REVIEW ARTICLE
Helper T cell differentiation

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CD4^{+} T helper cells are key regulators of host health and disease. In the original model, specialized subsets of T helper cells are generated following activation through lineage-specifying cytokines and transcriptional programs, but recent studies have revealed increasing complexities for CD4^{+} T-cell differentiation. Here, we first discuss CD4^{+} T-cell differentiation from a historical perspective by highlighting the major studies that defined the distinct subsets of T helper cells. We next describe the mechanisms underlying CD4^{+} T-cell differentiation, including cytokine-induced signaling and transcriptional networks. We then review current and emerging topics of differentiation, including the plasticity and heterogeneity of T cells, the tissue-specific effects, and the influence of cellular metabolism on cell fate decisions. Importantly, recent advances in cutting-edge approaches, especially systems biology tools, have contributed to new concepts and mechanisms underlying T-cell differentiation and will likely continue to advance this important research area of adaptive immunity.

Keywords: T cells; Treg; differentiation; plasticity; immunometabolism

INTRODUCTION

CD4^{+} T helper cells are an essential and complex component of the immune system. Upon recognition of antigen-major histocompatibility complex molecules and proper costimulation, naive CD4^{+} T cells exit from quiescence to undergo clonal expansion. Cytokines within the immediate milieu program the differentiating cells into a specific effector cell type. The mechanisms that govern these processes have been actively investigated for over 30 years, yielding many ground-breaking discoveries that have laid the foundation for the therapeutic approaches for cancer, autoimmunity, and infectious disease.

Following the initial discovery of T helper 1 (T$_H$1) and T$_H$2 cells, the identification of additional functionally distinct T cells, mainly via traditional immunological approaches, has significantly expanded our view from the dichotomous existence of these cells. More recently, technological advances have uncovered complexities underlying the mechanisms for CD4^{+} T-cell effector programming. Indeed, the heterogeneity and plasticity of T helper cells are becoming increasingly appreciated as major players in the adaptive immune system as they adapt and react to different stimuli.

The goal of this review is to provide both a historical perspective and an updated view of T helper cell differentiation. First, we summarize the landmark discoveries in T helper cell subsets and how cytokines shape their programming. Next, we discuss the signaling and transcriptional events underlying T helper cell differentiation. We then describe current research areas, including the plasticity and heterogeneity of T cells in inflammatory states and within tissues and the roles of metabolic reprogramming in cell fate decisions. We also highlight emerging concepts as revealed, in part, by cutting-edge systems biology approaches.

A BRIEF HISTORY OF T HELPER CELL SUBSETS

In 1986, the groundbreaking study describing T$_H$1 and T$_H$2 cells, classified according to differential cytokine production and surface marker expression, was published[1] (Fig. 1). T$_H$1 cells produce the cytokines interferon-γ (IFN-γ), interleukin (IL)-2, and tumor necrosis factor-α and are important for antiviral and antibacterial immunities. T$_H$2 cells are defined by the expression of their signature cytokines IL-4, IL-5, and IL-13 and are immunologically important against extracellular pathogens, such as worm infections. In 1995, nearly a decade after the first description of T$_H$1 and T$_H$2 cells, another landmark discovery identified an immunosuppressive T-cell type that constitutively expresses CD25, later termed regulatory T cells (T$_{regs}$).[2] The dichotomous paradigm of proinflammatory T helper cells reigned for nearly two decades until a third subset, T$_H$17 cells, were designated in 2005.[3,4] The discovery of T$_H$17 cells was based, in part, on earlier studies that clarified the roles of IL-12 and IL-23 that share the p40 subunit.[5,6] T$_H$17 cells are critical for anti-fungal responses and for host defense against bacterial infection (intracellular and extracellular)[7]; in addition, T$_H$17 cells are abundant within the gut to help regulate the gut microbiota. The signature cytokines of T$_H$17 cells include IL-17A, IL-17F, and IL-22. Another distinguished T helper subset of cells, T follicular helper (T$_{FH}$) cells, promotes humoral immunity within germinal centers (GCs).[8–10] These cells produce IL-21, which is critical for B-cell stimulation,[11] and can be defined by CXCR5 (C-X-C chemokine receptor type 5) expression[12,13] and coexpression with programmed cell death-1 (PD-1) and/or ICOS (Inducible T-cell COStimulator). T$_{FH}$ cells may also produce IL-4, which is important for immunoglobulin class switching in B cells.[14]

Additional "unconventional" T helper subsets have also been defined but will not be the focus of this review. T$_H$3 cells, first described in 1994, are an immunosuppressive subset marked by

high expression of transforming growth factor-β (TGF-β).[15] Discovered in 1997, another immunosuppressive subset named T_R1 cells secrete IL-10 and are distinct from T_{regs}.[16] T_H9 cells were designated as IL-9 producers that are distinct from IL-9-producing T_H2 cells in 2008.[17,18] Finally, T_H22 cells were described in 2009 as a subset with exclusive IL-22 expression, distinguishing them from IL-22-producing T_H17 cells.[19,20] Further information on these cells can be found in other reviews.[21,22]

Overall, the production of signature cytokines defines T helper cell subsets and functional capacities. Moreover, cytokine signals, typically produced by antigen presenting cells, orchestrate lineage decisions of activated $CD4^+$ T cells. Differentiation of T_H1 cells is promoted by the cytokine IL-12,[23] while IL-4 drives T_H2 cell differentiation.[24] T_{FH} cells are induced by IL-21 and IL-6.[25] Unlike other effector $CD4^+$ T-cell subsets, T_{regs} can be generated directly within the thymus (tT_{regs}) during thymocyte development.[2] T_{regs} may also be induced peripherally (pT_{regs}) and in vitro by the cytokines TGF-β and IL-2.[26] T_H17 cell differentiation is promoted by IL-1β, IL-6, IL-21, IL-23, IL-1β, and TGF-β.[27–34] Aberrant T_H17 responses are strongly associated with autoimmune disorders.[5] Interestingly, different T_H17-promoting cytokines can induce unique types of T_H17 cells as follows: TGF-β promotes an IL-10-producing and less pathogenic subtype, while IL-1β promotes a more proinflammatory subtype of T_H17 cells.[31,35,36] Thus, cytokines are intricately linked to $CD4^+$ T-cell differentiation due to polarizing capability and being lineage-defining products of differentiated cells.
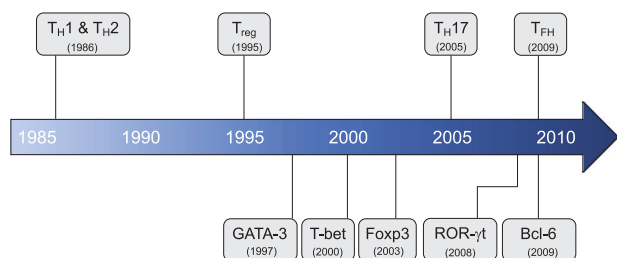

Fig. 1 Timeline of major T helper cell discoveries. T helper populations (top) and major transcriptional regulators (bottom) listed in chronological order of the first published observation and/or the commonly agreed upon period establishing a separate lineage

TRANSCRIPTIONAL NETWORKS REGULATING T HELPER CELL DIFFERENTIATION

Following cytokine stimulation, distinct signaling transducer and activator of transcription (STAT) family proteins are activated and drive T helper cell differentiation. STAT4 is activated downstream of IL-12 and is critical for T_H1 cell differentiation,[37,38] while STAT6 is induced downstream of IL-4 for T_H2 cell differentiation.[39–41] It was later shown that T_H1 cell differentiation is also influenced by IFN-γ signaling through STAT1,[42] thus revealing a possible autocrine regulation, similar to IL-4 in T_H2 cells. In agreement with the elevated expression of CD25, strong IL-2-STAT5 signaling is important for T_{reg} cell differentiation,[43,44] though it is also important in T_H2 cell differentiation.[45] The differentiation of both T_H17 and T_{FH} cells is dependent on STAT3 signaling[9,25,32] through IL-6/IL-23 and IL-21, respectively.

