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T-helper cells flexibility: the possibility of reprogramming T cells fate

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Various disciplines cooperate to find novel approaches to cure impaired body functions by repairing, replacing, or regenerating cells, tissues, or organs. The possibility that a stable differentiated cell can reprogram itself opens the door to new therapeutic strategies against a multitude of diseases caused by the loss or dysfunction of essential, irreparable, and specific cells. One approach to cell therapy is to induce reprogramming of adult cells into other functionally active cells. Understanding the factors that cause or contribute to T cell plasticity is not only of clinical importance but also expands the knowledge of the factors that induce cells to differentiate and improves the understanding of normal developmental biology. The present review focuses on the advances in the conversion of peripheral CD4+ T cells, the conditions of their reprogramming, and the methods proposed to control such cell differentiation.

KEYWORDS
CD4 T cells, Th17, Treg, plasticity, differentiation, reprogramming

Introduce

It has long been believed that mature T-helper (Th) lymphocytes are terminally differentiated cells of functionally distinct subpopulations that differ in transcriptional and cytokine profiles. However, recent studies have shown that T cells have varying degrees of plasticity, allowing them to adapt to specific challenges and acquire new characteristics and functions in immune responses, depending on the tissue microenvironment. Surprisingly, lymphocytes recognized as terminally differentiated Th1 and Th2 can change their functional specialization under certain microenvironmental conditions. Foxp3-expressing regulatory T cells (Tregs) was previously thought to be remarkably stable under both basal and inflammatory conditions (1, 2). But several recent studies have shown that changes in expression and stability FoxP3 during inflammation can alter the phenotypic properties of Tregs, converting Tregs effector function, thus confirming a certain degree of plasticity cells (3–7). T-helper 17 cells (Th17) are largely unstable and can reprogram into many types of T-helper lymphocytes. Determining the possible plasticity of T cell subpopulations in various tissues is highly relevant for future therapeutic interventions in such diverse immune pathologies as chronic viral infections, cancer,

and autoimmune diseases. In this review the authors will not discuss the processes occurring in the thymus during positive and negative selection, and stability and flexibility CD8+ cytotoxic T cells as they are widely analyzed in other publications (8–11). The present review focuses on the advances in the conversion of peripheral CD4+ T cells, the conditions of their reprogramming, and the methods proposed to control such cell differentiation.

The differentiation of T-helper lymphocyte subpopulations in the periphery.

T cells differ from most somatic cells due to continued differentiation in adulthood depending on the encountered antigen, as well as the ability to reprogram between different CD4+ T-helper cell lineages (12–18). CD4+ T-lymphocytes has the great potential to perceive various activating signals such as cytokines, chemokines, and other environmental factors, in response to which cascades of effector programs are triggered. Traditionally, the activation process of naïve T lymphocytes begins after 3 subsequent signals (19, 20): 1. T-cell receptors (TCRs) recognize antigens presented on the surface of cells in a complex with MHC. 2. Interaction of costimulatory molecules (CD4/CD8/CD28, etc.). 3. Creation of a cytokine microenvironment. Depending on the type of signal, the naive CD4+ T cell starts to express various transcription factors leading to the activation of transcription and translation of cytokine and chemokine genes necessary for eliminating specific pathogens or preventing immune-mediated pathologies (see Table 1).

TCR signaling plays a critical role in the selection of differentiation lineages of various CD4+ T-cell subpopulations. The T-cell receptor TCR belongs to the family of immunoreceptors. It consists of 2 chains: α/β or γ/δ. In this review, α/β TCRs will be described, and all further references to "TCR" will refer specifically to the αβ TCR. We will not discuss molecular models of TCR signaling initiation, which leads to different cellular responses, as they are widely analyzed in other publications (96–98). A brief description of intracellular events that occur during the interaction of TCR and pMHC is described in Box 1. The table summarizes some data on the role of different signaling pathways and transcription factors, in the differentiation of T lymphocyte subpopulations [adapted and added from 99)].

In doing so, the affinity strength of antigens to TCRs was shown to be sufficient to induce differences in the physiology of differentiated T cells. When naïve CD4+ T cells are subjected to strong TCR stimulation, differentiation of Th1 preferentially proceeds, both *in vitro* and *in vivo* (100–102). Conversely, weak TCR signaling favors Th2 cell differentiation (101, 103). Besides, the strength of TCRs signaling influence on initial cytokine production: low antigen concentrations trigger interleukin (IL)-4-independent IL-4 production during the first 24 hours after T cell engagement, whereas stimulation with high concentrations suppresses early IL-4 production but enhances interferon production (IFN)-γ (19). The issue of whether differences in the strength of TCRs signaling affect

TABLE 1 Role of signaling pathways and transcription factors in the differentiation of T lymphocyte subpopulations.

