedback inhibition by Suppressor of Cytokine Signaling (SOCS) proteins (Fig. 1a). Relatively little is known about the roles of PIAS proteins in immune regulation compared to SOCS proteins, which have been the focus of extensive investigation [reviewed in (Alexander and Hilton 2004; Shuai and Liu 2005)] (discussed below).

That both the initial and sustained activation of STAT proteins are tightly regulated allows cells to respond rapidly to fluctuations in extra-cellular cytokine concentrations. Mammals have 7 STAT proteins (STAT1-4, STAT5a, STAT5b, STAT6), and these proteins and their associated regulatory networks ultimately determine the fate of T cell differentiation (Adamson et al. 2009; Alexander and Hilton 2004; O'Shea and Murray 2008). STAT proteins play unique and determinant roles in T cell fate determinism. STAT1 and STAT4 are critical for T_H1 responses and the upregulation of T-bet, whereas STAT6 is integral to IL-4-mediated T_H2 differentiation and induction of GATA-3 expression (Adamson et al. 2009; Amsen et al. 2009; Elo et al. 2010; Schulz et al. 2009). Moreover, STAT5 activated downstream of IL-2 is essential for Foxp3 expression in Treg cells, and STAT3 is a key transcriptional regulator of T_H17 differentiation (Adamson et al. 2009; Burchill et al. 2007; Laurence et al. 2007; O'Shea and Murray 2008; Passerini et al. 2008; Wei et al. 2008).

Though many cytokines can promote its activation, IL-6 is the principle activator of STAT3 in naïve T cells (Chen et al. 2006; Kishimoto 2005; Nishihara et al. 2007) (Fig. 1a). The functional IL-6 receptor is expressed by a variety of cell types in most tissues, and is comprised of two sub-units; a unique cytokine-binding chain (IL-6Rα), and the IL-6 signal transducer (IL6ST; a.k.a. gp130), which is shared between multiple cytokine receptors, and (as its name implies) mediates IL-6 dependent signal transduction (Kishimoto 2005; Nishihara et al. 2007; Wang et al. 2009). IL-6 is a principle regulator of T_H17 differentiation in vitro, and T_H17 responses in vivo. Genetic ablation of IL-6 or IL-6R in mice, or anti-IL-6R monoclonal antibody treatment impairs T_H17 cell development and is protective in a variety of T_H17-driven disease models (Serada et al. 2008; Jones et al. 2010; Okuda et al. 1998).

Activation of naïve T cells in T_H17-polarizing conditions (i.e., IL-6 plus TGFβ) leads to STAT3-dependent upregulation of both *Il21* and *Il23r* gene expression (Yang et al. 2007; Zhou et al. 2007). Subsequent IL-21 and IL-23 production by activated T_H17 cells and APC sustains STAT3 phosphorylation (Kwon et al. 2009;

McGeachy et al. 2009), thereby maintaining its activity in developing T_H17 cells. Persistent STAT3 signaling may be critical to allow for cooperation with the T_H17-specific orphan nuclear receptors RORγt and RORα, which are STAT3 target genes themselves, in remodeling of the *Il17a/Il17f* locus and driving high-level IL-17 expression (Yang et al. 2007; Zhou et al. 2007).

TGFβ, the other classical T_H17-inducing cytokine, has been suggested to exert its effects, at least in part, by enhancing tyrosine phosphorylation of STAT3 (Qin et al. 2009) (Fig. 1a). The precise mechanism remains unclear, but TGFβ-dependent regulation of STAT3 activation is likely indirect, as the type 1 TGFβ receptor (TGFβR1) is a serine/threonine kinase. TGFβ also promotes T_H17 responses via negative regulation of IFNγ and IL-4 signaling (Das et al. 2009; Li et al. 2006), both of which inhibit T_H17 differentiation (Park et al. 2005) (Fig. 1a). TGFβ likely utilizes multiple downstream signaling pathways to control both T_H17 differentiation and Foxp3 expression (Derynck and Zhang 2003; Martinez et al. 2009; Yang et al. 2008b). Further understanding of how these distinct signaling modules control T cell differentiation may shed new light on the diverse biological activities of TGFβ in vivo.

STAT3 directly *trans*-activates most of the T_H17 signature genes, including *Il17a*, *Il17f*, *Rorc* (RORγt), and *Rora* (RORα) (Durant et al. 2010; Nishihara et al. 2007; Yang et al. 2007, 2008c; Zhou et al. 2007). As noted above, synergy between STAT3 and RORγt may be critical for T_H17 differentiation; whereas STAT3 activation does not induce high-level IL-17 expression in the absence of RORγt, and ectopic expression of RORγt is likewise insufficient to drive T_H17 differentiation in $Stat3^{-/-}$ T cells (Yang et al. 2007, 2008c; Zhou et al. 2007).

In addition to positively regulating T_H17 differentiation, STAT3 prevents developing T_H17 cells from diverging into the inducible T regulatory (iTreg) lineage. IL-6, IL-21, and IL-23 each inhibit TGFβ-induced expression of Foxp3, and do so in a STAT3-dependent manner (Bettelli et al. 2006; Korn et al. 2008; Yang et al. 2007; Zhou et al. 2007, 2008). STAT3 may also modulate the function of Foxp3 protein. Whereas Foxp3 has been shown to directly bind and inhibit the transcriptional activity of both RORγt and RORα, RORγt function is restored in the context of Foxp3 expression by stimulation of cells with STAT3-activating cytokines (Ichiyama et al. 2008; Martinez et al. 2009; Samanta et al. 2008; Zhang et al. 2008; Zhou et al. 2008). Thus, mechanisms underlying STAT3-mediated interference with Foxp3 expression or function may lead to novel therapeutic approaches to inhibit T_H17 differentiation, while enhancing iTreg cell development or function.

STAT3 also maintains the pro-inflammatory function of differentiated T_H17 cells. Effector/memory T_H17 cells have been shown to be inherently unstable both in vitro and in vivo (Janke et al. 2010; Martin-Orozco et al. 2009; McGeachy et al. 2007); likely requiring continued inflammatory cytokine signaling, particularly through IL-23 and IL-1β, to maintain IL-17 expression. In vitro, T_H17 cells differentiated with TGFβ and IL-6 to produce both IL-17 and the anti-inflammatory cytokine IL-10 (McGeachy et al. 2007). Transfer of in vitro-differentiated T_H17 cells generates inflammatory lesions, but disease is often associated with a phenotypic switch of the transferred cells from a T_H17 to a T_H1 phenotype (Martin-Orozco et al. 2009). Restimulation of previously differentiated T_H17 cells in vitro with TGFβ and IL-6

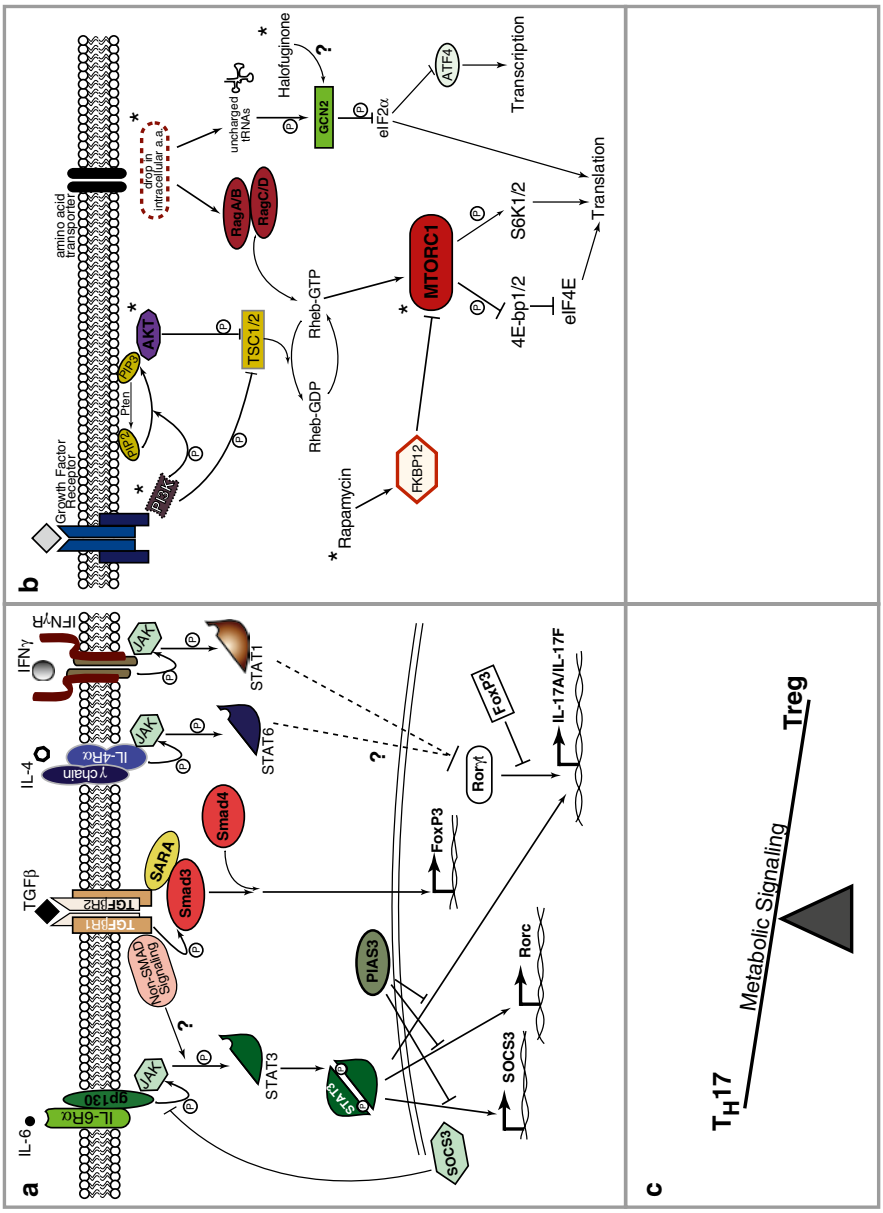
a
IL-6
IL-6Rα
gp130
JAK
STAT3
STAT3
SOCS3
SOCS3
Rorc
PIAS3
TGFβ
TGFβR2
TGFβR1
Non-SMAD Signaling
SARA
Smad3
Smad4
FoxP3
IL-4
γ chain
IL-4Rα
JAK
STAT6
IFNγ
IFNγR
JAK
STAT1
?
?
Rorγt
FoxP3
IL-17A/IL-17F
b
Growth Factor Receptor
PI3K
PIP3
Pten
PIP2
AKT
TSC1/2
Rheb-GTP
Rheb-GDP
Rapamycin
FKBP12
MTORC1
4E-bp1/2
eIF4E
S6K1/2
Translation
amino acid transporter
drop in intracellular a.a
RagA/B
RagC/D
uncharged tRNAs
GCN2
eIF2α
ATF4
Transcription
Halofuginone
?
c
TH17
Metabolic Signaling
Treg