The ability of naive $CD4^+$ T cells to undergo lineage polarization into distinct effector subsets is mediated by master transcription factors (Fig. 2). These multifunctional transcription factors are able to dictate cell fate by either (I) inducing the expression of lineage-specific genes and coactivators or (II) repressing the expression of genes that are associated with alternate lineages. These regulatory effects can be carried out by directly binding to the promoter and enhancer regions or indirectly by influencing epigenetic changes that are conducive to subset-specific gene programs. GATA-3 was the first master transcription factor identified to induce T helper cell differentiation, which promotes T_H2 cell differentiation downstream of IL-4-STAT6.[46,47] This finding was followed by the discovery of the master regulator of T_H1 cell differentiation, T-bet.[48] These master transcription factors play opposing roles in T_H1/T_H2 cell fate decisions; GATA-3 is induced during T_H2 cell differentiation and strongly suppresses T_H1-associated gene expression,[49] and vice versa for T-bet with T_H2-associated gene expression during T_H1 cell differentiation.[50] T-bet also directly binds to GATA-3 to mediate its suppression of T_H2 programs.[51] Furthermore, forced expression of GATA-3 in T_H1 cells can induce IL-4 expression,[49,52] while forced expression of T-bet in T_H2 cells can induce IFN-γ expression.[53,54] Interestingly, the absence of GATA-3 permits T_H1 cell differentiation independently of IL-12 and IFN-γ[53], suggesting a primary role for GATA-3 in regulating T_H1 vs. T_H2 cell differentiation. In fact, GATA-3 functions primarily as an epigenetic modifier of the T_H2 cytokine loci, and GATA-3 is not required for Il4 transcription in fully differentiated T_H2 cells.[55] T-bet also functions to suppress the production of IL-17A.[56]

Downstream of STAT3 signaling is the T_H17 master regulator ROR-γt (retinoic acid receptor-related orphan receptor-γt).[57] This
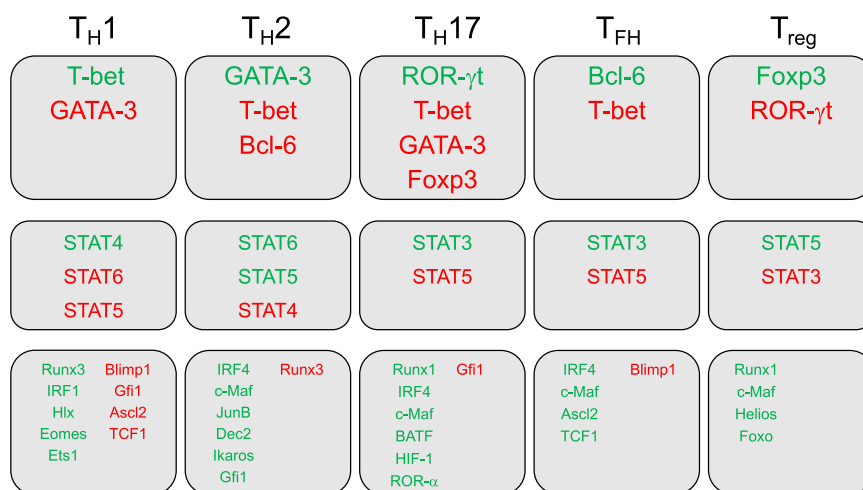

Fig. 2 Transcriptional regulators of T helper cells. T helper cell subsets and associated positive (green) and negative (red) transcriptional regulators are separated by master regulators (top), signaling transducer and activator of transcription (STAT) molecules (middle), and additional important transcription factors (bottom)

transcription factor directly regulates the expression of IL-17A and IL-17F, along with other T$_H$17-specific genes,[58] and T$_H$17 cytokine production is drastically reduced in ROR-γt-deficient cells.[57] The transcriptional regulator of T$_{FH}$ cells is B-cell lymphoma-6 (Bcl-6), which is a characteristic that is shared with GC B cells.[8,10] Mice with germline deficiency in Bcl-6 do not generate T$_{FH}$ cells and develop T$_H$2-dominant immune disease.[59–61] Interestingly, while Bcl-6 induces T$_{FH}$-associated surface molecules (e.g., CXCR5 and PD-1) and represses alternate T helper subset cytokines, such as IFN-γ and IL-17A,[61] it does not directly promote IL-21 expression.[59]

Research to identify a master transcriptional regulator and/or definitive markers for T$_{regs}$ was guided by genetic studies. These studies demonstrated that the lymphoproliferative disorder, known as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), is caused by mutations in the gene encoding FOXP3 (forkhead box P3),[62,63] while the mutation of the mouse homolog Foxp3 gene is responsible for the "Scurfy" phenotype.[64,65] Indeed, the function and identity of T$_{regs}$ are dependent upon Foxp3 expression.[66,67] Furthermore, a regulatory phenotype is imparted upon conventional T helper cells with enforced Foxp3 expression.[66–68] TGF-β can promote T$_{reg}$ and T$_H$17 cell differentiation, yet T$_H$17-associated factors suppress Foxp3 expression through ROR-γt binding or STAT3 signaling.[69] Though both tT$_{regs}$ and pT$_{regs}$ are categorically Foxp3-expressing T$_{regs}$, it is now understood that there are distinct functional properties and cis control elements between the two populations.[70,71] tT$_{regs}$ mediate self-tolerance and prevention of autoimmunity, while pT$_{regs}$ enforce peripheral immune tolerance and general suppression of inflammation.

Aside from their respective master regulators, additional transcription factors are also critical regulators of T helper cell differentiation. The runt-related transcription factor (Runx) family is important for T-cell development and function. Runx3 promotes IFN-γ expression and represses Il4 gene expression in T$_H$1 cells.[72,73] Runx1 is critical for T$_{reg}$ cell function and Foxp3 stability[74–76] and for the identity and function of T$_H$17 cells by promoting the expression of ROR-γt and IL-17A.[77] The interferon regulatory factor (IRF) family also regulates T helper cell differentiation. IFN-γ signaling induces IRF1, which assists T$_H$1 identity through the upregulation of IL-12Rα.[78] IRF4 upregulates GATA-3 and thus is important for T$_H$2 cell function.[79,80] Interestingly, T$_H$17 and T$_{FH}$ cells also utilize IRF4 for differentiation.[81,82] Transcription factors can also be part of negative-feedback mechanisms affecting differentiation. Both T$_H$1 and T$_{FH}$ generation are impaired by Blimp-1 expression, which is induced by IL-2 signaling.[60,83] In fact, IL-2-STAT5 signaling inhibits Bcl-6 due to similarities in binding sites near T$_{FH}$ genes.[84] c-Maf is another important transcription factor for T helper cell differentiation that has context-specific functions based on chromatin availability,[85] making it both a positive and negative regulator of cytokine genes within the same cell. Downstream of TCR signaling, c-Maf is a known positive regulator of Il10 expression,[58,86,87] yet it promotes Il4 expression in T$_H$2 cells[88,89] and is also involved in T$_H$17[58,87] and T$_{FH}$[90] cell differentiation. Furthermore, c-Maf is critical for the cellular function of T$_{regs}$ in the gut.[91] More comprehensive descriptions of additional transcription factors involved in T helper cell differentiation, including roles for ROR-α for T$_H$17 cell generation[92] and Ascl2 and T-cell factor 1 (TCF-1) for regulating T$_{FH}$ vs. T$_H$1 or T$_H$17 cell differentiation,[93–95] are reviewed elsewhere.[96,97] Future studies will continue to determine the transcriptional networks imparting context-specific functions of T helper cell subsets.

While master transcriptional regulators play critical roles in T helper cell differentiation, transcriptional mediators working in a coordinated network are required to drive cell fate decisions. The first reported descriptions of large-scale, transcriptional network-dependent control of CD4$^+$ T-cell differentiation were focused on T$_H$17 cells.[58,98] These studies used chromatin immunoprecipitation-sequencing (ChIP-seq)[58] and small interfering RNA[98] screening

methods, together with computational analyses, to reconstruct the dynamic regulatory network of T$_H$17 cell differentiation. Recently, using a combination of CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9) screening and next-generation sequencing, including RNA-seq, ChIP-seq, and ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing), researchers generated a quantitative "atlas" of T$_H$2 cell differentiation.[99] Complex data integration, via a new biocomputational methodology, grants novel insight into regulatory networks involving different transcription factors, metabolic regulators, and cytokine signaling pathways.[99] Future adaptation of these integrative and systems-level analyses to modern topics in immunology is an exciting prospect.

T-CELL PLASTICITY AND HETEROGENEITY
Differentiated T helper cells adapt to ever-changing microenvironments and surrounding molecular cues. Thus, these cells are able to continuously adapt to unique circumstances to provide proper immunity.[100] Earlier work hinted at this phenomenon by forced expression of master regulators in differentiated T cells.[49,52–54] Indeed, differentiated CD4$^+$ T cells can adopt alternate expression profiles and produce cytokines that are associated with alternate T-cell lineages,[101–105] including T$_{FH}$ cells[106] (Fig. 3a). This duality is due, in part, to bivalent histone modifications (e.g., H3K4me3 and H3K27me3) that are maintained in T helper cells near all master regulator genes and are independent of the differentiated status of the cell.[107] In other words, T helper cells remain "poised" to adopt alternative subset transcriptional programs upon receiving the appropriate signals. Extracellular cues that drive subset differentiation from naive T cells are also involved in this process. T$_H$1 cells can be repolarized to produce IL-4 under T$_H$2 conditions,[102] and T$_H$2 cells will express IFN-γ when cultured with IL-12, IFN-γ, and type I IFNs.[108] IL-12 can also cause T$_H$17 cells to express IFN-γ.[103,109,110] Interestingly, T$_{FH}$ cells can be made to coexpress IL-21 and any other subset-specific cytokines when cultured under respective conditions.[106] Both T$_H$17 and T$_{FH}$ cells demonstrate plasticity within peripheral tissues[111] and are discussed in more detail below.