Pathway	Role	References
Ras-ERK1/2-AP-1	early determination of the $CD8^+$ T cell memory	(21)
	proliferation and Th1 differentiation *in vitro;* inhibition of regulatory T cell (Treg) differentiation	(22)
	BATF regulate the differentiation of T helper (Th)17 cells and the conversion of $CD4^+Foxp3^+$ cells to $CD4^+IL\text{-}17^+$ cells.	(23)
	JunB activates the expression of Th17 lineage-specifying genes and coordinately represses genes controlling Th1 and regulatory T-cell fate.	(24)
IP_3 -Ca^{2+} -NFAT	Ca^{2+} signals control proliferation, differentiation, apoptosis, and a variety of transcriptional programs	(25–27)
	development and function of regulatory T cells	(28)
PKCθ-IKK-NF-κB	PKCθ is recruited to the immunologic synapse between APC and T cell, triggering T cell activation activation and proliferation of mature B and T cells	(29–34)
	cytoskeletal polarization in T cells	(35)
	PDK1 is essential for integrating the TCR and CD28 signals and activating NF-κB and PKCθ in T-cells.	(36–39)
TSC1/2-mTOR	mTOR is involved in the activation, differentiation, and function of effector T cells (Th1, Th2, Th17), but blocks Treg cell formation	(40–42)
Phosphatases	Phosphorylation and dephosphorylation of TCR signaling molecules affect the formation of signaling complexes and the propagation of TCR signals	(43–45)
	SHP-1 negatively regulates the differentiation process from naïve T cells to Th1 or Th2 effector T cells and/or the proliferation of differentiated T cells	(46)
	SHP2 is to suppress the differentiation of T cells to the Th2 phenotype.	(47)
	Forced expression of miR-181a enhances the TCR response in mature T cells, making activation by antagonistic ligands possible	(48)
	Suppression of *Ptpn22* (SHP2 gene) increases the expansion and function of effector/memory T cells	(49)

(Continued)

TABLE 1 Continued

Pathway	Role	References
	Recruitment of the inhibitor phosphatases PP2A and SHP2 is involved in the induction of partial anergy of T_{reg} cells in response to TCR and CD28 stimulation	(50)
	DUSP2 inhibits signaling through STAT3 and restricts Th17 differentiation	(51)
	PTPN2 may support memory CD4+ T-cell responses by shaping memory effector functions or prolonging lymphocyte survival acting on STAT1	(52)
Ubiquitination and degradation	Roquin1/2 ligases maintain immune tolerance and block differentiation of effector and Tfh cells	(53)
	LMP7 (part of the immunoproteasome) promotes Th1 and Th17 differentiation, has no effect on Th2 cells, and blocks Treg cells	(54)
	overexpression of Stub1 in Treg cells abrogated their ability to suppress inflammatory immune responses in vitro and in vivo and conferred a T1-like phenotype by promoting degradation of the Foxp3	(55)
	E3 ligases, including Cbl-b, the gene related to anergy in lymphocytes	(56)
DAG kinases	loss of DGKα and/or DGKζ leading to hyperactivation, impaired induction of anergy	(57, 58)
	loss of DGKζ enhanced TCR signaling, and increased generation of nT_{reg} cells in mice.	(59)
Ikaros (including Helios and Aiolos)	In mice, depending on the mutation in Ikaros, the number of T, B, and NK lymphocytes and their early precursors are absent or significantly reduced, but impaired hyperproliferation and differentiation of CD4 lymphocytes may be observed	(60, 61)
GATA-3	Th2 cell differentiation, deletion of Gata-3 in early or late stage thymocytes showed the arrest of the DN3 population with decreased DN4, DP, and SP populations or impaired differentiation into CD4+ T cells, respectively	(62–64)
	Gata-3 induces expression of the *Zbtb7b* gene, encoding a ThPOK transcription factor that inhibits differentiation of DP-thymocytes into CD8-SP cells and promotes differentiation into CD4-SP cells	(65)
Notch	Provides differentiation of common lymphoid precursors into T-lymphocytes. In the absence of Notch, B-lymphocytes are formed	(66, 67)

(Continued)