Fig. 1 Regulation of Th17 differentiation by cytokine and metabolic signaling pathways. (**a**) Cross-regulation of cytokine signaling pathways during T_H17 differentiation. Stimulation of activated T cells with IL-6 induces JAK activation, and subsequent recruitment, phosphorylation, and dimerization of STAT3. Active STAT3 dimers translocate to the nucleus where they *trans*-activate the expression of T_H17-signature genes (Rorc, *Il17a*, *Il17f*) as well as the feedback inhibitor of STAT3 signaling, SOCS3. SOCS3 binds the intracellular domain of gp130 and promotes its degradation. PIAS3 physically interacts with STAT3 and blocks its function by promoting tyrosinedephosphorylation and nuclear export. Co-stimulation of T cells with TGFβ leads to transcription of *FoxP3* in a Smad3/4-dependent pathway, which in turn interacts with and blocks Rorγt function. Smad3/4-independent TGFβ signaling may enhance IL-6-dependent phosphorylation of STAT3, possibly through repression of *Socs3* gene expression. IL-4 and IFNγ signaling inhibits *Rorc* upregulation and T_H17 differentiation. (**b**) Metabolic pathways and signal transduction. Activation of PI-3K downstream of growth factor stimulation promotes generation of the lipid second messenger PIP_3, which is regulated by the phosphatase Pten. Both PI-3K and Akt phosphorylate and inhibit the TSC1/2 complex, which in turn inhibits the small GTPase Rheb through its intrinsic GAP activity. Active (GTP-bound) Rheb, is required for the kinase activity of mTORC1 that drives cell metabolism in nutrient-rich conditions via phosphorylation of 4E-bp1/2 and S6K1. Amino acid deprivation, in contrast to nutrient-responsive pathways, inactivates the Rag family of small GTPases, which are necessary for Rheb-mediated activation of mTORC1. Amino acid limitation also leads to unaminoacylated (i.e., uncharged) tRNAs, which are non-discriminately recognized by the eIF2α kinase GCN2. Following tRNA binding, GCN2 undergoes autophosphorylation, subsequently activating the amino acid starvation response (AAR) pathway. Biochemical hallmarks of AAR pathway activation include phosphorylation of eIF2α and induction of ATF4 protein expression. Whereas inhibition of eIF2α reduces global protein synthesis, ATF4 activates a compensatory program of gene expression that confers relative stress resistance to cells. Furthermore, the macrolide rapamycin binds to and destabilizes mTORC1 in complex with its cellular ligand FKBP12. The small molecule halofuginone stimulates GCN2-dependent activation of the AAR. Asterisks indicate molecules or proteins shown to regulate the reciprocal differentiation of T_H17 and iTreg cells. (**c**) Metabolic stress promotes T cell tolerance by inhibiting T_H17 differentiation and enhancing iTreg cell development

for a second time results in cells that produce IL-10 but not IL-17 (McGeachy et al. 2007). This may represent the fact that IL-6R expression is down-regulated on T cells following activation (Betz and Muller 1998). In contrast, secondary stimulation of T_H17 cells with IL-23 confers stable expression of IL-17 and down-regulation of IL-10 (McGeachy et al. 2007). Upon transfer into mice, IL-23-stimulated T_H17 cells promote tissue inflammation, whereas T_H17 cells repeatedly stimulated with TGFβ and IL-6 actually protect against auto-immune tissue damage (McGeachy et al. 2007). Given that IL-23 is potent inducer of STAT3 activation, these findings collectively indicate that STAT3 initiates, amplifies, and stabilizes the pro-inflammatory function of T_H17 cells (Ahern et al. 2010; Korn et al. 2009).

Given these key roles of STAT3 in dictating T_H17 differentiation and function, a growing body of literature has been dedicated to investigating the regulation of STAT3 in T cells. As with all STAT proteins, STAT3 is controlled by the biology of the cytokine receptors that induce its activity. In the case of IL-6, both IL-6Rα and gp130 can be expressed either on the cell surface, or secreted as truncated proteins lacking their trans-membrane domains (Kishimoto 2005; Rose-John et al. 2006). Soluble IL-6Rα (sIL-6Rα) binds free extracellular IL-6; this complex in turn interacts with cell surface-expressed gp130 to initiate signaling. This mode of IL-6 signal transduction is termed trans-IL-6 signaling and has been implicated in diverse pathophysiologies such as auto-immunity, cancer, and tissue fibrosis (Igaz et al. 2000; Kishimoto 2005; McLoughlin et al. 2005; Nowell et al. 2003). Trans-IL-6 signaling generally amplifies IL-6 signaling by rendering otherwise non-responsive cells (i.e., cells that express gp130 but not IL-6Rα), responsive to IL-6:sIL6Rα complexes. Potentially, as a counter-balance to the generally pro-inflammatory actions of trans-IL-6 signaling, gp130 can also be expressed in a soluble form (sgp130), either via proteolytic cleavage or alternative splicing (Diamant et al. 1997; Graf et al. 2008). In contrast to soluble IL-6Rα, however, soluble gp130 is antagonistic in nature, binding to extracellular IL-6:sIL-6Rα complexes and preventing subsequent signaling through cell surface-expressed gp130 (Rose-John et al. 2006). Because it cannot bind to soluble IL-6 in the absence of sIL-6Rα, sgp130 only blocks trans-IL-6 signaling, not IL-6 signaling instigated by the binding of free IL-6 to cell surface receptors. Both sIL-6Rα and sgp130 are commonly elevated in the serum of auto-immune patients, as well as in affected tissue (Dayer and Choy 2010; Simon et al. 2008). In mice, recombinant sgp130 inhibits T_H17 differentiation and associated tissue inflammation via inhibition of STAT3 activation (Nowell et al. 2009). However, because gp130 is a shared component of all IL-6 family cytokine receptors, sgp130 is predicted to have broad effects on in vivo (Muller-Newen 2003).

The other STAT3-activating cytokines relevant for T_H17 cells, namely IL-21 and IL-23, signal through unique cell surface receptors (IL-21R and IL-23R). The IL-21R is comprised of IL-21Rα and the shared IL-2RG (a.k.a., gamma-common (γ_c)) (Rochman et al. 2009). IL-23 receptors consist of IL-23R and IL-12Rβ1, the latter of which is shared with the IL-12 receptor complex (Kastelein et al. 2007). In contrast to IL-6 receptors, the IL-21 and IL-23 receptors are expressed exclusively as transmembrane proteins and are regulated via gene expression. The IL-21 and IL-23 receptors are highly expressed on developing T_H17 cells (Yang et al. 2008a; Zhou et al. 2007). IL-23R transcripts are abundant in mouse and human IL-17-producing

memory T cells as well as some myeloid cells that express IL-17 in response to IL-23 stimulation (Awasthi et al. 2009). Temporal regulation of the IL-6, IL-21, and IL-23 receptors, rather than utilization of distinct signaling pathways, may explain why these cytokines seem to play non-redundant roles in initiating, amplifying, and stabilizing T_H17 cell development; each cytokine receptor may be used by T_H17 cells to maintain STAT3 activation at distinct stages in their maturation.

In addition to cytokine receptors, physiological STAT3 activation is under the cell-intrinsic control of SOCS proteins (Alexander and Hilton 2004; O'Shea and Murray 2008) (Fig. 1a). Eight SOCS proteins exist in mammals (SOCS1-7, and CIS) and their gene expression is directly *trans*-activated by STAT proteins themselves; they can utilize distinct biochemical mechanisms to inhibit STAT signaling. Inhibitory mechanisms of SOCS proteins include: (1) competing with STAT proteins for cytokine receptor docking, (2) serving as pseuosubstrates for JAK phosphorylation, and (3) degrading cytokine receptors or JAKs directly via recruitment of the E3 ubiquitin ligase complex (Alexander and Hilton 2004; O'Shea and Murray 2008). Even though SOCS proteins are generally conserved, each family member displays remarkably specialized functions in T cell biology, with SOCS3 specifically regulating STAT3-driven responses (Chen et al. 2006).

Germline deletion of SOCS3 in mice, or transgenic overexpression at an embryonic stage, is lethal (Marine et al. 1999). Conditional deletion of SOCS3 in T cells, however, is viable, and these mice have grossly normal T cell compartments (Chen et al. 2006). T cells lacking SOCS3 display longer and more exaggerated STAT3 phosphorylation kinetics in response to cytokine stimulation (Chen et al. 2006). The increased activation kinetics of STAT3 in the context of SOCS3 deficiency is accompanied by increases in STAT3 promoter occupancy (Chen et al. 2006). In contrast, cytokine-mediated phosphorylation of other STAT family members (e.g., STAT1, STAT4, STAT5, STAT6) is largely unaffected by SOCS3 ablation (Chen et al. 2006). SOCS3 has been suggested to regulate STAT3 activation via binding to gp130 and inducing its degradation via an ubiquitin- and proteosome-dependent pathway (Alexander and Hilton 2004; O'Shea and Murray 2008). As such, transgenic expression of a mutant gp130 protein (Y759F) in mice that cannot interact with SOCS3 exacerbates T_H17 cell development in vitro, similar to mice lacking SOCS3 (Atsumi et al. 2002; Nishihara et al. 2007). Naïve T cells isolated from gp130 ^{Y759F} transgenic mice activated in T_H17-polarizing conditions also express less Foxp3, and the mice themselves tend to develop spontaneous arthritis (Nishihara et al. 2007). The interplay between SOCS3, STAT3, and STAT3-activating receptors thus represents a seminal pathway in the development of T_H17 cells (Chen et al. 2006; McLoughlin et al. 2005; O'Shea and Murray 2008; Qin et al. 2009).

In addition to the SOCS, the PIAS protein family contains intrinsic phosphatase and E3-ubiquitin ligase activity and has also been shown to negatively regulate STAT signaling (Shuai and Liu 2005). PIAS1 acutely regulates innate inflammatory responses through repression of STAT1 activity, whereas PIAS3 targets STAT3 for dephosphorylation (Liu et al. 2004; Dabir et al. 2009) (Fig. 1a). PIAS3, like all PIAS family members, is phosphorylated in response to inflammation; it interacts and translocates with tyrosine-phosporylated STAT3 to the nucleus before inducing dephosphorylation and nuclear export of STAT3 (Dabir et al. 2009). The STAT3/PIAS3

complex can be further regulated, by TRIM8 for example, which relieves PIAS3-mediated repression of STAT3 signaling when overexpressed in cells (Okumura et al. 2010). In addition to promoting STAT dephosphorylation, some PIAS proteins function primarily by inducing ubiquitin-dependent proteosomal degradation of interacting molecules (Liu et al. 2004; Shuai and Liu 2005). PIAS proteins can also regulate non-STAT transcription factors, such as p53 and CBP/p300, and can bind the small ubiquitin-like protein SUMO (Tan et al. 2010). Whether PIAS3 regulates STAT3-driven T_H17 differentiation directly remains to be determined.