Diversity among individual T helper subsets was established with the observations that master transcription factors could be coexpressed within certain subpopulations. One common example is a T-bet$^+$ROR-γt$^+$ T-cell population found in the gut and within the inflamed central nervous system (CNS) that produces both IFN-γ and IL-17.[109] Arguably the most well-established example of functional plasticity are T$_{regs}$, which are reprogrammed to suppress specific types of inflammation (e.g., T$_H$1 vs. T$_H$2 inflammation).[112,113] For instance, in the context of T$_H$1 inflammation, T$_{regs}$ receiving IFN-γ and/or IL-12 signals will express T-bet and the T$_H$1-chemokine receptor CXCR3, which enables migration and accumulation within areas of T$_H$1 inflammation.[114,115] This "T$_H$1-like" capability is a critical part of the cell-mediated immune homeostasis of T$_{regs}$, as loss of T-bet$^+$ T$_{regs}$ (but not T-bet expression itself) results in T$_H$1-dominant immune disease.[116] Importantly, T$_{regs}$ must maintain Foxp3 expression for suppressive function, despite the extrinsic T$_H$1 influence. This regulation occurs by ongoing IL-2-STAT5 signaling, which reinforces Foxp3 expression[117] and limits the expression of IL-12Rβ2.[118] Similar functional effects in T$_{regs}$ are observed for T$_H$2 inflammation, where IL-4 induces IRF4 and GATA-3[119–121] or in T$_H$17 inflammation with IL-6-STAT3 signaling for ROR-γt upregulation.[122–124] T$_{FH}$ responses are controlled by a unique class of T$_{regs}$, termed T follicular regulatory (T$_{FR}$) cells, which express Bcl-6 and T$_{FH}$ surface markers CXCR5 and PD-1.[125–127] Notably, while the overall importance of effector-like transcriptional signatures in T$_{regs}$ is clear, T$_{regs}$ expressing effector-like transcriptional programs are also associated with aberrant immune diseases and show defective suppressive capacity ex vivo.
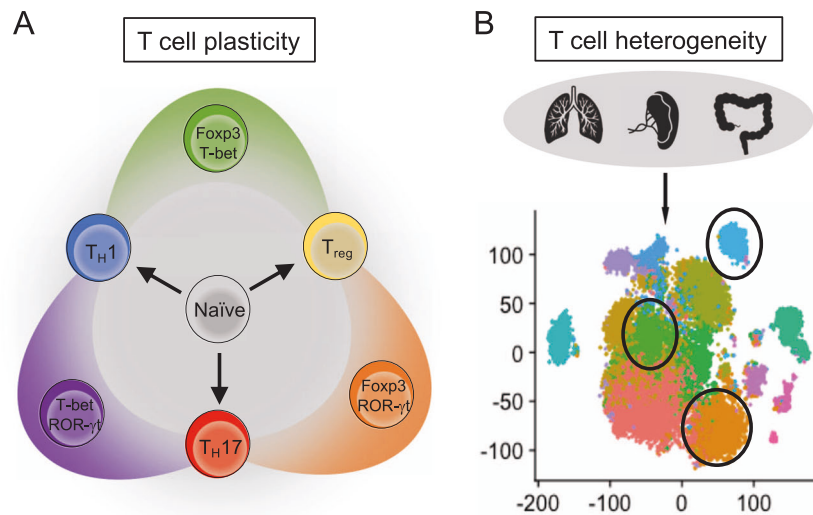
Fig. 3 T helper cell plasticity and heterogeneity. **a** Representative diagram of plasticity in selected T helper cells. Differentiated T helper cells can coexpress master transcriptional regulators from other lineages. **b** Diagram with example tSNE (T-distributed stochastic neighbor embedding) plot generated from single-cell RNA-sequencing (scRNA-seq) data. Single-cell analysis allows for the discovery of subpopulations with unique expression profiles

Increased frequencies of T_H1-like T_{regs} are observed in patients with type I diabetes[128] and multiple sclerosis.[115] Likewise, T_H2-like T_{regs} are increased in patients with food allergies,[121] and T_H17-like T_{regs} are enriched in the synovium of rheumatoid arthritis patients.[129] The mechanisms for this notable disparity between effector-like T_{regs} in healthy function and disease states remain unclear.

Heterogeneity has also become a key area of research due to the availability of powerful experimental methods, such as single-cell RNA-seq (scRNA-seq).[130] Single-cell resolution allows for the discovery of unique subpopulations of T cells that would otherwise be "lost" in bulk RNA-seq analyses (Fig. 3b). With the inference of multiple developmental or transitional "states" of the same cell type, this method can garner more information when combined with "pseudotime" analysis, which generates a visualized continuum of cells as they progress, regress, or transgress along a developmental trajectory. This analysis is especially applicable to T helper cell differentiation to provide a temporal dimension of gene regulation as opposed to a snap-shot of the end result.

Previous work in T_H17 cell differentiation demonstrated both pathogenic and regulatory-like T_H17 cell generation from different cytokine stimuli.[35] The combination of scRNA-seq and computational approaches shows that in vivo-derived T_H17 cells, isolated from an autoimmune environment, are also heterogeneous and have a spectrum of functional states.[131,132] Applying computational analysis with scRNA-seq also reveals novel targets for dictating the different cellular fates of T_H17 cells.[131–133] "Pseudotime" analyses have also demonstrated the relative temporal trajectory of IL-17-producing T_H17 cells and T_H1-like T_H17 cells[132] as well as differentiating bifurcations in T_H1 and T_{FH} cell fates in a mouse model of malaria.[134] In both cases, these cells originate from a common precursor, yet the programming of their differential fates is coincident with the expression of unique chemokine receptors, transcription factors, and functional profiles.[132,134] Applications of scRNA-seq and computational approaches have also recently been applied to T_{regs} in lymphoid tissues, identifying an important role for TCR signal strength in shaping the activation and heterogeneity of T_{regs}.[135] Furthermore, this approach has identified similarities and differences among nonlymphoid tissue-resident T_{regs}, known to be starkly different from lymphoid-resident cells.[136,137]

Another emerging concept in T helper cell heterogeneity is cells with stem-like qualities. Even in chronic viral infections that were thought to drive T-cell exhaustion and loss of memory potential, a stem-like population of $CD8^+$ T cells with an enhanced capacity for self-renewal and responsiveness to immune checkpoint therapy was recently identified and depends upon TCF-1–LEF-1 (lymphoid enhancer-binding factor 1) signaling.[138–140] Long-lived T_H17 cells with stem cell-like attributes of self-renewal and multipotency were also found.[141] Interest in stem-like T helper cells has recently increased by reports of their involvement in autoimmune diseases, including chronic colitis[142] and experimental autoimmune encephalomyelitis.[132] The functional identification of these cells has revealed another dimension in T helper cell differentiation that is only decipherable under specific disease contexts but is physiologically relevant. Furthermore, it remains to be seen if other T helper subsets, particularly highly plastic T_{regs}, also have stem-like properties during homeostasis or autoimmunity.

TISSUE T-CELL POPULATIONS

While cellular heterogeneity and plasticity occur during inflammation,[100] recent studies suggest that these phenomena also arise under steady-state conditions. Lineage-tracing genetic systems have been an invaluable tool to demonstrate the physiological relevance of T-cell plasticity.[109,143,144] For example, tissue-specific plasticity occurs in Peyer's patches (PPs) and gut-associated lymphoid tissues where GC B cells capable of producing IgA are generated. T_{FH} cells within the PPs have been shown to develop from gut-derived T_H17 cells or pT_{regs} that have been activated through MyD88-coupled receptors.[145–147] Furthermore, inflammatory T_H17 cells can transdifferentiate into T_{regs} to resolve inflammatory responses.[144] Thus, $CD4^+$ T-cell plasticity can play critical roles under homeostasis.

Both tT_{regs} and pT_{regs} reside in mucosal tissues, including gut-associated lymphoid tissues. $Foxp3^+$ T_{regs} found within these sites are inherently heterogeneous, as tissue T_{regs} must coexpress effector T-cell transcription factors for proper function. For instance, intestinal pT_{regs} express ROR-γt to suppress T_H1, T_H2, and T_H17 inflammation in the gut.[122,123] Similarly, T_{regs} migrate into PPs at steady-state conditions and differentiate into $Foxp3^+Bcl-6^+$ T_{FR} cells to regulate IgA antibody production.[148]

Tissue T_{regs} regulate type 2 immunity in the intestines, lung, and skin under steady-state conditions.[70,149–151] This function is supported by the inflammation-triggered upregulation of IRF4[120] or GATA-3 by TCR or IL-33 signals, which mediate the stability and function of T_{regs}.[152–154] Notably, functional redundancy of GATA-3 and T-bet in T_{regs} exists for the proper control of immune homeostasis[119] and potentially explains why the conditional deletion of GATA-3 in T_{regs} does not trigger excessive inflammation.[152,153,155] However, the hypercolonization of selective commensals induces potent type 2 inflammation within the skin of mice bearing GATA-3-deficient T_{regs}.[151] Thus, the heterogeneity and plasticity of T_{regs} are important for tissue homeostasis.