TABLE 1 Continued

Pathway	Role	References
STAT (signaling transducer and activator of transcription)		
STAT1	STAT1 Is Required for IL-6–Mediated Bcl6 Induction Tfh	(68)
	Inhibits differentiation of Th17 lymphocytes	(69)
	Together with Tbet, it is a key factor in Th1 lineage differentiation, IFNγ-STAT1-T-bet pathway	(70, 71)
STAT3	Required for RORγt induction and Th17 lymphocyte differentiation	(72)
STAT4	IL-12 activates STAT4, which is critical for Th1 lymphocytes. Stat4-deficient lymphocytes differentiate predominantly into Th2 lymphocytes	(73, 74)
	STAT4 is important for activating the function of IL-23-stimulated Th17 pathogenic lymphocytes	(75)
STAT5	Activation of STAT5 by IL-2 stimulates differentiation of Th1, Th2, and Th9 cells but suppresses the development of Th17 and Tfh cells	(76–82)
	Regulates FoxP3 expression and Treg differentiation	(83)
STAT6	Activation of STAT6 under the influence of IL-4 triggers Th2 lymphocyte differentiation	(84–86)
	STAT6 suppresses FoxP3 expression and differentiation of iTreg cells	(87, 88)
Blimp1	Blimp-1 suppresses Tfh cell differentiation through suppression of Bcl-6	(89, 90)
Bcl-6	Tfh cell differentiation, master regulator	(90–92)
Bcl-3	Suppresses Th9 differentiation through limiting glutathione availability	(93)
BATF	Binds to the *Il6* promoter and enhances IL-6 cytokine secretion	(94)
	Together with STAT3, it is required for RORγt induction and Th17 lymphocyte differentiation	(95)

Th17 cell differentiation remains controversial (104). It should be noted that the strength of TCR signaling also regulates the differentiation of regulatory T cells (Treg) (105, 106). A low density of high-affinity ligands is important for the stable induction of peripheral Treg cells (107, 108). Longer TCR-pMHC residence time as well as high-affinity TCRs are positively associated with follicular helper T cell (Tfh) differentiation (102, 109). Stable Th 9 function requires sustained TCR signaling and the IL-9 secretion (110, 111).

An important principle of CD4+ T-cell differentiation is that one of the characteristic cytokines produced by each differentiated cell

BOX 1 Intracellular events TCRs initiation.

The TCR consists of an extracellular region, a transmembrane region, and a shorter cytoplasmic tail. At the same time, none of the TCR chains has internal kinase activity or the ability to interact with non-receptor tyrosine kinases (Mariuzza et al., 2020). The TCR forms complexes with δ-, γ-, ε- and ζ-chains of CD3, essential for cell surface expression and intracellular signaling. The interaction between the extracellular domains of CD3 immunoglobulin subunits and TCR chains is necessary for the formation of the antigen-recognition complex of T cells. Despite the presence of longer cytoplasmic fragments, CD3 chains also lack the enzymatic activity that would support intracellular signal transduction during antigen recognition. For this purpose, co-receptor CD4 are located near the TCR chains, which bind the TCR to the Src family kinases Lck and Fyn, which engage and phosphorylate the CD3 immunoreceptor tyrosine-based activation motif (ITAM) complex and initiate the downstream signaling cascade, ultimately leading to T cell survival, differentiation, and effector functions (Walk et al., 1998). The combinatorial mutation of ITAM TCR demonstrated their central importance for T cell development and function (Bettini et al., 2017; Holst et al., 2008; Love & Hayes, 2010). Decreased ITAMs lead to increased Treg formation in mice (M. O. Li & Rudensky, 2016). Phosphorylation of ITAMs enables recruitment of the TCR-associated protein Zeta-chain-associated protein kinase 70 (Zap70), which is then phosphorylated by Lck. Zap70 then phosphorylates four key sites on the linker for activation of T cells (LAT), which allows the proteins to be recruited to the LAT signalosome. The subsequent effect is the activation of the Rat sarcoma (Ras)/extracellular signal-related kinase (Erk)/Activator protein 1 (AP-1) pathway, Protein kinase C-θ (PKCθ)/κB kinase (IKK)/nuclear factor-κB (Nf-κB) pathway, and the calcium-dependent Calcineurin/nuclear factor of activated T cells (NFAT) pathway (Hwang et al., 2020; Shah et al., 2021). Transcription factors downstream of these pathways, NFAT, Nf-κB, and AP-1 contribute to IL-2 transcription as well as to IL-2RA transcription, which encodes the α-chain of the IL-2 receptor (CD25) (Y. Li et al., 2022). Phosphorylation and dephosphorylation of TCR signaling molecules such as Syk and ZAP-70, as well as ubiquitination and degradation of CD3ζ, PKCθ, ZAP-70, phospholipase C-γ1, and phosphoinositide-3-kinase, negatively regulate TCR signaling pathways. Signal transducer and activator of transcription (STAT) proteins control clone-specific transcription factor expression and also control epigenetic changes, such as histone modifications or DNA methylation, which open specific DNA sites for transcription.

also plays a critical role in the induction of such cells, potentially providing a powerful positive feedback loop (Figure 1). These "feedback" cytokines are IFN-γ for Th1, IL-4 for Th2, IL-21 for Th17, and TGF-β for iTreg. Thus, exposure to IL-12 or IFN-γ activates STAT-1 and triggers the expression of the transcription factor T-bet, which is required for Th1 lymphocyte differentiation (112). IL-4 signals activate STAT6 and further Gata3 required for polarization toward Th2 lymphocytes (J. 113). However, if TGF-β is additionally present in the medium, other transcription factors, PU.1 and IRF4, are activated and direct differentiation toward Th9 lymphocytes (114). A combination of TGF-β and IL-6 or IL-21, via the STAT3 pathway (115), induces the expression of RORγt and directs CD4+ differentiation toward Th17 (116, 117). Besides, TGF-β is essential for Treg cell differentiation through its effect on the FoxP3