Each mechanism of STAT3 regulation discussed above, with the exception of PIAS3, has lent key insight into the biology of T_H17 cells. However, an important concept to remember is that the STAT3 signaling pathway does not exist in a vacuum. T cells activated in vivo are simultaneously bombarded with an array of cytokines, each at gradient concentrations. T cells exposed to IL-6, therefore, are simultaneously confronted by a broader milieu of cytokines in the local microenvironment; any or all of which may augment or repress IL-6R signaling. As touched on earlier, TGFβ signaling can both synergize with IL-6 to enhance STAT3 phosphorylation (Qin et al. 2009), and inhibit RORγt activity via induction of Foxp3 (Martinez et al. 2009; Zhang et al. 2008; Zhou et al. 2008). Still other examples of cytokines that negatively regulate T_H17 differentiation are the T_H1- and T_H2-associated cytokines, IFNγ and IL-4, respectively (Park et al. 2005) (Fig. 1a). IL-2 in the mouse also inhibits T_H17 differentiation (Laurence et al. 2007), though it remains unclear if this is through enhancing Foxp3 expression, or through more direct regulation of STAT3 signaling. Regardless, the inhibitory role of IL-2 during T_H17 differentiation is confounded by the fact that IL-2 is paradoxically *required* for the differentiation of human T_H17 cells (Manel et al. 2008). Lastly, IL-27, which is produced by activated APC, is another potent inhibitor of T_H17 differentiation (Diveu et al. 2009; Murugaiyan et al. 2009). However, IL-27 does not affect the pro-inflammatory function of established T_H17 cells (El-behi et al. 2009). In general, little is known as to how these cytokines repress T_H17 differentiation, although it is interesting to note that IL-27, like IL-6, IL-21, and IL-23, also induces the phosphorylation of STAT3 (Xu et al. 2009). However, STAT3 activation downstream of IL-27 fails to induce IL-17 expression, and instead leads to the differentiation of IL-10-producing type 1 regulatory (Tr1) cells (Apetoh et al. 2010; Xu et al. 2009). How STAT3 functions in a context-dependent manner to prompt either pro- or anti-inflammatory gene expression in T cells remains an open question.

3 Dynamic Transcription Factor Interactions Regulating T_H17 Cell Development

T_H17 differentiation should be viewed as a continuum, with STAT3 representing only the first of many nodal points that ultimately gives rise to a pro-inflammatory T_H17 cell. Chronologically, the second step in the, 'how to become a T_H17 cell' manual is the induction of a transcription factor network conducive for T_H17

differentiation that also prevents diversion into alternative effector or regulatory lineages. At the center of the T_H17 transcriptional regulatory web is the lineage-defining orphan nuclear receptor RORγt (Chen et al. 2007; Zhou and Littman 2009). The notion of RORγt as a lineage-defining transcription factor for T_H17 cells was first illustrated by gene expression profiling showing its selective expressed in T_H17 cells vis-à-vis T_H1, T_H2, or iTreg cells (Ivanov et al. 2006). RORγt was also demonstrated to be necessary for the in vitro differentiation of mouse and human naïve T cells into T_H17 cells (Ivanov et al. 2006; Manel et al. 2008; Zhou et al. 2007). In vivo, nearly all $CD4^+$ T_H17 cells present in the intestinal lamina propria express RORγt (Ivanov et al. 2006). Furthermore, T cells from *Rorc*$^{-/-}$ mice fail to generate T_H17 cells upon antigen challenge, and these mice are largely resistant to EAE (Ivanov et al. 2006; Yang et al. 2008c). The related orphan nuclear receptor RORα (specifically Rora4) is also expressed in T_H17 cells; it is responsible for residual T_H17 differentiation observed in the absence of RORγt (Yang et al. 2008c). Collectively, this data demonstrates a pivotal role for RORγt in the developmental program of both mouse and human T_H17 cells.

RORγt, like other lineage-defining transcription factors in T cells (e.g., T-bet, Foxp3), is not expressed in conventional (i.e., non-Treg) naïve T cells, but is rapidly upregulated under T_H17-priming conditions (Ivanov et al. 2006; Yang et al. 2008c; Zhou et al. 2007) (Fig. 1a). As with STAT3, both the initial induction and subsequent function of RORγt are subject to strict regulation. Whereas STAT3 *trans*-activates *Rorc* gene expression, PPARγ, a broad-acting anti-inflammatory nuclear receptor, inhibits *Rorc* expression (Klotz et al. 2009). PPARγ does not bind directly to the *Rorc* promoter, but instead prevents clearance of silencing mediator for retinoid and thyroid hormone receptors (SMRT) from the *Rorc* promoter upon T cell activation (Klotz et al. 2009). Accordingly, mice lacking PPARγ display enhanced *Rorc* expression and increased T_H17 differentiation, whereas pharmacologic activation of PPARγ represses *Rorc* gene expression in wild-type, but not PPAR$^{-/-}$ T cells (Klotz et al. 2009).

MicroRNAs have also been implicated in controlling RORγt expression (Wei and Pei 2010). miRNA-326 is highly expressed in human and mouse T_H17 cells, and is elevated in inflamed CNS tissue from multiple sclerosis patients (Du et al. 2009). Regulation of RORγt expression by mir-326 is indirect, presumably functioning through its bio-informatically-predicted target, the transcription factor Ets-1 (Du et al. 2009). The mechanism by which Ets-1 regulates *Rorc* expression and T_H17 differentiation has yet to be clarified. In a broader sense, the regulation of T_H17 differentiation by miRNAs remains open for exploration (Wei and Pei 2010). Indeed, most of the signaling molecules discussed herein have conserved miRNA binding sites in their 3′ UTRs; identification of T_H17-regulating miRNAs, and the function of their target molecules in this context could have broad therapeutic implications.

Once expressed, RORγt interacts with a host of transcription factors (physically or functionally) to control T_H17 lineage commitment and specify patterns of cytokine expression within T_H17 cells. Most notable of the RORγt-interacting partners is Foxp3 (Zhou et al. 2008). This transcriptional complex is particularly

intriguing because both RORγt and Foxp3 are lineage-defining transcription factors that are co-regulated by TGFβ signaling. Other examples of transcription factor interactions critical for T cell fate determinism include the interaction between T-bet and GATA-3, which regulates the T_H1/T_H2 balance, and the Foxp3/NFAT interaction that guides Treg cell development (Hwang et al. 2005; Wu et al. 2006). Several groups have now confirmed that Foxp3 directly interacts with RORγt (also RORα) via co-immuno-precipitation experiments (Du et al. 2008; Ichiyama et al. 2008; Yang et al. 2008b; Zhang et al. 2008). Such steady-state interactions between transcription factors are relatively uncommon, as many ternary complexes are only stabilized upon DNA binding (e.g., Foxp3:NFAT, NFAT:AP-1) (Jain et al. 1993; Wu et al. 2006). An N-terminal portion of Foxp3 encoded by exon 2, and the ligand-binding domain of RORγt mediate the Foxp3/RORγt interaction (Du et al. 2008; Ichiyama et al. 2008; Yang et al. 2008b; Zhou et al. 2008).

Functionally, the Foxp3/RORγt prevents RORγt transcriptional activity on target genes, either in primary T cells or in transiently-transfected cell lines (Du et al. 2008; Ichiyama et al. 2008; Yang et al. 2008b; Zhou et al. 2008) (Fig. 1a). While some details remain unclear, exon 2 of Foxp3 contains a conserved LQALL motif, which matches the LxxLL core motif used by transcriptional co-activators (NCoA) or co-repressors (NCoR) to bind to nuclear receptors such as RORγt and RORα (Du et al. 2008; Yang et al. 2008b). Thus, it is conceivable that Foxp3 passively interferes with RORγt transcriptional activity by competing with necessary co-factors for RORγt binding. However, this model of Foxp3-mediated RORγt antagonism is not without caveats. One perplexing factor is that Foxp3-mediated inhibition of RORγt function is relieved by pro-inflammatory cytokine stimulation, notably IL-6 or IL-23 (Yang et al. 2008b; Zhou et al. 2008). As detailed above, these cytokines are indispensible for STAT3-mediated induction of RORγt expression. How Foxp3 antagonizes RORγt function in vivo if these factors are only co-expressed in the context of inflammation remains to be elucidated.

TGFβ signaling may also utilize additional mechanisms to antagonize RORγt function. One specific example is the transcription factor Smad3, which is recruited to and directly phosphorylated by active TGFβ receptors. Smad3, particularly phosphorylated Smad3, has been shown capable of directly interacting with RORγt (Martinez et al. 2009). Similar to the Foxp3/RORγt interaction, Smad3/RORγt complexes antagonize RORγt transcriptional activity on transfected reporter constructs. In line with this model, conditional deletion of Smad3 in T cells leads to enhanced T_H17 differentiation and impaired Foxp3 upregulation (Martinez et al. 2009). Whether the T_H17-enhancing effects of Smad3 deficiency is due to increased RORγt activity or is secondary to impaired Foxp3 upregulation is presently unclear. Nonetheless, the interactions between RORγt, Foxp3, and Smad3 represent novel putative mechanisms by which TGFβ maintains peripheral T cell tolerance.

RORγt also interacts with a broader network of more ubiquitous transcription factors to coordinate T_H17 differentiation and to specify patterns of cytokine gene expression [reviewed in (Sundrud and Nolan 2010)]. A number of transcription factors have been shown to play key synergistic roles downstream of RORγt to control T_H17 differentiation. Notable among these 'secondary' T_H17 transcription factors are IRF4, the AP-1 transcription factor BATF, and STAT3 itself (Brustle et al. 2007;

Huber et al. 2008; Schraml et al. 2009; Yang et al. 2007; Zhou et al. 2007). All of these factors share two key functional features within the T_H17 framework: (1) unlike RORγt, they are broadly expressed in multiple T cell lineages, and (2) ectopic expression of RORγt does not compensate for their absence. With regard to broad expression in T cell lineages, each of these factors also regulates non-T_H17 T cell functions. For example, STAT3 regulates development of $CXCR5^+$ T follicular helper (Tfh) cells, as well as IL-10-producing Tr1 cells (Apetoh et al. 2010; Nurieva et al. 2008; Vogelzang et al. 2008). Likewise, IRF4 is important for the development of both T_H2, and IL-9-secreting 'T_H9' cells (Rengarajan et al. 2002; Staudt et al. 2010). BATF is more generally involved in adaptive immunity, regulating both T and B cell function (Betz et al. 2010). Thus, IRF4, BATF, and STAT3 constitute an integral network of secondary transcription factors usurped by RORγt to specify T_H17 differentiation. These findings indicate that transcriptional regulation of T_H17 differentiation is fundamentally synergistic, and that interactions between transcription factors (be they functional or physical) are key for achieving the diversity seen in adaptive immune responses.