Cytokines and transcriptional networks regulate the differentiation of several memory $CD4^+$ and $CD8^+$ T-cell subsets. Among these memory T-cell subsets are tissue-resident memory T cells (T_{RM}), which were first shown to be induced by pathogens. T_{RM} cells share transcriptional signatures with other effector and memory subsets but also have distinct transcriptional programs, as reviewed elsewhere.[156] However, host antigens, such as commensal bacteria, can also induce T_{RM}-like T cells that produce IFN-γ or IL-17 in the skin.[157,158] IL-17-producing $CD8^+$ and $CD4^+$ T cells within the skin can coexpress ROR-γt and GATA-3 in the presence of the "alarmins" IL-18, IL-33, or IL-1. This regulation is essential for generating type 2 inflammation that promotes wound healing.[151] These studies establish tissue T-cell heterogeneity as a critical node for tissue homeostasis and repair.

How immune cells regulate the tissue microenvironment through cell–cell interactions and signaling networks is an emerging concept in immunological research. For example, T_{regs} support stem cell homeostasis in the intestines, hair follicle, and bone marrow.[159–161] Moreover, T_{regs} regulate microbiota and metabolite diversity, which controls inflammatory processes at both local and distal sites.[70,162,163] Thus, the molecular dissection of how resident and infiltrating T cells modulate the overall tissue microenvironment will likely uncover new regulations of disease etiology and progression.

METABOLIC CONTROL OF T HELPER CELL FUNCTION
Metabolic control of differentiation
The discovery that different T helper cells have shared, yet distinct, metabolic programs has established that metabolism can influence $CD4^+$ T-cell fates.[164–166] Specifically, T_H1, T_H2 and especially T_H17 cells maintain high levels of glycolysis compared with in vitro-derived T_{regs}.[167–169] These observations have since been extended to T_{FH} cells, which have increased glycolytic metabolism relative to non-T_{FH} cells; however, cell-autonomous IL-2 dampens the differentiation and metabolic programs of T_{FH} cells at the expense of T_H1 programs through the induction of Blimp-1.[170–172] Additionally, glutaminolysis programs increase in T_H17 cells compared with T_H1 or in vitro-derived T_{regs},[173] while fatty acid synthesis is a critical regulator of T_H17 and, to a lesser extent, T_H1 cell generation and function.[174,175] In contrast, fatty acid synthesis opposes the differentiation of T_{regs},[174] demonstrating that this pathway also balances T_H17 vs. T_{reg} cell programming. T_{regs} require high levels of mitochondrial activity to support their differentiation, homeostasis, and function in vitro and in vivo.[149,150,167,169,176] Using in vitro systems with the drug etomoxir,[167,169] T_{regs} were found to rely on fatty acid oxidation to induce mitochondrial oxidative metabolism, but this model has recently been challenged in vivo.[177] Moreover, genetic studies show that metabolic programs are altered in accordance with the requirements for the activation, functional reprogramming, and migration of tT_{regs} and likely pT_{regs},[149,150,176,178–182] thus establishing layers of complexity remaining to be fully addressed.

The kinase mammalian target of rapamycin (mTOR), which functions via one of the two distinct complexes mTORC1 or mTORC2, and the transcription factors c-Myc, hypoxia-inducible factor-1α (HIF-1α), and nuclear factor of activated T-cells (NFAT) cooperatively induce the expression of metabolic enzymes and nutrient transporters (e.g., glucose, amino acids, fatty acids, and minerals) that promote anabolic metabolism.[168,183–187] Activation programs also lead to increased expression of the high-affinity IL-2 receptor and induction of its downstream signaling, which can further amplify Akt- and mTOR-dependent signaling to tune cellular metabolism.[188] Also downstream of mTORC1, HIF-1α signaling is a critical determinant of the reciprocal differentiation of T_H17 and T_{regs}.[168,189] HIF-1α activity induces glycolysis under hypoxic conditions. This oxygen-sensing function is enforced by the von Hippel-Lindau (VHL) complex and opposed by prolyl-4-hydroxylase domain (PHD) proteins. Upon activation, VHL-deficient $CD8^+$ T cells have augmented glycolytic function and reduced mitochondrial oxidative metabolism, which are associated with increased effector programming, terminal effector-memory T-cell differentiation, and increased cell death.[190,191] In contrast, PHD-deficient $CD4^+$ T cells have increased and decreased differentiation of T_H1 and T_{regs}, respectively, which can boost anti-tumor immunity in hyperoxic environments, such as the lung.[192] These results implicate oxygen sensing by upstream regulators of HIF-1α as determinants of T_H1 and T_{reg} cell fate decisions.

Metabolic signaling can also impart functional reprogramming of effector T cells by epigenetic mechanisms (Fig. 4). mTOR signaling promotes T_H1 and T_H2 cell differentiation, perhaps in part by inducing epigenetic-related metabolites.[183,193–195] The lactate dehydrogenase A (LDHA)-dependent aerobic glycolytic flux maintains high levels of cytosolic acetyl-coenzyme A,[196] which can posttranslationally modify proteins via the enzymatic activities of histone acetyltransferases.[164] Indeed, LDHA-deficient T cells have reduced T_H1 responses owing to reduced H3K9Ac on the Ifng locus.[196] A recent study has shown that glutaminolysis can regulate the differentiation and function of T_H1 and T_H17 cells through discrete mechanisms. T_H17 cell differentiation is reduced in the absence of glutaminolytic metabolism due to an accumulation of reactive oxygen species in part alter H3K27me3 levels and chromatin accessibility in T_H17-related genes.[167,173] In contrast, T_H1 cell proliferation and function are only temporarily impaired when glutaminolysis is suppressed and are associated with a loss of the α-ketoglutarate (α-KG)-dependent suppression of H3K27me3 that is reversed and elevated by increased IL-2-mTORC1 signaling.[173] Additional studies will continue to dissect how mTOR and metabolic networks orchestrate the differentiation and function of $CD4^+$ T cells at different stages of effector programming.

Metabolic programs are also key regulators of the activation of naive $CD4^+$ T cells. During antigen-driven quiescence exit, naive T cells rapidly rewire their metabolic programs, switching from a catabolic to anabolic state by upregulating glycolysis, glutaminolysis, and mitochondrial metabolism.[197] Defects in metabolic reprogramming during quiescence exit manifest in reduced T-cell expansion by suppressing cell proliferation and/or survival, as well as impaired T_H1, T_H2, T_H17, and T_{FH} responses as reviewed elsewhere.[164–166] It remains unclear whether alterations in effector $CD4^+$ T-cell programming are distinct from, or a consequence of, proliferation defects. However, a recent study suggested that the demethylase-inducing effects of α-KG mediate the CTCF-dependent induction of T_H1 signature genes separate from effects on proliferation.[198] These results thus provide evidence that metabolic networks can prime $CD4^+$ T-cell differentiation independently of effects on proliferation.

Metabolic control of plasticity and heterogeneity
The metabolic landscape also shapes T-cell plasticity and heterogeneity. Metabolic disruptions due to excessive phosphoinositide 3-kinase (PI3K) and mTOR activities can lead to increased
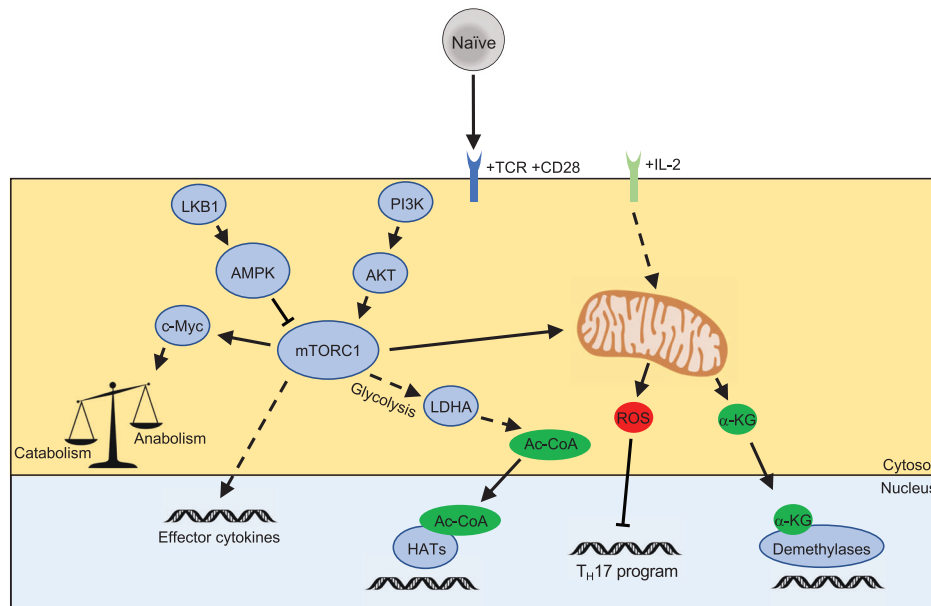
Fig. 4 Metabolic control of T helper cell differentiation. Activated T cells undergo metabolic reprogramming, which leads to permissive epigenetic alterations of T effector genes. Much of this pathway is coordinated by mammalian target of rapamycin (mTOR) signaling and mitochondrial function to generate metabolic co-factors of epigenetic-modifying enzymes

methylation of the *Foxp3* locus and reduce stability of T_{regs}, leading to uncontrolled T_H1 and T_{FH} cell responses.[180,181] Defects in metabolic reprogramming are also associated with an exhausted-like state in T_{regs}, characterized by the hyperexpression of coinhibitory molecules that impairs their stability and suppression of T_H2 immunity.[150] High levels of glycolytic metabolism limit Foxp3 induction to promote T_H17 cell conversion at the expense of T_{regs}.[167,168] Strong glycolytic signals also reduce the stability of tT_{regs}, leading to the generation of IFN-γ- or IL-17-producing ex-T_{regs},[179,199] and these observations further establish the link between metabolic programming and plasticity or instability of T_{regs}.