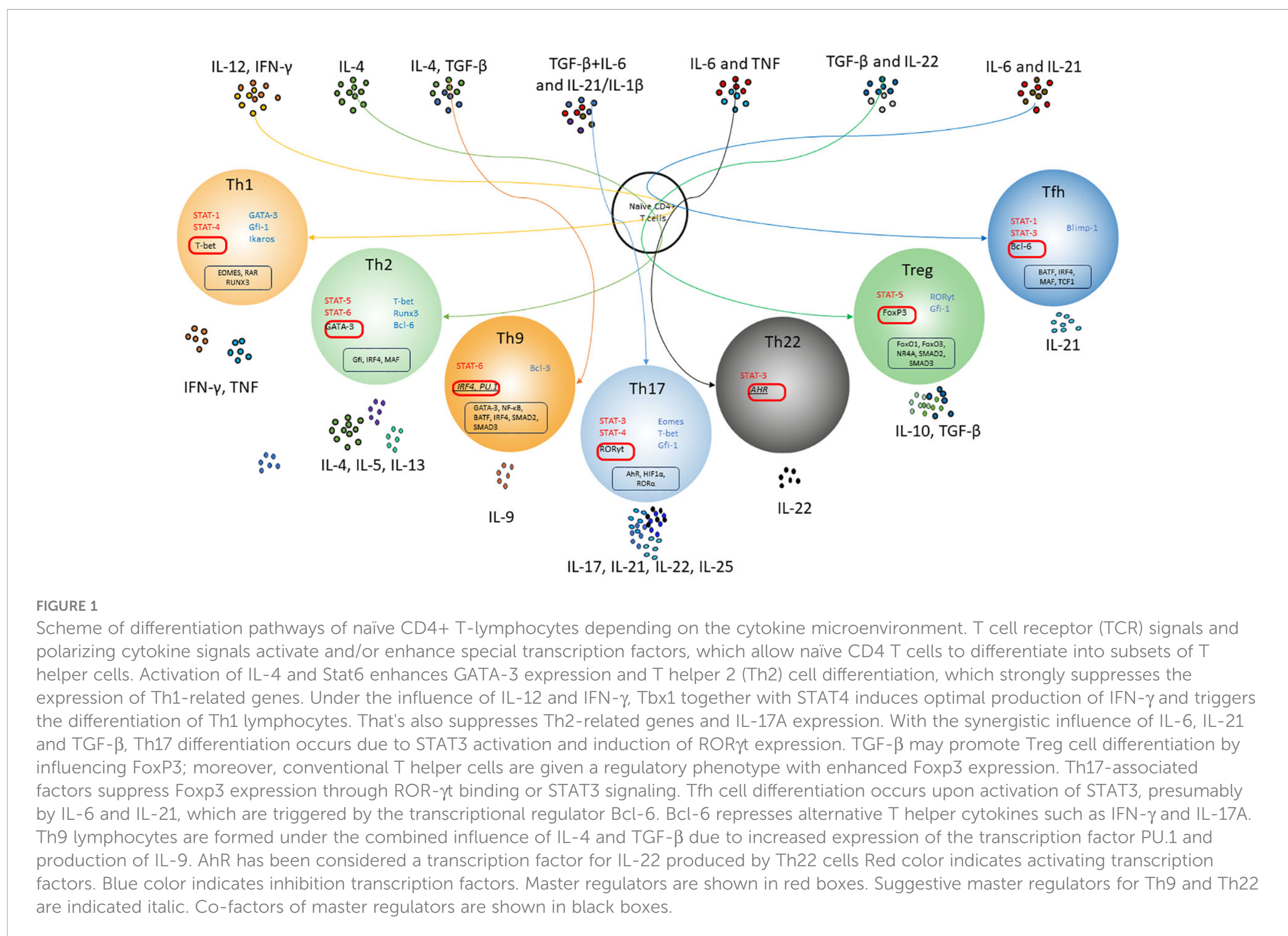

FIGURE 1
Scheme of differentiation pathways of naïve CD4+ T-lymphocytes depending on the cytokine microenvironment. T cell receptor (TCR) signals and polarizing cytokine signals activate and/or enhance special transcription factors, which allow naïve CD4 T cells to differentiate into subsets of T helper cells. Activation of IL-4 and Stat6 enhances GATA-3 expression and T helper 2 (Th2) cell differentiation, which strongly suppresses the expression of Th1-related genes. Under the influence of IL-12 and IFN-γ, Tbx1 together with STAT4 induces optimal production of IFN-γ and triggers the differentiation of Th1 lymphocytes. That's also suppresses Th2-related genes and IL-17A expression. With the synergistic influence of IL-6, IL-21 and TGF-β, Th17 differentiation occurs due to STAT3 activation and induction of RORγt expression. TGF-β may promote Treg cell differentiation by influencing FoxP3; moreover, conventional T helper cells are given a regulatory phenotype with enhanced Foxp3 expression. Th17-associated factors suppress Foxp3 expression through ROR-γt binding or STAT3 signaling. Tfh cell differentiation occurs upon activation of STAT3, presumably by IL-6 and IL-21, which are triggered by the transcriptional regulator Bcl-6. Bcl-6 represses alternative T helper cytokines such as IFN-γ and IL-17A. Th9 lymphocytes are formed under the combined influence of IL-4 and TGF-β due to increased expression of the transcription factor PU.1 and production of IL-9. AhR has been considered a transcription factor for IL-22 produced by Th22 cells Red color indicates activating transcription factors. Blue color indicates inhibition transcription factors. Master regulators are shown in red boxes. Suggestive master regulators for Th9 and Th22 are indicated italic. Co-factors of master regulators are shown in black boxes.

transcription factor (118). In the absence of TGF-β, IL-6 and IL-21 also induce the expression of another transcription factor, Bcl6, the major transcription factor of Tfh cells, via STAT3 (119). STAT3, stimulated by IL-6 and TNF, is required for the differentiation of Th22 cells expressing AhR as their major transcription factor (120).

Thus, CD4+ T-cell plasticity is influenced by a number of external (e.g., cytokines, various metabolites) and internal factors. Effector CD4+ T-lymphocytes depending on the cytokine microenvironment can develop into different subpopulations of cells with specific functions. It is worth noting that a meta-analysis of genes previously associated with Th1, Th2, Th17, and Treg populations showed that except for master regulator genes (T-bet, GATA-3, Bcl-6, FoxP3 and RORγt), all other genes considered specific showed significant variability (121). This confirms the ability of previously polarized T cells to change their phenotype and repolarize toward other differentiation lineages.