All of the T_H17 regulatory mechanisms described above are defined by their influence on IL-17 expression. Yet still other transcription factors form a more peripheral regulatory network during T_H17 differentiation, specifying the pattern of cytokine gene expression in T_H17 cells without regulating IL-17 expression per se. The aryl hydrocarbon receptor (AHR) and c-maf are examples of transcription factors that regulate auxiliary cytokine expression in T_H17 cells. AHR is a cellular receptor for a variety of synthetic and naturally-occurring aromatic hydrocarbons (Esser et al. 2009). More recently, AHR has been found to be an important regulator of IL-22 expression in T_H17 cells (Alam et al. 2010; Ramirez et al. 2010; Veldhoen et al. 2008). Given the broad role of IL-22 in cutaneous biology (Wolk et al. 2004, 2010), AHR may play a more specialized role in tuning the functions of skin-homing T_H17 cells. AHR-deficient T cells can still generate IL-17A-producing T_H17 cells, albeit with slightly reduced efficiency compared with wild-type cells. However, IL-22 expression in $AHR^{-/-}$ T cells is profoundly impaired (Alam et al. 2010; Ramirez et al. 2010; Veldhoen et al. 2008). Further illustration is provided by experiments in which either RORγt or AHR are retrovirally overexpressed in T cells; RORγt strongly induces IL-17 expression without much affect on IL-22, whereas AHR expression in T cells has the opposite effect, inducing IL-22 but not IL-17 expression (Alam et al. 2010; Ramirez et al. 2010; Veldhoen et al. 2008).

C-maf also regulates auxiliary cytokine expression in T_H17 cells. C-maf was originally characterized as a T_H2-promoting transcription factor due to its direct regulation of the *Il4* locus (Kim et al. 1999). More recently, however, c-maf has been shown to be highly expressed in T_H17 cells, again in a STAT3-dependent manner, and to activate the expression of IL-10 (Xu et al. 2009). IL-10 displays potent anti-inflammatory properties in vivo, even in instances where cells are co-expressing IL-17 (Apetoh et al. 2010; McGeachy et al. 2007). Thus, AHR and c-maf have little impact on classical T_H17 differentiation (as defined by expression of IL-17), but rather serve to further specify whether or not T_H17 cells will make additional pro- or anti-inflammatory cytokines. From the parenchymal perspective, factors that dictate the balance of pro- and anti-inflammatory cytokine expression in

T_H17 cells are equally as important as those that regulate a single 'signature' cytokine. Indeed, a more holistic view of T cell responses could greatly impact the way we think about inflammation and immunity.

4 Tuning T Cell Responses by Metabolic Signaling Pathways

Although cytokines get the most publicity, they are not the only means of immunoregulation. Nutrients (i.e., vitamins, hormones, growth factors) and amino acids pervade all tissues, and their local concentrations are constantly read-out by cells via conserved metabolic signaling pathways (Fig. 1b). Metabolic signaling has historically been considered a means of ensuring that surrounding tissues have the nutrients necessary to support cellular function; inducing a state of cellular hyporesponsiveness to conserve nutrients and maintain homeostasis when nutrient starvation (i.e., metabolic stress) occurs (Glick et al. 2010; Lin et al. 2008; Reiling and Sabatini 2006). More nuanced effects of metabolic pathways on cell physiology, however, have begun to emerge. It is now clear that more modest fluctuations in nutrient and amino acid levels (as opposed to complete starvation) can shape, rather than abort, cellular responses to growth and differentiation cues (Delgoffe and Powell 2009; Maciver et al. 2008). Moreover, numerous plant and microbe-derived natural products target evolutionarily-conserved metabolic signaling pathways and these molecules have proven to be invaluable tools for dissecting cell physiology, while also providing a framework for rational drug design (Haustedt et al. 2006; Li and Vederas 2009).

A historical focus of cancer research, metabolic signaling within the immune system has emerged as a broad and relatively unexplored signal transduction network that has profound impacts on the differentiation and effector function of T cells. During the last 15 years, investigations into the effects of calorie restriction (CR) and small molecules such as the fungal macrolide rapamycin have led to the general principle that metabolic stress promotes T cell tolerance (Barshes et al. 2004; Delgoffe and Powell 2009; Piccio et al. 2008; Wu and Mohan 2009). More recent studies, particularly those involving gene targeting in mice, have taken this concept a step further, showing that metabolic signaling pathways, particularly those involved in amino acid and energy homeostasis, influence both T_H17 and Treg cells in a cell-intrinsic manner (Delgoffe and Powell 2009; Mellor and Munn 2008; Webb et al. 2008; Wu and Mohan 2009) (Fig. 1c).

5 mTORC1 in Control of T Cell Differentiation

One of the best-characterized metabolic signaling pathways that exert broad tolerogenic effects on T cell function is the mammalian target of rapamycin (mTOR) pathway [reviewed in (Huang and Manning 2008; Ma and Blenis 2009; Reiling and

Sabatini 2006)] (Fig. 1b). A serine/threonine kinase belonging to the phosphatidylinositol 3-kinase (PI-3K) family, mTOR can be found in two distinct macromolecular complexes at steady-state; mTOR complex 1 (mTORC1), which is comprised of mTOR bound to the regulatory proteins Raptor, mLST8/GβL, PRAS40, and DEPTOR, and mTORC2, in which mTOR is bound to Rictor, GβL, and mSIN1 (Huang and Manning 2008; Laplante and Sabatini 2009; Ma and Blenis 2009; Sarbassov et al. 2004). Whereas mTORC1 is a key cellular nutrient sensor that is directly regulated by intracellular amino acids, ATP, and growth factor signaling, mTORC2 is not directly regulated by nutrients. The two complexes also differ in their substrate specificity and their sensitivity to rapamycin (Laplante and Sabatini 2009; Reiling and Sabatini 2006).

When nutrients and amino acid levels are at a surplus, mTORC1 is maintained as an active kinase, phosphorylating substrates in a raptor-dependent manner (Kim et al. 2002). Substrates of mTORC1 include eIF4E-bp1/2 and S6K1/2 (Proud 2004; Schalm and Blenis 2002). These substrates control metabolic processes such as ribosome biogenesis, cap-dependent translation, and energy production through the regulation of glycolytic enzymes (Ma and Blenis 2009; Reiling and Sabatini 2006). Nutrient starvation, limited amino acid availability, or exposure to rapamycin, all inactivate mTORC1 by causing its disassociation with Raptor, and all have been demonstrated to reduce T_H17 differentiation and enhance Treg differentiation or function (Kopf et al. 2007; Locke et al. 2009; Sundrud et al. 2009) (Fig. 1b).

Importantly, distinct types of metabolic stress negatively regulate mTORC1 via unique upstream mechanisms (Huang and Manning 2008; Laplante and Sabatini 2009). Nutrients and amino acids control mTORC1 via the small GTPase Rheb, which activates mTORC1 when in the GTP-bound state (Huang and Manning 2008; Ma and Blenis 2009; Reiling and Sabatini 2006). Rapamycin bypasses Rheb by binding to the immunophilin FKBP12, which directly destabilizes mTORC1 (Yip et al. 2010). Furthermore, nutrients regulate Rheb activity via tuberous sclerosis complex (TSC)1/2, which are also called hamartin and tuberin. TSC1/2 inhibit Rheb function through its intrinsic GTPase activating protein (GAP) activity (Kwiatkowski 2003; Manning et al. 2005). Phosphorylation of the TSC1/2 complex by nutrient and energy-responsive kinases (e.g., PI-3K, Akt/PKB, Erk1/2, AMPK) inhibits TSC1/2 to maintain mTORC1 activity in nutrient-rich environments (Huang and Manning 2008; Kwiatkowski 2003). In contrast to nutrients, amino acid levels control Rheb-dependent mTORC1 activity through the Rag family of GTPases, not TCS1/2 (Sancak et al. 2008, 2010).

T cell-specific deletion of mTOR results in defective naïve T cell differentiation into T_H1, T_H2, or T_H17 effector lineages, while promoting Foxp3 upregulation and Treg suppressive function (Delgoffe et al. 2009). The diversion of mTOR-deficient T cells from effector to regulatory lineages is associated with dysregulated STAT activation downstream of multiple cytokine receptors, including IL-12, IL-4 and IL-6. $mTOR^{-/-}$ T cells also display increased TGFβ responsiveness, as seen by enhanced Smad3 phosphorylation (Delgoffe et al. 2009). In line with these genetic experiments, transient inhibition of mTORC1 signaling by either amino acid limitation or treatment with sub-immuno-suppressive doses of rapamycin also inhibits

T_H17 differentiation in vitro (Kopf et al. 2007; Sundrud et al. 2009). At least for amino acid depletion, the inhibition of T_H17 differentiation is selective, as it does not influence T_H1 or T_H2 differentiation (Sundrud et al. 2009).

Further implicating mTORC1 as a key regulator of the T_H17/Treg balance are studies showing that both PI-3K and Akt activation downstream of the TCR strongly reduces Foxp3 expression in recently activated T cells, even in the presence of TGFβ (Sauer et al. 2008). Reducing the strength or duration of TCR-activation in vitro strongly enhances Foxp3 expression while inhibiting T_H17 differentiation (Sauer et al. 2008). Furthermore, spontaneous antigen-specific iTreg differentiation is observed in vivo following low-dose soluble antigen challenge (Apostolou and von Boehmer 2004; Kretschmer et al. 2005). Collectively, this data suggest that the PI-3K/Akt/mTORC1 signaling axis modifies peripheral T cell responses to TGFβ signaling in a way that dominantly inhibits Foxp3 induction when active, allowing for synergy with IL-6 to induce T_H17 differentiation. In addition to driving mTORC1 activity, active Akt phosphorylates and inhibits Foxo family transcription factors, which have recently been shown to activate *Foxp3* gene expression (Harada et al. 2010; Ouyang et al. 2010). As a potential compensatory mechanism, natural thymic-derived Treg (nTreg) cells fail to activate Akt in response to TCR-agonists and this feature appears key for maintaining Treg cell suppressive function despite strong TCR engagement (Zeiser et al. 2008). Indeed, ectopic expression of a constitutively active Akt mutant in developing thymocytes or mature nTregs represses Foxp3 expression and extinguishes suppressive function (Haxhinasto et al. 2008).