Using a lineage-tracing system and scRNA-seq, we recently found that IL-17-producing T_H17 cells can adopt different metabolic states in accordance with their pathogenicity; a stem-like signature of IL-17-producing cells has low mTORC1 activity and downstream anabolic programs and an IFN-γ-producing state has high mTORC1 activity and anabolic metabolism (glycolytic and mevalonate-related pathways).[132] This metabolic heterogeneity is mediated by a balance of TCF-1 and T-bet-dependent programs, and it is also associated with altered histone acetylation and chromatin accessibility at the *Ifng* locus. Thus, metabolic and functional flexibility, together with the dynamic regulation of transcriptional and epigenetic networks, mediate heterogeneity within specific effector T-cell subsets. Exploring the mechanisms for effector T-cell heterogeneity will be crucial as more precision-based therapies are used to treat various diseases.

Metabolic control of tissue T-cell responses
One emerging downstream consequence of metabolic reprogramming is the capacity to modulate T-cell accumulation within tissues at steady-state conditions,[149,170,182] where extracellular nutrients further tune their function. This phenomenon is perhaps best exemplified within the tumor microenvironment, where T cells compete for limited nutrient sources, including glutamine and glucose.[200] Many in vitro studies have been informative for understanding the role of nutrients in T helper cell differentiation and function. For instance, defects in T_H1 and T_H17 cell differentiation occur when glutamine or leucine are limited,

owing in part to the modulation of mTORC1 or AMPK (5' adenosine monophosphate-activated protein kinase) activity,[173,201–203] though the precise mechanisms remain elusive. The manipulation of extracellular glucose concentrations can also alter effector T-cell function through multiple mechanisms in vitro. First, AMPK, which is activated when glucose levels are low, regulates the function of T_H1 and T_H17 cells under contexts of nutrient limitations in vitro and following bacterial and viral challenges.[203] Second, glycolytic flux promotes the translation of *Ifng* mRNA by disengaging the glycolytic enzyme GAPDH (glyceraldehyde 3-phosphate dehydrogenase) from its 3'-untranslated region,[204] in addition to mediating the acetylation of H3K9 at the *Ifng* promoter.[196] Third, glucose deprivation diminishes the production of phosphoenolpyruvate, which helps to sustain Ca^{2+}/NFAT signaling that can itself promote glycolytic reprogramming in T cells.[187,205] Fourth, glucose and glutamine metabolism regulate Myc expression by inducing *O*-linked *N*-acetylglucosaminylation.[206] Thus, one functional consequence of glucose and glutamine metabolism is the feedforward reinforcement of transcriptional networks that promote metabolic reprogramming.

While changes in nutrient concentrations are likely to have impacts beyond the tumor microenvironment, we lack a complete understanding of the role of nutrient sensing under physiologically relevant conditions, especially under steady-state conditions. Indeed, the deletion of transporters or anabolic-related enzymes or the in vitro manipulation of these programs (where other nutrients are still likely to be in excess), as described above, cannot fully mimic the dynamics of nutrient sensing and metabolic programming of the tissue microenvironment. Moreover, in vitro systems cannot fully recapitulate the cell–cell interactions or other factors (e.g., cytokines and microbiota) of specific tissues, or if complex microenvironments may shape how T cells respond to nutrients. Therefore, much remains to be discovered about the functional roles of nutrients and metabolic pathways in T helper cell responses, especially in vivo.

HUMAN T HELPER CELLS
Though most of the seminal work in T helper cell differentiation was performed in murine cells, the basic tenants of human CD4+

T-cell differentiation are well conserved across species. On the whole, mouse and human T helper cell subsets are similar with respect to definitive cytokines, master transcriptional regulators, and signaling pathways, as reviewed elsewhere.[207] In contrast to laboratory mice, the human immune system is subjected to continuous and diverse pathogen exposure, which is reflected in the relatively more substantial degree of heterogeneity and plasticity in T helper cells. Additional factors, such as age, genetics, microbiota, and location (among many other factors), further add to this complexity and are an active area of investigation.

The elucidation of human T-cell heterogeneity and plasticity is emerging as a powerful tool to discover new mechanistic targets and to optimize current therapies for patient-specific and/or disease-specific purposes. Techniques, such as scRNA-seq, performed on primary tissues from melanoma patients have demonstrated differential cellular mechanisms of action caused by checkpoint therapies[208] and have enabled the characterization of T-cell subsets that best respond to these therapies.[209] Thus, from a meta-analysis or clinical perspective, the heterogeneity within human T helper cells can be viewed as an opportunity, instead of a caveat, to discover novel mechanisms of T-cell differentiation and function as reviewed in more detail elsewhere.[210,211]

CONCLUDING REMARKS
Over the past three decades, the field of T helper cell differentiation has seen exponential growth. Starting from a relatively simplistic view of T_H1 and T_H2 cells, T helper cell differentiation has become one of the most actively studied areas in immunology. The seminal studies described in this review have laid the foundations for our current understanding of the mechanisms that both prevent and potentially cause immune-mediated diseases. Despite the significant advancements, many important questions remain to be answered.

T helper cells are receptive to many extrinsic and intrinsic signals that influence transcriptional programs and cell identity throughout their lifespan. Master transcriptional networks are cross-regulatory but can also be coexpressed in unique subpopulations. We are beginning to unravel the complex mechanisms of T helper cell plasticity and heterogeneity, which are highly integrated with metabolic regulation and tissue homeostasis. As scRNA-seq and other next-generation techniques become more accessible, these techniques can be used to address large-scale and systems-level questions about the crosstalk between different tissues and the regulation of T helper cell differentiation. Thus, the generation and integration of "big data" will be an integral component in future investigations. Overall, the outlook for this field remains bright, and we look forward in anticipation to what the next 30 years will bring.

ADDITIONAL INFORMATION
Competing interests: The authors declare no competing interests.

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REFERENCES
1. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348–2357 (1986).
2. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. & Toda, M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**, 1151–1164 (1995).
3. Harrington, L. E. et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* **6**, 1123–1132 (2005).
4. Park, H. et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* **6**, 1133–1141 (2005).
5. Cua, D. J. et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421**, 744–748 (2003).
6. Oppmann, B. et al. Novel p19 Protein Engages IL-12p40 to Form a Cytokine, IL-23, with Biological Activities Similar as Well as Distinct from IL-12. *Immunity* **13**, 715–725 (2000).
7. Patel, D. D. & Kuchroo, V. K. Th17 cell pathway in human immunity: lessons from genetics and therapeutic interventions. *Immunity* **43**, 1040–1051 (2015).
8. Chtanova, T. et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J. Immunol.* **173**, 68–78 (2004).
9. Nurieva, R. I. et al. 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 (2008).
10. Kim, C. H. et al. Unique gene expression program of human germinal center T helper cells. *Blood* **104**, 1952–1960 (2004).
11. Bryant, V. L. et al. Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5(+) T follicular helper cells. *J. Immunol.* **179**, 8180–8190 (2007).
12. Schaerli, P. et al. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* **192**, 1553–1562 (2000).
13. Breitfeld, D. et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* **192**, 1545–1551 (2000).
14. Reinhardt, R. L., Liang, H. –E. & Locksley, R. M. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nature Immunology* **10**, 385–393 (2009).
15. Chen, Y., Kuchroo, V. K., Inobe, J., Hafler, D. A. & Weiner, H. L. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* **265**, 1237–1240 (1994).
16. Groux, H. et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742 (1997).
17. Veldhoen, M. et al. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat. Immunol.* **9**, 1341–1346 (2008).
18. Dardalhon, V. et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. *Nat. Immunol.* **9**, 1347–1355 (2008).
19. Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A. & Sallusto, F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* **10**, 857–863 (2009).
20. Trifari, S., Kaplan, C. D., Tran, E. H., Crellin, N. K. & Spits, H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat. Immunol.* **10**, 864–871 (2009).
21. Zeng, H., Zhang, R., Jin, B. & Chen, L. Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. *Cell. Mol. Immunol.* **12**, 566–571 (2015).
22. Kaplan, M. H., Hufford, M. M. & Olson, M. R. The development and in vivo function of T helper 9 cells. *Nat. Rev. Immunol.* **15**, 295–307 (2015).
23. Hsieh, C. S. et al. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* **260**, 547–549 (1993).
24. Seder, R. A. & Paul, W. E. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu. Rev. Immunol.* **12**, 635–673 (1994).
25. Nurieva, R. et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* **448**, 480–483 (2007).
26. Chen, W. et al. Conversion of peripheral CD4+ CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* **198**, 1875–1886 (2003).
27. Bettelli, E. et al. Reciprocal developmental pathways for the generation of pathogenic effector T(H)17 and regulatory T cells. *Nature* **441**, 235–238 (2006).
28. Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M. & Stockinger, B. TGF beta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* **24**, 179–189 (2006).
29. Mangan, P. R. et al. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* **441**, 231–234 (2006).