Possibilities of reprogramming CD4+ lymphocyte subpopulations

For a long time, it was believed that different subpopulations of CD4+ T cells were terminal lines of differentiation of T-helper cells. However, accumulating data show the plasticity of CD4+ T cells and their ability to acquire different properties and functions (122, 123). T-helper lymphocytes can express not only their clonally specific cytokines and Transcription factors but also simultaneously markers of other T helper lineages, making them similar to hybrid cells (124). This is especially shown for Th17-Treg pairs (125, 126) and Th17-Th1 cells (127, 128). Still, there is also evidence for reciprocal reprogramming of other Th lines, which will be discussed further.

Mutual plasticity of Th1-Th2 lymphocytes

The earliest discovered and most studied subpopulations of helper T lymphocytes are Th1 and Th2 cells. Th1 and Th2 subsets seem to be the most stable, as they are regulated by mutually repressive and self-amplifying transcription and signaling factors (T-bet and IFN-γ for Th1 and GATA-3 and IL-4 for Th2) (19, 100–103). However, it was soon shown that forced expression of the GATA3 regulator GATA3 in Th1-lymphocytes using the viral vector Th2-master induced the production of Th2-cytokines (IL-4 and IL-5), as well as CCR4. At the same time, transduced cells partially preserve their Th1-specific profile (expression of IFN-γ and IL-12Rβ2) (129). Conversely, expression of T-bet (Tbx21) in Th2 cells promoted a Th1 phenotype. It was also shown that memory CD4+ T cells under repeated stimulation *in vivo* were able to produce cytokines of the oppositional lineage, indicating the existing functional plasticity in T helper cell responses (130). However, this flexibility decreases as CD4+ lymphocytes mature (131), which, in general, is characteristic of the differentiation dynamics of any multipotent progenitor cells. This is thought to occur due to chromatin remodeling in cytokine gene loci to increase the efficiency of effector cytokine production and inhibition of opposing cytokine programs (132).

Chromatin modification is responsible for the suppression of the differentiation program of oppositional Th cell lines. Thus, T-bet activation in Th1 causes loss of HDAC-Sin3A at the *Ifng* locus and promotes IFN-γ expression (133). In Th2 cells, IFN-g production is suppressed due to the deposition of the repressive histone mark H3K27me3 to the *Ifng* locus (134). Demethylase Jmjd3 changes in histone methylation (H3K27me3 and H3K4me3 levels) in target genes and regulates Th1, Th2, and Th17 lymphocyte differentiation. Deletion of Jmjd3 results in Th2 and Th17 differentiation and blocks Th1 differentiation (135). After activation and differentiation of human Th2 cells, the permissive marks H3K9 acetylation and H3K4me3 were increased in the respective loci of the Th2-specific genes *Il-4, Il-5, and Il-13* (77).