6 Metabolic Stress Negatively Regulates T_H17 Cell Development

Amino acids are cellular multi-taskers. In addition to regulating mTORC1 activity, free intracellular amino acids are covalently linked onto cognate tRNA molecules for use in protein synthesis. Amino acid starvation thus blocks mTORC1 signaling and concomitantly prompts the accumulation of un-aminoacylated (i.e., uncharged) tRNAs (Deval et al. 2009; Staschke et al. 2010). Uncharged tRNAs are collectively recognized and bound by the protein kinase general control nonrepressed 2 (GCN2), which in turn inhibits cap-dependent protein synthesis through serine phosphorylation of the alpha sub-unit of the translation initiation factor eIF2 (eIF2α) (Harding et al. 2003; Wek et al. 2006). Whereas protein synthesis is globally reduced upon eIF2 inhibition in order to preserve amino acids, translation of a select set of stress-responsive mRNAs is actually induced. Among these stress-activated proteins is the transcription factor ATF4, which directly *trans*-activates expression of genes involved in amino acid transport and biosynthesis. This stress response system, termed the amino acid starvation response (AAR; also called general amino acid control (GAAC) in yeast), allows cells to compensate for amino acid-limiting environments (Harding et al. 2000, 2003) (Fig. 1b). The AAR pathway is biochemically linked to a broader network of stress response pathways, including those responsible for responses to hypoxia and ER stress, which is collectively termed

integrated stress response (ISR) (Harding et al. 2003; Ron and Walter 2007; Wek et al. 2006). ISR pathways, including the AAR, share the feature of inhibiting eIF2-dependent translation as a means to maintain cell homeostasis, but can be distinguished by the activation of unique upstream eIF2 kinases as well as distinct transcriptional responses. Recent work indicates that the AAR pathway selectively inhibits T_H17 differentiation in vitro and reduces T_H17-mediated inflammation in vivo (Sundrud et al. 2009).

Activation of the AAR pathway is actively regulated in vivo in order to stem T cell-mediated inflammation. Indoleamine 2,3-dioxygenase (IDO) and arginase 1 (Arg1) are enzymes secreted by APC subsets in response to inflammation; they mediate the catabolic depletion of tryptophan and arginine, resulting in local amino acid starvation (Huang et al. 2010; Nicholson et al. 2009). Several studies have reported that IDO-expressing DC subsets dominantly induce activation of the AAR in bystander T cells, promote Treg-mediated tolerance, and suppress the development of T_H17 cells in vivo (Baban et al. 2009; Munn et al. 2005). In a more recent study of HIV-infected individuals, elevated IDO expression has been associated with fewer circulating T_H17 cells, increased frequency of Treg cells, and disease progression (Favre et al. 2010). By virtue of its catabolic activity on tryptophan, IDO also generates small molecules known collectively as kynurenines (Constantino 2009). Interestingly, kynurenines have also recently been shown to suppress T_H17 differentiation and enhance Foxp3 expression in the absence of tryptophan deprivation (Desvignes and Ernst 2009; Mezrich et al. 2010). Whether IDO-mediated effects on T_H17 and Treg cells in vivo are mediated through tryptophan depletion, kynurenine production, or some combination thereof, remains to be determined.

Amino acid starvation and subsequent inhibition of T_H17 differentiation can also be pharmacologically induced, by treatment with the small molecule halofuginone (HF). HF is a derivative of the naturally-occurring plant alkaloid, febrifugine [reviewed in (Pines and Nagler 1998)]. Treatment of T cells with HF rapidly induces GCN2 activation, eIF2α phosphorylation, ATF expression, and a transcriptional response similar to that of amino acid starvation (Sundrud et al. 2009). Functionally, HF treatment impairs T_H17 differentiation selectively, without affecting the development of T_H1 or T_H2 cells. These selective effects of HF on T_H17 differentiation are mirrored by amino acid deprivation, but differ from the more broad effects of mTORC1 deficiency on all effector lineages. HF also modulates T_H17 cell differentiation selectively in vivo; C57/B6 mice treated with HF are protected against T_H17-, but not T_H1-driven EAE (Sundrud et al. 2009). Accordingly, HF reduces the proportion of T_H17 cells in the periphery and CNS of diseased mice, but has no effect on the development of T_H1 cells. HF-treated T cells cultured in T_H17 polarizing cytokine conditions display elevated levels of Foxp3 concomitant with repression of IL-17. However, the upregulation of Foxp3 expression by HF is likely a bystander effect of inhibiting T_H17 lineage commitment, as HF treatment impairs IL-6-dependent STAT3 activation, and also strongly represses T_H17 differentiation in T cells lacking Foxp3 (Sundrud et al. 2009). Importantly, although amino acid starvation induced by HF is both necessary and sufficient for repressing T_H17 differentiation, the downstream pathways that link HF-induced stress to T cell regulation are unclear.

Collectively, this data highlights that T_H17 cells are uniquely sensitive to metabolic stress and nutrient availability. The obvious question is why? Although more work is needed to address this question, it is interesting to speculate that T_H17 cells are subject to a "metabolic checkpoint". Because T_H17 cells drive chronic and progressive tissue inflammation which involves activation and proliferation of resident cells as well as massive infiltration of metabolically active mononuclear cells, T_H17-targeted tissues are likely to require significant stores of nutrients and amino acids to maintain homeostasis. Thus, much in the way cell cycle checkpoints prevent unwarranted or malignant proliferation, a metabolic checkpoint for T_H17 differentiation would reduce the chance of a local inflammatory response spiraling into pathologic tissue damage. It would also stand to reason that such a checkpoint would involve mechanisms to induce or activate local regulatory cells, and leave intact other host-protective aspects of adaptive immunity (i.e., T_H1, T_H2 cells).

Interestingly, it seems not all forms of cellular stress are specialized to regulate T_H17 responses. For example, the unfolded protein response (UPR), which is a distinct stress response induced by increased ER load, has no effect on the T_H17/Treg balance (Sundrud et al. 2009). It will be interesting to determine whether additional forms of cellular stress (e.g., genotoxic stress, hypoxia) regulate T_H17 differentiation specifically, or T cell responses more broadly. Certainly future investigation into the link between metabolic stress and T_H17 cells is needed, but if harnessed, this concept and the underlying biology offers vast therapeutic potential for the treatment of T_H17-driven immune pathologies.

7 Concluding Remarks

Through all the data and discussion presented in this chapter, the central theme is a simple one: T cell responses to growth and differentiation signals are inherently shaped by the context in which they are seen. That context is not static or one-dimensional. Rather, the physiologic context of T cells in vivo is dynamic and multi-dimensional. Of course, T_H17 cell differentiation involves the establishment of a core, cell-intrinsic, and well-defined developmental program involving STAT3, RORγt, and a series of interacting transcription factors. However, as laid out herein, this core program is subject to synergy or antagonism by auxiliary cytokines, miRNAs, and even the metabolic state of cells. By example, IL-2, all-trans retinoic acid, and metabolic stress all create a cellular context in which TGFβ signaling drives Foxp3 expression and development of regulatory characteristics. Yet in other contexts, such as inflammation and active metabolic signaling, the response of cells to TGFβ signaling is molded from inducing tolerance to promoting T_H17 differentiation and inflammation. Indeed, great strides have been made in our understanding of T_H17 cell biology. Yet much more work is needed to translate these and future discoveries into medicines. The challenge now is to define these cellular contexts, determine how they are established or may be manipulated, and interrogate their molecular interactions with the core T_H17 differentiation program. Answers to these questions

will no doubt provide new insight into T_H17 cell biology and may lead to novel strategies for the regulation of T_H17 cells in clinical disease settings.

References

Adamson, A. S., Collins, K., Laurence, A., and O'Shea, J. J. (2009). The Current STATus of lymphocyte signaling: new roles for old players. Curr Opin Immunol *21*, 161–166.

Adema, G. J. (2009). Dendritic cells from bench to bedside and back. Immunol Lett *122*, 128–130.

Ahern, P. P., Schiering, C., Buonocore, S., McGeachy, M. J., Cua, D. J., Maloy, K. J., and Powrie, F. (2010). Interleukin-23 Drives Intestinal Inflammation through Direct Activity on T Cells. Immunity *33*, 279–288.

Alam, M. S., Maekawa, Y., Kitamura, A., Tanigaki, K., Yoshimoto, T., Kishihara, K., and Yasutomo, K. (2010) Notch signaling drives IL-22 secretion in CD4+ T cells by stimulating the aryl hydrocarbon receptor. Proc Natl Acad Sci USA *107*, 5943–5948.

Alexander, W. S., and Hilton, D. J. (2004). The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. Annu Rev Immunol *22*, 503–529.

Amsen, D., Spilianakis, C. G., and Flavell, R. A. (2009). How are T(H)1 and T(H)2 effector cells made? Curr Opin Immunol *21*, 153–160.

Apetoh, L., Quintana, F. J., Pot, C., Joller, N., Xiao, S., Kumar, D., Burns, E. J., Sherr, D. H., Weiner, H. L., and Kuchroo, V. K. (2010). The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol *11*, 854–861.

Apostolou, I., and von Boehmer, H. (2004). In vivo instruction of suppressor commitment in naive T cells. J Exp Med *199*, 1401–1408.

Atsumi, T., Ishihara, K., Kamimura, D., Ikushima, H., Ohtani, T., Hirota, S., Kobayashi, H., Park, S. J., Saeki, Y., Kitamura, Y., and Hirano, T. (2002). A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. J Exp Med *196*, 979–990.

Awane, M., Andres, P. G., Li, D. J., and Reinecker, H. C. (1999). NF-kappa B-inducing kinase is a common mediator of IL-17-, TNF-alpha-, and IL-1 beta-induced chemokine promoter activation in intestinal epithelial cells. J Immunol *162*, 5337–5344.

Awasthi, A., Riol-Blanco, L., Jager, A., Korn, T., Pot, C., Galileos, G., Bettelli, E., Kuchroo, V. K., and Oukka, M. (2009). Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. J Immunol *182*, 5904–5908.

Baban, B., Chandler, P. R., Sharma, M. D., Pihkala, J., Koni, P. A., Munn, D. H., and Mellor, A. L. (2009). IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. J Immunol *183*, 2475–2483.

Barshes, N. R., Goodpastor, S. E., and Goss, J. A. (2004). Pharmacologic immuno-suppression. Front Biosci *9*, 411–420.

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

Betz, B. C., Jordan-Williams, K. L., Wang, C., Kang, S. G., Liao, J., Logan, M. R., Kim, C. H., and Taparowsky, E. J. (2010). Batf coordinates multiple aspects of B and T cell function required for normal antibody responses. J Exp Med *207*, 933–942.

Betz, U. A., and Muller, W. (1998). Regulated expression of gp130 and IL-6 receptor alpha chain in T cell maturation and activation. Int Immunol *10*, 1175–1184.

Brustle, A., Heink, S., Huber, M., Rosenplanter, C., Stadelmann, C., Yu, P., Arpaia, E., Mak, T. W., Kamradt, T., and Lohoff, M. (2007). The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. Nat Immunol *8*, 958–966.

Burchill, M. A., Yang, J., Vogtenhuber, C., Blazar, B. R., and Farrar, M. A. (2007). IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells. J Immunol *178*, 280–290.

Chen, Z., Laurence, A., Kanno, Y., Pacher-Zavisin, M., Zhu, B. M., Tato, C., Yoshimura, A., Hennighausen, L., and O'Shea, J. J. (2006). Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci USA *103*, 8137–8142.