30. McGeachy, M. J. et al. 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 (2009).

31. Zielinski, C. E. et al. Pathogen-induced human T(H)17 cells produce IFN-gamma or IL-10 and are regulated by IL-1 beta. *Nature* **484**, 514–U139 (2012).

32. Zhou, L. et al. 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 (2007).

33. Korn, T. et al. IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. *Nature* **448**, 484–487 (2007).

34. Chung, Y. et al. Critical Regulation of Early Th17 Cell Differentiation by Inter-leukin-1 Signaling. *Immunity* **30**, 576–587 (2009).

35. Ghoreschi, K. et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature* **467**, 967–U144 (2010).

36. McGeachy, M. J. et al. 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 (2007).

37. Kaplan, M. H., Sun, Y. L., Hoey, T. & Grusby, M. J. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* **382**, 174–177 (1996).

38. Thierfelder, W. E. et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. *Nature* **382**, 171–174 (1996).

39. Kaplan, M. H., Schindler, U., Smiley, S. T. & Grusby, M. J. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* **4**, 313–319 (1996).

40. Takeda, K. et al. Essential role of Stat6 in IL-4 signalling. *Nature* **380**, 627–630 (1996).

41. Shimoda, K. et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* **380**, 630–633 (1996).

42. Afkarian, M. et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. *Nat. Immunol.* **3**, 549–557 (2002).

43. Davidson, T. S., DiPaolo, R. J., Andersson, J. & Shevach, E. M. Cutting edge: IL-2 is essential for TGF-beta-mediated induction of Foxp(3+) T regulatory cells. *J. Immunol.* **178**, 4022–4026 (2007).

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

45. Zhu, J. F., Cote-Sierra, J., Guo, L. Y. & Paul, W. E. Stat5 activation plays a critical role in Th2 differentiation. *Immunity* **19**, 739–748 (2003).

46. Zheng, W. & Flavell, R. A. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* **89**, 587–596 (1997).

47. Zhang, D. H., Cohn, L., Ray, P., Bottomly, K. & Ray, A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. *J. Biol. Chem.* **272**, 21597–21603 (1997).

48. Szabo, S. J. et al. A novel transcription factor, T-bet, directs Th1 lineage com-mitment. *Cell* **100**, 655–669 (2000).

49. Ouyang, W. et al. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* **9**, 745–755 (1998).

50. Usui, T. et al. T-bet regulates Th1 responses through essential effects on GATA-3 function rather than on IFNG gene acetylation and transcription. *J. Exp. Med.* **203**, 755–766 (2006).

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

52. Ouyang, W. et al. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* **12**, 27–37 (2000).

53. Mullen, A. C. et al. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. *Science* **292**, 1907–1910 (2001).

54. Mullen, A. C. et al. Hlx is induced by and genetically interacts with T-bet to promote heritable T(H)1 gene induction. *Nat. Immunol.* **3**, 652–658 (2002).

55. Zhu, J. F. et al. Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses. *Nat. Immunol.* **5**, 1157–1165 (2004).

56. Mukasa, R. et al. Epigenetic instability of cytokine and transcription factor gene loci underlies plasticity of the T helper 17 cell lineage. *Immunity* **32**, 616–627 (2010).

57. Ivanov, II et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* **126**, 1121–1133 (2006).

58. Ciofani, M. et al. A validated regulatory network for Th17 cell specification. *Cell* **151**, 289–303 (2012).

59. Nurieva, R. I. et al. Bcl6 mediates the development of T follicular helper cells. *Science* **325**, 1001–1005 (2009).

60. Johnston, R. J. et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* **325**, 1006–1010 (2009).

61. Yu, D. et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* **31**, 457–468 (2009).

62. Bennett, C. L. et al. The immune dysregulation, polyendocrinopathy, entero-pathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* **27**, 20–21 (2001).

63. Wildin, R. S. et al. X-linked neonatal diabetes mellitus, enteropathy and endo-crinopathy syndrome is the human equivalent of mouse scurfy. *Nature Genetics* **27**, 18–20 (2001).

64. Brunkow, M. E. et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* **27**, 68–73 (2001).

65. Chatila, T. A. et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J. Clin. Invest.* **12**, R75–81 (2000).

66. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003).

67. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003).

68. Wan, Y. Y. & Flavell, R. A. Regulatory T-cell functions are subverted and con-verted owing to attenuated Foxp3 expression. *Nature* **445**, 766–770 (2007).

69. Zhou, L. et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing ROR gamma t function. *Nature* **453**, 236–U214 (2008).

70. Josefowicz, S. Z. et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. *Nature* **482**, 395–399 (2012).

71. Zheng, Y. et al. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature* **463**, 808–812 (2010).

72. Djuretic, I. M. et al. Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. *Nat. Immunol.* **8**, 145–153 (2007).

73. Naoe, Y. et al. Repression of interleukin-4 in T helper type 1 cells by Runx/Cbf beta binding to the Il4 silencer. *J. Exp. Med.* **204**, 1749–1755 (2007).

74. Ono, M. et al. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature* **446**, 685–689 (2007).

75. Rudra, D. et al. Runx-CBF beta complexes control expression of the transcription factor Foxp3 in regulatory T cells. *Nat. Immunol.* **10**, 1170–U1153 (2009).

76. Kitoh, A. et al. Indispensable role of the Runx1-Cbf beta transcription complex for in vivo-suppressive function of FoxP3(+) regulatory T cells. *Immunity* **31**, 609–620 (2009).

77. Zhang, F. P., Meng, G. X. & Strober, W. Interactions among the transcription factors Runx1, ROR gamma t and Foxp3 regulate the differentiation of inter-leukin 17-producing T cells. *Nat. Immunol.* **9**, 1297–1306 (2008).

78. Kano, S. I. et al. The contribution of transcription factor IRF1 to the interferon-gamma-interleukin 12 signaling axis and T(H)1 versus T-H-17 differentiation of CD4(+) T cells. *Nat. Immunol.* **9**, 34–41 (2008).

79. Rengarajan, J. et al. Interferon regulatory factor 4 (IRF4) interacts with NFATc2 to modulate interleukin 4 gene expression. *J. Exp. Med.* **195**, 1003–1012 (2002).

80. Lohoff, M. et al. Dysregulated T helper cell differentiation in the absence of interferon regulatory factor 4. *Proc. Natl. Acad. Sci. USA* **99**, 11808–11812 (2002).

81. Brustle, A. et al. The development of inflammatory T-H-17 cells requires interferon-regulatory factor 4. *Nat. Immunol.* **8**, 958–966 (2007).

82. Bollig, N. et al. Transcription factor IRF4 determines germinal center formation through follicular T-helper cell differentiation. *Proc. Natl. Acad. Sci. USA* **109**, 8664–8669 (2012).

83. Martins, G. A. et al. Transcriptional repressor Blimp-1 regulates T cell home-ostasis and function. *Nat. Immunol.* **7**, 457–465 (2006).

84. Johnston, R. J., Choi, Y. S., Diamond, J. A., Yang, J. A. & Crotty, S. STAT5 is a potent negative regulator of T-FH cell differentiation. *J. Exp. Med.* **209**, 243–250 (2012).

85. Gabrysova, L. et al. c-Maf controls immune responses by regulating disease-specific gene networks and repressing IL-2 in CD4(+) T cells. *Nat. Immunol.* **19**, 497–507 (2018).

86. Rutz, S. et al. Transcription factor c-Maf mediates the TGF-beta-dependent suppression of IL-22 production in T(H)17 cells. *Nat. Immunol.* **12**, 1238–1245 (2011).

87. Xu, J. et al. c-Maf regulates IL-10 expression during Th17 polarization. *J. Immunol.* **182**, 6226–6236 (2009).

88. Ho, I. C., Hodge, M. R., Rooney, J. W. & Glimcher, L. H. The proto-oncogene c-maf is responsible for tissue-specific expression of interleukin-4. *Cell* **85**, 973–983 (1996).