Experimental studies showed that differentiated Th1 cells under the influence of IL-4 or helminths can be converted into an IFN-γ-producing Th2 lineage (136, 137). Conversely, treatment of Th2 lymphocytes with IL-12 and anti-IL-4 induces repolarization toward Th1 cells (138, 139). However, it is possible that reprogramming of the Th1 phenotype to the Th2 phenotype may reflect the growth of the Uncommitted Precursors population rather than the growth of the Th subpopulation (138). Another study shows that infections of Th2 lymphocytes with lymphocytic choriomeningitis virus, known for its strong induction of type I and type II interferons, results in the reprogramming toward GATA-3 + T-bet + Th1-lymphocytes capable of producing Th1 and Th2 cytokines (140). It was also shown that deletion of the IL-10 gene in naïve CD4+ lymphocytes led to Th1 lymphocyte differentiation even under Th2 differentiation conditions (141).

As mentioned previously, TCR and costimulatory signals can also influence plasticity between CD4+ T-cell subsets. TCR stimulation is ubiquitously required for cytokines to reprogram T cell subpopulations (140). The weaker stimulation of TCRs during priming *in vivo* makes possible significant plasticity in their cytokine production upon reactivation (142). Depending on signal strength and recall, the antigen dose also redirects subsets of T helper cells: higher antigen concentration promotes Th2 cell phenotypes in memory Th1 cells (143). At low antigen concentrations, Th2-lymphocytes differentiate with the participation of Itk kinase. Cells deprived of Itk kinase, even under conditions of stimulation with low levels of antigen, show increased expression of T-bet and differentiate into Th1 (144).

Plasticity of Th1/Th2 to other Th lymphocytes

There are published data on mutual transitions of Th9, Tfh, and Th22 into Th1/Th2 lymphocytes. The flexibility of all the above populations with Th17 cells will be considered separately due to the great plasticity of Th17 lymphocytes.

Treatment of Th2 lymphocytes with TGF-β causes the loss of their characteristic profile and induces the secretion of IL-9, and in combination with IL-4, it drives the differentiation of Th9 cells (145). On the other hand, under Th1 culture conditions (in the presence of IL-12), Th9 lymphocytes can acquire a Th1 phenotype and produce IFN-γ *in vivo* (146, 147). Polyamines play a critical role in the regulation of Th2/Th9 balance. Endogenously generated

polyamines enhance GATA-3 expression and promote Th2 cell differentiation (148). Under polyamine deficiency, even under Th2 environmental conditions, expression of Th9-related genes (*Il9, Irf4, and Batf3*) is enhanced through suppression of GATA-3.

Helminth load results in reprogramming of Th2 lymphocytes into the Tfh population, via the Smad3/Smad4 and IRF-4 activation pathway (149, 150), and expresses the canonical Tfh markers Bcl6 and IL-21, as well as GATA3, a master regulator of Th2 cell differentiation (151). On the other hand, the formation of Th2 lymphocytes from IL-4-producing Tfh lymphocytes was shown in allergic asthma *in vivo*. Impairment of Tfh cell responses during the sensitization phase or Tfh cell depletion prevented Th2 cell-mediated responses following the challenge (152). In addition, pre-differentiated Tfh cells in Th1-, Th2-, or Th17-conditions acquire the ability to produce IFN-γ, IL-4, or IL-17, respectively, while retaining their Tfh potential (capacity to produce IL-21) (153).

Tfh can also be reprogrammed toward Tr1 cells *in vivo* upon Blimp1 upregulation (154). It was also shown that deletion of the IL-6 gene in naïve CD4+ lymphocytes led to Tr1 differentiation even under Th2 differentiation conditions (141). Th1 lymphocytes under IL-12 hyperstimulation and under conditions of high TCR ligation/chronic infection can also switch from proinflammatory effector cells to Tr1 cells producing IL-10 (155–157). It is important to note that IL-10 production by CD4+FoxP3- T-lymphocytes is considered to be the main marker of the Tr1 cell population. To date, data on cytokine production by different subpopulations of Th cells (Th1, Th2, and Th17) were obtained. Such IL-10+Th cells can control their own effector functions by turning IL-10 production on and off. Thus, Tr1 cells can be very heterogeneous and do not represent a separate lineage.

Taken together, the present data demonstrate the existing flexibility of Th1 and Th2, capable of acquiring different functional and phenotypic properties upon repeated antigen stimulation and under the influence of the respective microenvironment.