Chen, Z., Laurence, A., and O'Shea, J. J. (2007). Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. Semin Immunol *19*, 400–408.

Chung, D. R., Kasper, D. L., Panzo, R. J., Chitnis, T., Grusby, M. J., Sayegh, M. H., and Tzianabos, A. O. (2003). CD4+ T cells mediate abscess formation in intra-abdominal sepsis by an IL-17-dependent mechanism. J Immunol *170*, 1958–1963.

Constantino, G. (2009). New promises for manipulation of kynurenine pathway in cancer and neurological diseases. Expert Opin Ther Targets *13*, 247–258.

Crome, S. Q., Clive, B., Wang, A. Y., Kang, C. Y., Chow, V., Yu, J., Lai, A., Ghahary, A., Broady, R., and Levings, M. K. (2010). Inflammatory Effects of Ex Vivo Human Th17 Cells Are Suppressed by Regulatory T Cells. J Immunol *185*, 3199–3208.

Dabir, S., Kluge, A., and Dowlati, A. (2009). The association and nuclear translocation of the PIAS3-STAT3 complex is ligand and time dependent. Mol Cancer Res *7*, 1854–1860.

Das, J., Ren, G., Zhang, L., Roberts, A. I., Zhao, X., Bothwell, A. L., Van Kaer, L., Shi, Y., and Das, G. (2009). Transforming growth factor beta is dispensable for the molecular orchestration of Th17 cell differentiation. J Exp Med *206*, 2407–2416.

Dayer, J. M., and Choy, E. (2010). Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor. Rheumatology (Oxford) *49*, 15–24.

Delgoffe, G. M., Kole, T. P., Zheng, Y., Zarek, P. E., Matthews, K. L., Xiao, B., Worley, P. F., Kozma, S. C., and Powell, J. D. (2009). The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. Immunity *30*, 832–844.

Delgoffe, G. M., and Powell, J. D. (2009). mTOR: taking cues from the immune microenvironment. Immunology *127*, 459–465.

Derynck, R., and Zhang, Y. E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. Nature *425*, 577–584.

Desvignes, L., and Ernst, J. D. (2009). Interferon-gamma-responsive non-hematopoietic cells regulate the immune response to Mycobacterium tuberculosis. Immunity *31*, 974–985.

Deval, C., Chaveroux, C., Maurin, A. C., Cherasse, Y., Parry, L., Carraro, V., Milenkovic, D., Ferrara, M., Bruhat, A., Jousse, C., and Fafournoux, P. (2009). Amino acid limitation regulates the expression of genes involved in several specific biological processes through GCN2-dependent and GCN2-independent pathways. Febs J *276*, 707–718.

Diamant, M., Rieneck, K., Mechti, N., Zhang, X. G., Svenson, M., Bendtzen, K., and Klein, B. (1997). Cloning and expression of an alternatively spliced mRNA encoding a soluble form of the human interleukin-6 signal transducer gp130. FEBS Lett *412*, 379–384.

Diveu, C., McGeachy, M. J., Boniface, K., Stumhofer, J. S., Sathe, M., Joyce-Shaikh, B., Chen, Y., Tato, C. M., McClanahan, T. K., de Waal Malefyt, R., *et al.* (2009). IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells. J Immunol *182*, 5748–5756.

Du, C., Liu, C., Kang, J., Zhao, G., Ye, Z., Huang, S., Li, Z., Wu, Z., and Pei, G. (2009). MicroRNA miR-326 regulates TH-17 differentiation and is associated with the pathogenesis of multiple sclerosis. Nat Immunol *10*, 1252–1259.

Du, J., Huang, C., Zhou, B., and Ziegler, S. F. (2008). Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3. J Immunol *180*, 4785–4792.

Dubin, P. J., and Kolls, J. K. (2008). Th17 cytokines and mucosal immunity. Immunol Rev *226*, 160–171.

Durant, L., Watford, W. T., Ramos, H. L., Laurence, A., Vahedi, G., Wei, L., Takahashi, H., Sun, H. W., Kanno, Y., Powrie, F., and O'Shea, J. J. (2010). Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity *32*, 605–615.

El-behi, M., Ciric, B., Yu, S., Zhang, G. X., Fitzgerald, D. C., and Rostami, A. (2009). Differential effect of IL-27 on developing versus committed Th17 cells. J Immunol *183*, 4957–4967.

Elo, L. L., Jarvenpaa, H., Tuomela, S., Raghav, S., Ahlfors, H., Laurila, K., Gupta, B., Lund, R. J., Tahvanainen, J., Hawkins, R. D., *et al.* (2010). Genome-wide profiling of interleukin-4 and STAT6 transcription factor regulation of human Th2 cell programming. Immunity *32*, 852–862.

Esser, C., Rannug, A., and Stockinger, B. (2009). The aryl hydrocarbon receptor in immunity. Trends Immunol *30*, 447–454.

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., Cianfarani, F., Odorisio, T., Traidl-Hoffmann, C., Behrendt, H., *et al.* (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Invest *119*, 3573–3585.

Favre, D., Mold, J., Hunt, P. W., Kanwar, B., Loke, P., Seu, L., Barbour, J. D., Lowe, M. M., Jayawardene, A., Aweeka, F., *et al.* (2010). Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of TH17 to regulatory T cells in HIV disease. Sci Transl Med *2*, 32ra36.

Glick, D., Barth, S., and Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. J Pathol *221*, 3–12.

Graf, D., Haselow, K., Munks, I., Bode, J. G., and Haussinger, D. (2008). Caspase-mediated cleavage of the signal-transducing IL-6 receptor subunit gp130. Arch Biochem Biophys *477*, 330–338.

Harada, Y., Harada, Y., Elly, C., Ying, G., Paik, J. H., DePinho, R. A., and Liu, Y. C. (2010). Transcription factors Foxo3a and Foxo1 couple the E3 ligase Cbl-b to the induction of Foxp3 expression in induced regulatory T cells. J Exp Med *207*, 1381–1391.

Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., and Ron, D. (2000). Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell *6*, 1099–1108.

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., Sadri, N., Yun, C., Popko, B., Paules, R., *et al.* (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell *11*, 619–633.

Haustedt, L. O., Mang, C., Siems, K., and Schiewe, H. (2006). Rational approaches to natural-product-based drug design. Curr Opin Drug Discov Devel *9*, 445–462.

Haxhinasto, S., Mathis, D., and Benoist, C. (2008). The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. J Exp Med *205*, 565–574.

Holland, S. M., DeLeo, F. R., Elloumi, H. Z., Hsu, A. P., Uzel, G., Brodsky, N., Freeman, A. F., Demidowich, A., Davis, J., Turner, M. L., *et al.* (2007). STAT3 mutations in the hyper-IgE syndrome. N Engl J Med *357*, 1608–1619.

Hu, X., and Ivashkiv, L. B. (2009). Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and auto-immune diseases. Immunity *31*, 539–550.

Huang, J., and Manning, B. D. (2008). The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. Biochem J *412*, 179–190.

Huang, L., Baban, B., Johnson, B. A., 3rd, and Mellor, A. L. (2010). Dendritic cells, indoleamine 2,3 dioxygenase and acquired immune privilege. Int Rev Immunol *29*, 133–155.

Huang, W., Na, L., Fidel, P. L., and Schwarzenberger, P. (2004). Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J Infect Dis *190*, 624–631.

Huber, M., Brustle, A., Reinhard, K., Guralnik, A., Walter, G., Mahiny, A., von Low, E., and Lohoff, M. (2008). IRF4 is essential for IL-21-mediated induction, amplification, and stabilization of the Th17 phenotype. Proc Natl Acad Sci USA *105*, 20846–20851.

Hwang, E. S., Szabo, S. J., Schwartzberg, P. L., and Glimcher, L. H. (2005). T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science *307*, 430–433.

Ichiyama, K., Yoshida, H., Wakabayashi, Y., Chinen, T., Saeki, K., Nakaya, M., Takaesu, G., Hori, S., Yoshimura, A., and Kobayashi, T. (2008). Foxp3 inhibits RORgammat-mediated IL-17A mRNA transcription through direct interaction with RORgammat. J Biol Chem *283*, 17003–17008.

Igaz, P., Horvath, A., Horvath, B., Szalai, C., Pallinger, E., Rajnavolgyi, E., Toth, S., Rose-John, S., and Falus, A. (2000). Soluble interleukin-6 receptor (sIL-6R) makes IL-6R negative T cell line respond to IL-6; it inhibits TNF production. Immunol Lett *71*, 143–148.

Ivanov, II, Atarashi, K., Manel, N., Brodie, E. L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K. C., Santee, C. A., Lynch, S. V., *et al.* (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485–498.

Ivanov, II, Frutos Rde, L., Manel, N., Yoshinaga, K., Rifkin, D. B., Sartor, R. B., Finlay, B. B., and Littman, D. R. (2008). Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe *4*, 337–349.

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

Jain, J., McCaffrey, P. G., Miner, Z., Kerppola, T. K., Lambert, J. N., Verdine, G. L., Curran, T., and Rao, A. (1993). The T-cell transcription factor NFATp is a substrate for calcineurin and interacts with Fos and Jun. Nature *365*, 352–355.

Janke, M., Peine, M., Nass, A., Morawietz, L., Hamann, A., and Scheffold, A. (2010). In-vitro-induced Th17 cells fail to induce inflammation in vivo and show an impaired migration into inflamed sites. Eur J Immunol *40*, 1089–1098.

Jones, G. W., McLoughlin, R. M., Hammond, V. J., Parker, C. R., Williams, J. D., Malhotra, R., Scheller, J., Williams, A. S., Rose-John, S., Topley, N., and Jones, S. A. (2010). Loss of CD4+ T cell IL-6R expression during inflammation underlines a role for IL-6 trans signaling in the local maintenance of Th17 cells. J Immunol *184*, 2130–2139.

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

Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell *110*, 163–175.

Kim, J. I., Ho, I. C., Grusby, M. J., and Glimcher, L. H. (1999). The transcription factor c-Maf controls the production of interleukin-4 but not other Th2 cytokines. Immunity *10*, 745–751.

Kishimoto, T. (2005). Interleukin-6: from basic science to medicine--40 years in immunology. Annu Rev Immunol *23*, 1–21.

Klotz, L., Burgdorf, S., Dani, I., Saijo, K., Flossdorf, J., Hucke, S., Alferink, J., Nowak, N., Beyer, M., Mayer, G.*, et al.* (2009). The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS auto-immunity. J Exp Med *206*, 2079–2089.

Kopf, H., de la Rosa, G. M., Howard, O. M., and Chen, X. (2007). Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. Int Immunopharmacol *7*, 1819–1824.

Korn, T., Bettelli, E., Oukka, M., and Kuchroo, V. K. (2009). IL-17 and Th17 Cells. Annu Rev Immunol *27*, 485–517.