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

90. Bauquet, A. T. et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat. Immunol.* **10**, 167–175 (2009).

91. Wheaton, J. D., Yeh, C. H. & Ciofani, M. Cutting edge: c-Maf is required for regulatory T cells to adopt RORgammat(+) and follicular phenotypes. *J. Immunol.* **199**, 3931–3936 (2017).

92. Yang, X. O. et al. T Helper 17 Lineage Differentiation Is Programmed by Orphan Nuclear Receptors RORα and RORγ³. *Immunity* **28**, 29–39 (2008).

93. Liu, X. et al. Transcription factor achaete-scute homologue 2 initiates follicular T-helper-cell development. *Nature* **507**, 513–518 (2014).

94. Choi, Y. S. et al. LEF-1 and TCF-1 orchestrate T(FH) differentiation by regulating differentiation circuits upstream of the transcriptional repressor Bcl6. *Nat. Immunol.* **16**, 980–990 (2015).

95. Xu, L. et al. The transcription factor TCF-1 initiates the differentiation of T(FH) cells during acute viral infection. *Nat. Immunol.* **16**, 991–999 (2015).

96. Basu, R., Hatton, R. D. & Weaver, C. T. The Th17 family: flexibility follows function. *Immunol. Rev.* **252**, 89–103 (2013).

97. Dominguez-Villar, M. & Hafler, D. A. Regulatory T cells in autoimmune disease. *Nat. Immunol.* **19**, 665–673 (2018).

98. Yosef, N. et al. Dynamic regulatory network controlling T(H)17 cell differentiation. *Nature* **496**, 461–468 (2013).

99. Henriksson, J. et al. Genome-wide CRISPR screens in T helper cells reveal pervasive crosstalk between activation and differentiation. *Cell* **176**, 882–896.e18 (2019).

100. DuPage, M. & Bluestone, J. A. Harnessing the plasticity of CD4(+) T cells to treat immune-mediated disease. *Nat. Rev. Immunol.* **16**, 149–163 (2016).

101. Harbour, S. N., Maynard, C. L., Zindl, C. L., Schoeb, T. R. & Weaver, C. T. Th17 cells give rise to Th1 cells that are required for the pathogenesis of colitis. *Proc. Natl. Acad. Sci. USA* **112**, 7061–7066 (2015).

102. Panzer, M. et al. Rapid in vivo conversion of effector T cells into Th2 cells during helminth infection. *J. Immunol.* **188**, 615–623 (2012).

103. Bending, D. et al. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J. Clin. Invest.* **119**, 565–572 (2009).

104. Jager, A., Dardalhon, V., Sobel, R. A., Bettelli, E. & Kuchroo, V. K. Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J. Immunol.* **183**, 7169–7177 (2009).

105. Lohning, M. et al. Long-lived virus-reactive memory T cells generated from purified cytokine-secreting T helper type 1 and type 2 effectors. *J. Exp. Med.* **205**, 53–61 (2008).

106. Lu, K. T. et al. Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. *Immunity* **35**, 622–632 (2011).

107. Wei, G. et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity* **30**, 155–167 (2009).

108. Hegazy, A. N. et al. Interferons direct Th2 cell reprogramming to generate a stable GATA-3(+)T-bet(+) cell subset with combined Th2 and Th1 cell functions. *Immunity* **32**, 116–128 (2010).

109. Hirota, K. et al. Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat. Immunol.* **12**, 255–263 (2011).

110. Lee, Y. K. et al. Late Developmental Plasticity in the T Helper 17 Lineage. *Immunity* **30**, 92–107 (2009).

111. Weinstein, J. S. et al. TFH cells progressively differentiate to regulate the germinal center response. *Nat. Immunol.* **17**, 1197–1205 (2016).

112. Liston, A. & Gray, D. H. Homeostatic control of regulatory T cell diversity. *Nat. Rev. Immunol.* **14**, 154–165 (2014).

113. Sakaguchi, S., Vignali, D. A., Rudensky, A. Y., Niec, R. E. & Waldmann, H. The plasticity and stability of regulatory T cells. *Nat. Rev. Immunol.* **13**, 461–467 (2013).

114. Koch, M. A. et al. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. *Nat. Immunol.* **10**, 595–U557 (2009).

115. Dominguez-Villar, M., Baecher-Allan, C. M. & Hafler, D. A. Identification of T helper type 1-like, Foxp3(+) regulatory T cells in human autoimmune disease. *Nat. Med.* **17**, 673–675 (2011).

116. Levine, A. G. et al. Stability and function of regulatory T cells expressing the transcription factor T-bet. *Nature* **546**, 421–425 (2017).

117. Feng, Y. Q. et al. Control of the inheritance of regulatory T cell identity by a cis element in the Foxp3 locus. *Cell* **158**, 749–763 (2014).

118. Koch, M. A. et al. T-bet(+) Treg cells undergo abortive Th1 cell differentiation due to impaired expression of IL-12 receptor beta 2. *Immunity* **37**, 501–510 (2012).

119. Yu, F., Sharma, S., Edwards, J., Feigenbaum, L. & Zhu, J. F. Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance. *Nat. Immunol.* **16**, 197–206 (2015).

120. Zheng, Y. et al. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. *Nature* **458**, 351–U116 (2009).

121. Noval Rivas, M. et al. Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy. *Immunity* **42**, 512–523 (2015).

122. Sefik, E. et al. Individual intestinal symbionts induce a distinct population of ROR gamma(+) regulatory T cells. *Science* **349**, 993–997 (2015).

123. Ohnmacht, C. et al. The microbiota regulates type 2 immunity through ROR gamma t(+) T cells. *Science* **349**, 989–993 (2015).

124. Chaudhry, A. et al. CD4(+) regulatory T cells control T(H)17 responses in a Stat3-dependent manner. *Science* **326**, 986–991 (2009).

125. Chung, Y. et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat. Med.* **17**, 983–988 (2011).

126. Wollenberg, I. et al. Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells. *J. Immunol.* **187**, 4553–4560 (2011).

127. Linterman, M. A. et al. Foxp3+ follicular regulatory T cells control the germinal center response. *Nat. Med.* **17**, 975–982 (2011).

128. McClymont, S. A. et al. Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. *J. Immunol.* **186**, 3918–3926 (2011).

129. Komatsu, N. et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. *Nat. Med.* **20**, 62–68 (2014).

130. Tanay, A. & Regev, A. Scaling single-cell genomics from phenomenology to mechanism. *Nature* **541**, 331–338 (2017).

131. Gaublomme, J. T. et al. Single-cell genomics unveils critical regulators of Th17 cell pathogenicity. *Cell* **163**, 1400–1412 (2015).

132. Karmaus, P. W. F. et al. Metabolic heterogeneity underlies reciprocal fates of TH17 cell stemness and plasticity. *Nature* **565**, 101–105 (2019).

133. Wang, C. et al. CD5L/AIM regulates lipid biosynthesis and restrains Th17 cell pathogenicity. *Cell* **163**, 1413–1427 (2015).

134. Lonnberg, T. et al. Single-cell RNA-seq and computational analysis using temporal mixture modeling resolves T(H)1/T-FH fate bifurcation in malaria. *Sci. Immunol.* **2**, pii: eaal2192 (2017).

135. Zemmour, D. et al. Single-cell gene expression reveals a landscape of regulatory T cell phenotypes shaped by the TCR. *Nat. Immunol.* **19**, 291–301 (2018).

136. Li, C. et al. TCR transgenic mice reveal stepwise, multi-site acquisition of the distinctive fat-Treg phenotype. *Cell* **174**, 285.e12–299.e12 (2018).

137. DiSpirito, J. R. et al. Molecular diversification of regulatory T cells in non-lymphoid tissues. *Sci Immunol* **3**, pii: eaat5861 (2018).

138. Im, S. J. et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* **537**, 417–421 (2016).

139. He, R. et al. Follicular CXCR5- expressing CD8(+) T cells curtail chronic viral infection. *Nature* **537**, 412–428 (2016).

140. Leong, Y. A. et al. CXCR5(+) follicular cytotoxic T cells control viral infection in B cell follicles. *Nat. Immunol.* **17**, 1187–1196 (2016).

141. Muranski, P. et al. Th17 cells are long lived and retain a stem cell-like molecular signature. *Immunity* **35**, 972–985 (2011).

142. Shin, B. et al. Effector CD4 T cells with progenitor potential mediate chronic intestinal inflammation. *J. Exp. Med.* **215**, 1803–1812 (2018).

143. Wilhelm, C. et al. An IL-9 fate reporter demonstrates the induction of an innate IL-9 response in lung inflammation. *Nat. Immunol.* **12**, 1071–U1073 (2011).

144. Gagliani, N. et al. TH17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* **523**, 221–U225 (2015).

145. Hirota, K. et al. Plasticity of Th17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. *Nat. Immunol.* **14**, 372–379 (2013).