Mutual plasticity of Treg and Th17 lymphocytes

Th17 and peripheral Treg differentiation are closely linked and are likely necessary to maintain tolerance and prevent the development of inflammatory diseases (158). Data from fate-mapping experiments in mouse models identified transdifferentiation events of Th17-to-T regulatory cells (12). In humans with autoimmune diseases, an increase in the number of IL-17-producing FoxP3+ Treg cells in peripheral blood correlates with disease severity (4, 159–162). A better understanding of the mechanisms underlying Th17-Treg transdifferentiation in the human condition may be critical for resolving inflammation in autoimmune diseases.

Upon stimulation with TGF-β, naive CD4+ T cells undergo a dual expression stage of the transcription factors Foxp3 and RORγT (163–165) and, depending on the microenvironmental factors, differentiate into Treg or Th17 cells. High levels of TGF-β, retinoic acid, and IL-2 activate Foxp3 and support the differentiation of Treg cells (6). Combination of TGF-β and IL-6 or IL-21, via the pyruvate kinase M2 and STAT3 pathway (115), induces the expression of RORγt and enhances CD4+ differentiation toward Th17 (116, 117, 166, 167), by inhibiting the expression of Foxp3 (X. 168). Decreased STAT3 activation upon treatment of T cells with CK2 inhibitors results in decreased expression of the IL-23 receptor, required for optimal differentiation and maintenance of Th17, and increased differentiation of FoxP3+ Treg cells (169, 170). Enhancement of aryl hydrocarbon receptor (AHR) expression under the influence of TGF-β promotes phosphorylation of SMAD2/3 and STAT3, enhances Th17 lymphocyte differentiation, and blocks Th1 phenotype cell differentiation (IL-2 and T-bet) (167). On the other hand, AhR promotes transactivation of *Il10*, and could potentially reprogram inflammatory Th17 lymphocytes toward IL-10+ Tr1 and termination of the immune response (12). In addition, the master regulators themselves are in reciprocal interactions: RORγT interacts directly with exon 2 of the Foxp3 gene to suppress development and activate Th17 cytokine transcription. Similarly, Foxp3 can bind RORγT to suppress IL-17 production (168).

The Tec family kinase, Itk, plays an important role in Treg/Th17 differentiation (144, 171, 172). Itk deletion, even in a Th17-conditioned microenvironment, decreases Th17 lymphocyte differentiation but increases expression (171, 173). In addition, Itk -/- CD4 T cells gave an increase in the number of Treg cells when cultured under inducible Treg conditions. This was associated with decreased activation of the Akt/mammalian target of the rapamycin (mTOR) pathway and increased sensitivity to IL-2 (171). mTOR and HIF-1 are well-known integrators of metabolic signals responsible for initiating an adaptive cellular response, and in particular promote the development of Th17, but inhibit Treg differentiation. Optimal activation of mTOR leads to increased glycolysis and STAT signaling, promotes the development of Th17, while inhibiting the Treg differentiation (42). Constitutive activation of mTORC1 in mouse T cells by deletion of the mTORC1 tuberous sclerosis 1 (TSC1) gene-negative regulator enhances Th1 and Th17 cell differentiation and blocked Tregs differentiation (174). mTOR, in turn, stimulates HIF-1 to support glycolysis and is required to control the Th17 phenotype (175). Inhibition of mTOR can lead to increased fatty acid oxidation (FAO) and promote Treg differentiation (176).

High-NaCl conditions regulate the expression of FoxP3 and IL-17A through the p38/MAPK pathway involving NFAT5 and SGK1 and promote the differentiation of a stable, pathogen-specific, anti-inflammatory Th17 cell in human T cells *in vitro* in the presence of TGF-b (177). Butyrate promotes IL-10 Treg production (178–181) and promoted IFN-γ production under Th1 conditions, but not Th17 lymphocytes *in vitro* (182, 183). Inhibition of histone deacetylases (HDACs) in T cells by butyrate increased acetylation of p70 S6 kinase and phosphorylation of rS6, regulating the mTOR pathway required for Th17, Th1, and IL-10(+) Treg cell formation in the respective cytokine microenvironment (181). Interestingly, under butyrate exposure, IL-10 production increases in any CD4+ T-cell lineage due to the expression of B-lymphocyte-induced maturation protein 1 (Blimp1) (182). In addition, butyrate enhances histone acetylation at the Foxp3 promoter, promoting stable Foxp3 expression (178, 179). Neutralization of RAGE by the soluble receptor for advanced glycation end products (sRAGE)

inhibits fatty acid synthesis and promotes polarization of CD4+ T cells toward Treg rather than Th17 (184).

In vitro and *in vivo*, biotin deficiency decreased FoxP3 expression and the number of Treg cell, but increased the expression of T-bet and RORγt and differentiation of CD4+ T cells into Th1 and Th17 cells with a significant increase in the production of proinflammatory cytokines IFN-γ, TNF, and IL-17 (185). Culturing in the presence of lactate reprograms Th17 lymphocytes toward Treg cells, significantly reducing IL-17A production and increasing FoxP3 expression through ROS-driven IL-2 secretion (186).