Korn, T., Mitsdoerffer, M., Croxford, A. L., Awasthi, A., Dardalhon, V. A., Galileos, G., Vollmar, P., Stritesky, G. L., Kaplan, M. H., Waisman, A.*, et al.* (2008). IL-6 controls Th17 immunity in vivo by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. Proc Natl Acad Sci USA *105*, 18460–18465.

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

Kwiatkowski, D. J. (2003). Rhebbing up mTOR: new insights on TSC1 and TSC2, and the pathogenesis of tuberous sclerosis. Cancer Biol Ther *2*, 471–476.

Kwon, H., Thierry-Mieg, D., Thierry-Mieg, J., Kim, H. P., Oh, J., Tunyaplin, C., Carotta, S., Donovan, C. E., Goldman, M. L., Tailor, P.*, et al.* (2009). Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. Immunity *31*, 941–952.

Laplante, M., and Sabatini, D. M. (2009). mTOR signaling at a glance. J Cell Sci *122*, 3589–3594.

Laurence, A., Tato, C. M., Davidson, T. S., Kanno, Y., Chen, Z., Yao, Z., Blank, R. B., Meylan, F., Siegel, R., Hennighausen, L.*, et al.* (2007). Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity *26*, 371–381.

Li, J. W., and Vederas, J. C. (2009). Drug discovery and natural products: end of an era or an endless frontier? Science *325*, 161–165.

Li, M. O., Wan, Y. Y., Sanjabi, S., Robertson, A. K., and Flavell, R. A. (2006). Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol *24*, 99–146.

Lin, J. H., Walter, P., and Yen, T. S. (2008). Endoplasmic reticulum stress in disease pathogenesis. Annu Rev Pathol *3*, 399–425.

Liu, B., Mink, S., Wong, K. A., Stein, N., Getman, C., Dempsey, P. W., Wu, H., and Shuai, K. (2004). PIAS1 selectively inhibits interferon-inducible genes and is important in innate immunity. Nat Immunol *5*, 891–898.

Locke, N. R., Patterson, S. J., Hamilton, M. J., Sly, L. M., Krystal, G., and Levings, M. K. (2009). SHIP regulates the reciprocal development of T regulatory and Th17 cells. J Immunol *183*, 975–983.

Ma, X. M., and Blenis, J. (2009). Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol *10*, 307–318.

Maciver, N. J., Jacobs, S. R., Wieman, H. L., Wofford, J. A., Coloff, J. L., and Rathmell, J. C. (2008). Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. J Leukoc Biol *84*, 949–957.

Manel, N., Unutmaz, D., and Littman, D. R. (2008). The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol *9*, 641–649.

Mangan, P. R., Harrington, L. E., O'Quinn, D. B., Helms, W. S., Bullard, D. C., Elson, C. O., Hatton, R. D., Wahl, S. M., Schoeb, T. R., and Weaver, C. T. (2006). Transforming growth factor-beta induces development of the T(H)17 lineage. Nature *441*, 231–234.

Manning, B. D., Logsdon, M. N., Lipovsky, A. I., Abbott, D., Kwiatkowski, D. J., and Cantley, L. C. (2005). Feedback inhibition of Akt signaling limits the growth of tumors lacking Tsc2. Genes Dev *19*, 1773–1778.

Marine, J. C., McKay, C., Wang, D., Topham, D. J., Parganas, E., Nakajima, H., Pendeville, H., Yasukawa, H., Sasaki, A., Yoshimura, A., and Ihle, J. N. (1999). SOCS3 is essential in the regulation of fetal liver erythropoiesis. Cell *98*, 617–627.

Martin-Orozco, N., Chung, Y., Chang, S. H., Wang, Y. H., and Dong, C. (2009). Th17 cells promote pancreatic inflammation but only induce diabetes efficiently in lymphopenic hosts after conversion into Th1 cells. Eur J Immunol *39*, 216–224.

Martinez, G. J., Zhang, Z., Chung, Y., Reynolds, J. M., Lin, X., Jetten, A. M., Feng, X. H., and Dong, C. (2009). Smad3 differentially regulates the induction of regulatory and inflammatory T cell differentiation. J Biol Chem *284*, 35283–35286.

McGeachy, M. J., Bak-Jensen, K. S., Chen, Y., Tato, C. M., Blumenschein, W., McClanahan, T., and Cua, D. J. (2007). TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol *8*, 1390–1397.

McGeachy, M. J., Chen, Y., Tato, C. M., Laurence, A., Joyce-Shaikh, B., Blumenschein, W. M., McClanahan, T. K., O'Shea, J. J., and Cua, D. J. (2009). The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol *10*, 314–324.

McLoughlin, R. M., Jenkins, B. J., Grail, D., Williams, A. S., Fielding, C. A., Parker, C. R., Ernst, M., Topley, N., and Jones, S. A. (2005). IL-6 trans-signaling via STAT3 directs T cell infiltration in acute inflammation. Proc Natl Acad Sci USA *102*, 9589–9594.

Mellor, A. L., and Munn, D. H. (2008). Creating immune privilege: active local suppression that benefits friends, but protects foes. Nat Rev Immunol *8*, 74–80.

Mezrich, J. D., Fechner, J. H., Zhang, X., Johnson, B. P., Burlingham, W. J., and Bradfield, C. A. (2010). An Interaction between Kynurenine and the Aryl Hydrocarbon Receptor Can Generate Regulatory T Cells. J Immunol *185*, 3190–3198.

Miller, S. A., and Weinmann, A. S. (2009). Common themes emerge in the transcriptional control of T helper and developmental cell fate decisions regulated by the T-box, GATA and ROR families. Immunology *126*, 306–315.

Milner, J. D., Brenchley, J. M., Laurence, A., Freeman, A. F., Hill, B. J., Elias, K. M., Kanno, Y., Spalding, C., Elloumi, H. Z., Paulson, M. L., *et al.* (2008). Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature *452*, 773–776.

Minegishi, Y., Saito, M., Tsuchiya, S., Tsuge, I., Takada, H., Hara, T., Kawamura, N., Ariga, T., Pasic, S., Stojkovic, O., *et al.* (2007). Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature *448*, 1058–1062.

Muller-Newen, G. (2003). The cytokine receptor gp130: faithfully promiscuous. Sci STKE *2003*, PE40.

Munn, D. H., Sharma, M. D., Baban, B., Harding, H. P., Zhang, Y., Ron, D., and Mellor, A. L. (2005). GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity *22*, 633–642.

Murugaiyan, G., Mittal, A., Lopez-Diego, R., Maier, L. M., Anderson, D. E., and Weiner, H. L. (2009). IL-27 is a key regulator of IL-10 and IL-17 production by human CD4+ T cells. J Immunol *183*, 2435–2443.

Nicholson, L. B., Raveney, B. J., and Munder, M. (2009). Monocyte dependent regulation of autoimmune inflammation. Curr Mol Med *9*, 23–29.

Nishihara, M., Ogura, H., Ueda, N., Tsuruoka, M., Kitabayashi, C., Tsuji, F., Aono, H., Ishihara, K., Huseby, E., Betz, U. A., *et al.* (2007). IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state. Int Immunol *19*, 695–702.

Nowell, M. A., Richards, P. J., Horiuchi, S., Yamamoto, N., Rose-John, S., Topley, N., Williams, A. S., and Jones, S. A. (2003). Soluble IL-6 receptor governs IL-6 activity in experimental arthritis: blockade of arthritis severity by soluble glycoprotein 130. J Immunol *171*, 3202–3209.

Nowell, M. A., Williams, A. S., Carty, S. A., Scheller, J., Hayes, A. J., Jones, G. W., Richards, P. J., Slinn, S., Ernst, M., Jenkins, B. J., *et al.* (2009). Therapeutic targeting of IL-6 trans-signaling counteracts STAT3 control of experimental inflammatory arthritis. J Immunol *182*, 613–622.

Nurieva, R. I., Chung, Y., Hwang, D., Yang, X. O., Kang, H. S., Ma, L., Wang, Y. H., Watowich, S. S., Jetten, A. M., Tian, Q., and Dong, C. (2008). Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity *29*, 138–149.

O'Shea, J. J., and Murray, P. J. (2008). Cytokine signaling modules in inflammatory responses. Immunity *28*, 477–487.

Okuda, Y., Sakoda, S., Bernard, C. C., Fujimura, H., Saeki, Y., Kishimoto, T., and Yanagihara, T. (1998). IL-6-deficient mice are resistant to the induction of experimental auto-immune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein. Int Immunol *10*, 703–708.

Okumura, F., Matsunaga, Y., Katayama, Y., Nakayama, K.I., and Hatakeyama, S. (2010). TRIM8 modulates STAT3 activity through negative regulation of PIAS3. J Cell Sci *123*, 2238–2245.

Ouyang, W., Beckett, O., Ma, Q., Paik, J. H., DePinho, R. A., and Li, M. O. (2010). Foxo proteins cooperatively control the differentiation of Foxp3+ regulatory T cells. Nat Immunol *11*, 618–627.

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y. H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol *6*, 1133–1141.

Passerini, L., Allan, S. E., Battaglia, M., Di Nunzio, S., Alstad, A. N., Levings, M. K., Roncarolo, M. G., and Bacchetta, R. (2008). STAT5-signaling cytokines regulate the expression of FOXP3 in CD4+CD25+ regulatory T cells and CD4+CD25- effector T cells. Int Immunol *20*, 421–431.

Piccio, L., Stark, J. L., and Cross, A. H. (2008). Chronic calorie restriction attenuates experimental auto-immune encephalomyelitis. J Leukoc Biol *84*, 940–948.

Pines, M., and Nagler, A. (1998). Halofuginone: a novel antifibrotic therapy. Gen Pharmacol *30*, 445–450.

Proud, C. G. (2004). mTOR-mediated regulation of translation factors by amino acids. Biochem Biophys Res Commun *313*, 429–436.

Qin, H., Wang, L., Feng, T., Elson, C. O., Niyongere, S. A., Lee, S. J., Reynolds, S. L., Weaver, C. T., Roarty, K., Serra, R., *et al.* (2009). TGF-beta promotes Th17 cell development through inhibition of SOCS3. J Immunol *183*, 97–105.

Ramirez, J. M., Brembilla, N. C., Sorg, O., Chicheportiche, R., Matthes, T., Dayer, J. M., Saurat, J. H., Roosnek, E., and Chizzolini, C. (2010). Activation of the aryl hydrocarbon receptor reveals distinct requirements for IL-22 and IL-17 production by human T helper cells. Eur J Immunol *40*, 2450–2459.

Reboldi, A., Coisne, C., Baumjohann, D., Benvenuto, F., Bottinelli, D., Lira, S., Uccelli, A., Lanzavecchia, A., Engelhardt, B., and Sallusto, F. (2009). C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol *10*, 514–523.

Reiling, J. H., and Sabatini, D. M. (2006). Stress and mTORture signaling. Oncogene *25*, 6373–6383.