146. Tsuji, M. et al. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. *Science* **323**, 1488–1492 (2009).

147. Wang, S. et al. MyD88 adaptor-dependent microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. *Immunity* **43**, 289–303 (2015).

148. Kawamoto, S. et al. Foxp3(+) T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity* **41**, 152–165 (2014).

149. Chapman, N. M. et al. mTOR coordinates transcriptional programs and mitochondrial metabolism of activated Treg subsets to protect tissue homeostasis. *Nat. Commun.* **9**, 2095 (2018).

150. Yang, K. et al. Homeostatic control of metabolic and functional fitness of Treg cells by LKB1 signalling. *Nature* **548**, 602–606 (2017).

151. Harrison, O. J. et al. Commensal-specific T cell plasticity promotes rapid tissue adaptation to injury. *Science* **363**, pii: eaat6280 (2019).

152. Rudra, D. et al. Transcription factor Foxp3 and its protein partners form a complex regulatory network. *Nat. Immunol.* **13**, 1010–1019 (2012).

153. Wohlfert, E. A. et al. GATA3 controls Foxp3(+) regulatory T cell fate during inflammation in mice. *J. Clin. Invest.* **121**, 4503–4515 (2011).

154. Schiering, C. et al. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* **513**, 564–568 (2014).

155. Wang, Y., Su, M. A. & Wan, Y. Y. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity* **35**, 337–348 (2011).

156. Amsen, D., van Gisbergen, K., Hombrink, P. & van Lier, R. A. W. Tissue-resident memory T cells at the center of immunity to solid tumors. *Nat. Immunol.* **19**, 538–546 (2018).

157. Naik, S. et al. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* **520**, 104–108 (2015).

158. Linehan, J. L. et al. Non-classical immunity controls microbiota impact on skin immunity and tissue repair. *Cell* **172**, 784.e18–796.e18 (2018).

159. Biton, M. et al. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. *Cell* **175**, 1307.e22–1320.e22 (2018).

160. Ali, N. et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell* **169**, 1119.e11–1129.e11 (2017).

161. Hirata, Y. et al. CD150(high) bone marrow tregs maintain hematopoietic stem cell quiescence and immune privilege via adenosine. *Cell Stem Cell* **22**, 445.e5–453.e5 (2018).

162. Scharschmidt, T. C. et al. A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity* **43**, 1011–1021 (2015).

163. Campbell, C. et al. Extrathymically generated regulatory T cells establish a niche for intestinal border-dwelling bacteria and affect physiologic metabolite balance. *Immunity* **48**, 1245.e9–1257.e9 (2018).

164. Mehta, M. M., Weinberg, S. E. & Chandel, N. S. Mitochondrial control of immunity: beyond ATP. *Nat. Rev. Immunol.* **17**, 608–620 (2017).

165. Geltink, R. I. K., Kyle, R. L. & Pearce, E. L. Unraveling the complex interplay between T cell metabolism and function. *Annu. Rev. Immunol.* **36**, 461–488 (2018).

166. Bantug, G. R., Galluzzi, L., Kroemer, G. & Hess, C. The spectrum of T cell metabolism in health and disease. *Nat. Rev. Immunol.* **18**, 19–34 (2018).

167. Gerriets, V. A. et al. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J. Clin. Invest.* **125**, 194–207 (2015).

168. Shi, L. Z. et al. HIF1a-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J. Exp. Med.* **208**, 1367–1376 (2011).

169. Michalek, R. D. et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J. Immunol.* **186**, 3299–3303 (2011).

170. Zeng, H. et al. mTORC1 and mTORC2 kinase signaling and glucose metabolism drive follicular helper T cell differentiation. *Immunity* **45**, 540–554 (2016).

171. Ray, J. P. et al. The interleukin-2-mTORc1 kinase axis defines the signaling, differentiation, and metabolism of T helper 1 and follicular B helper T cells. *Immunity* **43**, 690–702 (2015).

172. DiToro, D. et al. Differential IL-2 expression defines developmental fates of follicular versus nonfollicular helper T cells. *Science* **361**, pii: eaao2933 (2018).

173. Johnson, M. O. et al. Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. *Cell* **175**, 1780.e19–1795.e19 (2018).

174. Berod, L. et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat. Med.* **20**, 1327–1333 (2014).

175. Endo, Y. et al. Obesity drives Th17 cell differentiation by inducing the lipid metabolic kinase, ACC1. *Cell Rep.* **12**, 1042–1055 (2015).

176. Weinberg, S. E. et al. Mitochondrial complex III is essential for suppressive function of regulatory T cells. *Nature* **565**, 495–499 (2019).

177. Raud, B. et al. Etomoxir actions on regulatory and memory T cells are independent of Cpt1a-mediated fatty acid oxidation. *Cell Metab.* **28**, 504–515 (2018).

178. Zeng, H. et al. mTORC1 couples immune signals and metabolic programming to establish T-reg-cell function. *Nature* **499**, 485–490 (2013).

179. Wei, J. et al. Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. *Nat. Immunol.* **17**, 277–285 (2016).

180. Shrestha, S. et al. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. *Nat. Immunol.* **16**, 178–187 (2015).

181. Huynh, A. et al. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. *Nat. Immunol.* **16**, 188–196 (2015).

182. Kishore, M. et al. Regulatory T cell migration is dependent on glucokinase-mediated glycolysis. *Immunity* **47**, 875.e10–889.e10 (2017).

183. Yang, K. et al. T cell exit from quiescence and differentiation into Th2 cells depend on Raptor-mTORC1-mediated metabolic reprogramming. *Immunity* **39**, 1043–1056 (2013).

184. Tan, H. Y. et al. Integrative proteomics and phosphoproteomics profiling reveals dynamic signaling networks and bioenergetics pathways underlying T cell activation. *Immunity* **46**, 488–503 (2017).

185. Wang, R. N. et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* **35**, 871–882 (2011).

186. Frauwirth, K. A. et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* **16**, 769–777 (2002).

187. Vaeth, M. et al. Store-operated Ca(2+) entry controls clonal expansion of T cells through metabolic reprogramming. *Immunity* **47**, 664.e6–679.e6 (2017).

188. Ross, S. H. et al. Phosphoproteomic analyses of interleukin 2 signaling reveal integrated JAK kinase-dependent and -independent networks in CD8(+) T cells. *Immunity* **45**, 685–700 (2016).

189. Dang, E. V. et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. *Cell* **146**, 772–784 (2011).

190. Doedens, A. L. et al. Hypoxia-inducible factors enhance the effector responses of CD8(+) T cells to persistent antigen. *Nat. Immunol.* **14**, 1173–1182 (2013).

191. Phan, A. T. et al. Constitutive glycolytic metabolism supports CD8(+) T cell effector memory differentiation during viral infection. *Immunity* **45**, 1024–1037 (2016).

192. Clever, D. et al. Oxygen sensing by T cells establishes an immunologically tolerant metastatic niche. *Cell* **166**, 1117.e14–1131.e14 (2016).

193. Delgoffe, G. M. et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* **30**, 832–844 (2009).

194. Delgoffe, G. M. et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat. Immunol.* **12**, 295–303 (2011).

195. Lee, K. et al. Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* **32**, 743–753 (2010).

196. Peng, M. et al. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* **354**, 481–484 (2016).

197. Chapman, N. M. & Chi, H. Hallmarks of T-cell exit from quiescence. *Cancer Immunol. Res.* **6**, 502–508 (2018).

198. Chisolm, D. A. et al. CCCTC-binding factor translates interleukin 2- and alpha-ketoglutarate-sensitive metabolic changes in T cells into context-dependent gene programs. *Immunity* **47**, 251.e7–267.e7 (2017).

199. Gerriets, V. A. et al. Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat. Immunol.* **17**, 1459–1466 (2016).

200. Buck, M. D., Sowell, R. T., Kaech, S. M. & Pearce, E. L. Metabolic instruction of immunity. *Cell* **169**, 570–586 (2017).

201. Sinclair, L. V. et al. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat. Immunol.* **14**, 500–508 (2013).

202. Nakaya, M. et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* **40**, 692–705 (2014).

203. Blagih, J. et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* **42**, 41–54 (2015).

204. Chang, C. H. et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* **153**, 1239–1251 (2013).

205. Ho, P. C. et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* **162**, 1217–1228 (2015).

206. Swamy, M. et al. Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. *Nat. Immunol.* **17**, 712–720 (2016).

207. Schmitt, N. & Ueno, H. Regulation of human helper T cell subset differentiation by cytokines. *Curr. Opin. Immunol.* **34**, 130–136 (2015).

208. Wei, S. C. et al. Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. *Cell* **170**, 1120.e17–1133.e17 (2017).

209. Sade-Feldman, M. et al. Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* **176**, 404 (2019).

210. Sallusto, F. Heterogeneity of human CD4(+) T cells against microbes. *Annu. Rev. Immunol.* **34**, 317–334 (2016).

211. Davis, M. M. & Brodin, P. Rebooting human immunology. *Annu. Rev. Immunol.* **36**, 843–864 (2018).