Rengarajan, J., Mowen, K. A., McBride, K. D., Smith, E. D., Singh, H., and Glimcher, L. H. (2002). Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. J Exp Med *195*, 1003–1012.

Rochman, Y., Spolski, R., and Leonard, W. J. (2009). New insights into the regulation of T cells by gamma(c) family cytokines. Nat Rev Immunol *9*, 480–490.

Ron, D., and Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol *8*, 519–529.

Rose-John, S., Scheller, J., Elson, G., and Jones, S. A. (2006). Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. J Leukoc Biol *80*, 227–236.

Samanta, A., Li, B., Song, X., Bembas, K., Zhang, G., Katsumata, M., Saouaf, S. J., Wang, Q., Hancock, W. W., Shen, Y., and Greene, M. I. (2008). TGF-beta and IL-6 signals modulate chromatin binding and promoter occupancy by acetylated FOXP3. Proc Natl Acad Sci USA *105*, 14023–14027.

Sancak, Y., Bar-Peled, L., Zoncu, R., Markhard, A. L., Nada, S., and Sabatini, D. M. (2010). Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell *141*, 290–303.

Sancak, Y., Peterson, T. R., Shaul, Y. D., Lindquist, R. A., Thoreen, C. C., Bar-Peled, L., and Sabatini, D. M. (2008). The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science *320*, 1496–1501.

Sarbassov, D. D., Ali, S. M., Kim, D. H., Guertin, D. A., Latek, R. R., Erdjument-Bromage, H., Tempst, P., and Sabatini, D. M. (2004). Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol *14*, 1296–1302.

Sauer, S., Bruno, L., Hertweck, A., Finlay, D., Leleu, M., Spivakov, M., Knight, Z. A., Cobb, B. S., Cantrell, D., O'Connor, E.*, et al.* (2008). T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proc Natl Acad Sci USA *105*, 7797–7802.

Schalm, S. S., and Blenis, J. (2002). Identification of a conserved motif required for mTOR signaling. Curr Biol *12*, 632–639.

Schraml, B. U., Hildner, K., Ise, W., Lee, W. L., Smith, W. A., Solomon, B., Sahota, G., Sim, J., Mukasa, R., Cemerski, S.*, et al.* (2009). The AP-1 transcription factor Batf controls T(H)17 differentiation. Nature *460*, 405–409.

Schulz, E. G., Mariani, L., Radbruch, A., and Hofer, T. (2009). Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-gamma and interleukin-12. Immunity *30*, 673–683.

Serada, S., Fujimoto, M., Mihara, M., Koike, N., Ohsugi, Y., Nomura, S., Yoshida, H., Nishikawa, T., Terabe, F., Ohkawara, T.*, et al.* (2008). IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental auto-immune encephalomyelitis. Proc Natl Acad Sci USA *105*, 9041–9046.

Sharpe, A. H. (2009). Mechanisms of costimulation. Immunol Rev *229*, 5–11.

Shuai, K., and Liu, B. (2005). Regulation of gene-activation pathways by PIAS proteins in the immune system. Nat Rev Immunol *5*, 593–605.

Simon, D., Denniston, A. K., Tomlins, P. J., Wallace, G. R., Rauz, S., Salmon, M., Murray, P. I., and Curnow, S. J. (2008). Soluble gp130, an antagonist of IL-6 transsignaling, is elevated in uveitis aqueous humor. Invest Ophthalmol Vis Sci *49*, 3988–3991.

Staschke, K. A., Dey, S., Zaborske, J. M., Palam, L. R., McClintick, J. N., Pan, T., Edenberg, H. J., and Wek, R. C. (2010). Integration of general amino acid control and target of rapamycin

(TOR) regulatory pathways in nitrogen assimilation in yeast. J Biol Chem *285*, 16893–16911.

Staudt, V., Bothur, E., Klein, M., Lingnau, K., Reuter, S., Grebe, N., Gerlitzki, B., Hoffmann, M., Ulges, A., Taube, C., *et al.* (2010). Interferon-Regulatory Factor 4 Is Essential for the Developmental Program of T Helper 9 Cells. Immunity *33*, 192–202.

Sundrud, M. S., Koralov, S. B., Feuerer, M., Calado, D. P., Kozhaya, A. E., Rhule-Smith, A., Lefebvre, R. E., Unutmaz, D., Mazitschek, R., Waldner, H., *et al.* (2009). Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. Science *324*, 1334–1338.

Sundrud, M. S., and Nolan, M. A. (2010). Synergistic and combinatorial control of T cell activation and differentiation by transcription factors. Curr Opin Immunol *22*, 286–292.

Tan, J. A., Song, J., Chen, Y., and Durrin, L. K. (2010). Phosphorylation-dependent interaction of SATB1 and PIAS1 directs SUMO-regulated caspase cleavage of SATB1. Mol Cell Biol *30*, 2823–2836.

Vance, R. E., Isberg, R. R., and Portnoy, D. A. (2009). Patterns of pathogenesis: discrimination of pathogenic and non-pathogenic microbes by the innate immune system. Cell Host Microbe *6*, 10–21.

Veldhoen, M., Hirota, K., Westendorf, A. M., Buer, J., Dumoutier, L., Renauld, J. C., and Stockinger, B. (2008). The aryl hydrocarbon receptor links TH17-cell-mediated auto-immunity to environmental toxins. Nature *453*, 106–109.

Vogelzang, A., McGuire, H. M., Yu, D., Sprent, J., Mackay, C. R., and King, C. (2008). A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity *29*, 127–137.

Wang, X., Lupardus, P., Laporte, S. L., and Garcia, K. C. (2009). Structural biology of shared cytokine receptors. Annu Rev Immunol *27*, 29–60.

Webb, A., Johnson, A., Fortunato, M., Platt, A., Crabbe, T., Christie, M. I., Watt, G. F., Ward, S. G., and Jopling, L. A. (2008). Evidence for PI-3K-dependent migration of Th17-polarized cells in response to CCR2 and CCR6 agonists. J Leukoc Biol *84*, 1202–1212.

Wei, B., and Pei, G. (2010). microRNAs: critical regulators in Th17 cells and players in diseases. Cell Mol Immunol *7*, 175–181.

Wei, L., Laurence, A., and O'Shea, J. J. (2008). New insights into the roles of Stat5a/b and Stat3 in T cell development and differentiation. Semin Cell Dev Biol *19*, 394–400.

Wek, R. C., Jiang, H. Y., and Anthony, T. G. (2006). Coping with stress: eIF2 kinases and translational control. Biochem Soc Trans *34*, 7–11.

Wilson, C. B., Rowell, E., and Sekimata, M. (2009). Epigenetic control of T-helper-cell differentiation. Nat Rev Immunol *9*, 91–105.

Wolk, K., Kunz, S., Witte, E., Friedrich, M., Asadullah, K., and Sabat, R. (2004). IL-22 increases the innate immunity of tissues. Immunity *21*, 241–254.

Wolk, K., Witte, E., Witte, K., Warszawska, K., and Sabat, R. (2010). Biology of interleukin-22. Semin Immunopathol *32*, 17–31.

Wu, H. J., Ivanov, II, Darce, J., Hattori, K., Shima, T., Umesaki, Y., Littman, D. R., Benoist, C., and Mathis, D. (2010). Gut-residing segmented filamentous bacteria drive auto-immune arthritis via T helper 17 cells. Immunity *32*, 815–827.

Wu, T., and Mohan, C. (2009). The AKT axis as a therapeutic target in auto-immune diseases. Endocr Metab Immune Disord Drug Targets *9*, 145–150.

Wu, Y., Borde, M., Heissmeyer, V., Feuerer, M., Lapan, A. D., Stroud, J. C., Bates, D. L., Guo, L., Han, A., Ziegler, S. F., *et al.* (2006). FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell *126*, 375–387.

Xu, J., Yang, Y., Qiu, G., Lal, G., Wu, Z., Levy, D. E., Ochando, J. C., Bromberg, J. S., and Ding, Y. (2009). c-Maf regulates IL-10 expression during Th17 polarization. J Immunol *182*, 6226–6236.

Yang, L., Anderson, D. E., Baecher-Allan, C., Hastings, W. D., Bettelli, E., Oukka, M., Kuchroo, V. K., and Hafler, D. A. (2008a). IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. Nature *454*, 350–352.

Yang, X. O., Nurieva, R., Martinez, G. J., Kang, H. S., Chung, Y., Pappu, B. P., Shah, B., Chang, S. H., Schluns, K. S., Watowich, S. S., *et al.* (2008b). Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity *29*, 44–56.

Yang, X. O., Panopoulos, A. D., Nurieva, R., Chang, S. H., Wang, D., Watowich, S. S., and Dong, C. (2007). STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem *282*, 9358–9363.

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., Ma, L., Shah, B., Panopoulos, A. D., Schluns, K. S., *et al.* (2008c). T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity *28*, 29–39.

Yip, C. K., Murata, K., Walz, T., Sabatini, D. M., and Kang, S. A. (2010). Structure of the human mTOR complex I and its implications for rapamycin inhibition. Mol Cell *38*, 768–774.

Zeiser, R., Leveson-Gower, D. B., Zambricki, E. A., Kambham, N., Beilhack, A., Loh, J., Hou, J. Z., and Negrin, R. S. (2008). Differential impact of mammalian target of rapamycin inhibition on CD4+CD25+Foxp3+ regulatory T cells compared with conventional CD4+ T cells. Blood *111*, 453–462.

Zelante, T., De Luca, A., Bonifazi, P., Montagnoli, C., Bozza, S., Moretti, S., Belladonna, M. L., Vacca, C., Conte, C., Mosci, P., *et al.* (2007). IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. Eur J Immunol *37*, 2695–2706.

Zhang, F., Meng, G., and Strober, W. (2008). Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat Immunol *9*, 1297–1306.

Zheng, Y., Valdez, P. A., Danilenko, D. M., Hu, Y., Sa, S. M., Gong, Q., Abbas, A. R., Modrusan, Z., Ghilardi, N., de Sauvage, F. J., and Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med *14*, 282–289.

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

Zhou, L., and Littman, D. R. (2009). Transcriptional regulatory networks in Th17 cell differentiation. Curr Opin Immunol *21*, 146–152.

Zhou, L., Lopes, J. E., Chong, M. M., Ivanov, II, Min, R., Victora, G. D., Shen, Y., Du, J., Rubtsov, Y. P., Rudensky, A. Y., *et al.* (2008). TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature *453*, 236–240.

Zhu, J., and Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. Blood *112*, 1557–1569.

Zrioual, S., Toh, M. L., Tournadre, A., Zhou, Y., Cazalis, M. A., Pachot, A., Miossec, V., and Miossec, P. (2008). IL-17RA and IL-17RC receptors are essential for IL-17A-induced ELR+ CXC chemokine expression in synoviocytes and are overexpressed in rheumatoid blood. J Immunol *180*, 655–663.