It is worth noting that co-expression of FoxP3 and master regulators of other Th cells was shown for peripheral FoxP3+ Treg. Thus, in the intestine, up to 40% of Treg cells simultaneously express FoxP3 and RORγT and show enhanced immunosuppressive function, effectively restraining intestinal inflammation (5). RORγT is thought to maintain stable FoxP3 expression in Treg cells by blocking T-bet activation, which prevents Treg from reprogramming into a Th1-like effector phenotype. Treg in B-follicles of secondary lymphoid organs similar to Tfh express Bcl6, CXCR5, and PD-1 (7). Other Treg, similar to Th1 lymphocytes, express T-bet and CXCR3 (187) and may originate from Foxp3+ T cells under the influence of IFN-γ and STAT1 (187), and may also develop from T-bet+CD4+Th1 cells through the action of the immunosuppressive cytokine TGFβ (3). A high frequency of such T-bet+FoxP3+ Treg cells is observed in oncology and potently inhibits T helper 1 (Th1) cell responses (188). Similar to Th2 lymphocytes, Treg located in barrier sites, including the gastrointestinal tract and skin, can co-express GATA3, CCR8, and ST2 (IL-33 receptor α-chain) (189). These double-positive Treg, unlike their Th counterparts, are unable to produce proinflammatory cytokines. It was suggested that by expressing these Th cell-associated molecules, they accumulate in the same immune environments as their Th cell counterparts and selectively inhibit specific Th response modules.

Plasticity of Th17 and other Th lymphocytes

Th17 lymphocytes that express IL-17 and IFN-γ and that are transcriptionally similar to murine pathogenic Th17 lymphocytes were found at sites of inflammation in humans (158). In mice, it was shown that although both types of Th17 cells produced IL-17, non-pathogenic Th17 lymphocytes additionally expressed the immunoregulatory genes Il10, Il9, Maf, and Ahr, whereas expression of pro-inflammatory genes including *Csf2, Ifng, Tbx21, IL23r, and Gzmb* was upregulated in pathogenic Th17 (190, 191). IL-6 induces early expression of IL-1R required for early differentiation of Th17 cells *in vivo* (192). In addition, they differ in generation conditions: highly pathogenic Th17 lymphocytes are generated using a combination of IL-6 and IL-23 with IL-1β or TGFβ (190, 193).

Pathogenic Th17 lymphocytes exhibit dual Th1/Th17 positive characteristics, co-express RORγt and T-bet, as well as IL-12Rβ2 and IL-23R (194, 195). They are characterized by co-expression of pro-inflammatory cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-26, CCL20, and IL-22 (196–198). Phenotypic instability is thought to predispose Th17 cells to acquire a pro-inflammatory phenotype in chronically inflamed tissues.

Mycobacterium tuberculosis induces a Th1 population that co-expresses T-bet and RORγt, CXCR3, and CCR6 and produces IFN-γ (but not IL-17) (199). In addition, patients with RORC mutations lack Th1 (199), suggesting that they may originate from Th17 cells via plastic events in an IL-12-, TNF-α-, and/or IL-1β-dependent manner (200). Reverse trans-differentiation was shown. Non-classical CD161+CCR6+ Th1 lymphocytes of rheumatoid arthritis patients, unlike classical CD161-CCR6- Th1 cells, are reprogrammed into pathogenic Th17 lymphocytes in Th17-inducing conditions (127), which may contribute to their pathogenicity during the course of rheumatoid arthritis.

Circulating CD4+ memory T cells producing both IL-4 and IL-17, as well as IL-23R and CCR6, GATA3, and RORγt, were identified in patients with allergic asthma (201, 202) and with palmoplantar pustulosis (203). However, it is still unclear whether Th17/Th2 cells originate from Th17 or Th2 and whether IL-4 (Th17 to Th2 cell translation) or IL-1β, IL-6, IL-21 (Th2 to Th17 cell translation) occurs or whether additional cytokines are required.

A fraction of circulating human memory Tfh cells express CXCR5 and CCR6, Bcl6, and RORγt and produce IL-21, IL-22, and IL-17 (hence termed Tfh17) (204, 205). It remains to be clarified whether Tfh17 cells originate from Tfh or Th17 cells. Th17-to-Tfh plasticity may be relevant for Th17-mediated autoantibody generation.

Many different T cells with specific cytokine profiles are required for defense against various pathogenic exo- and endogenous influences. CD4+ T-lymphocyte subpopulations have varied degrees of plasticity and the ability to acquire new characteristics during the immune response. Even recognized terminally differentiated Th1/Th2 lymphocytes under certain conditions can transdifferentiate not only into each other but also into other subpopulations of Th lymphocytes. Th17 lymphocytes are characterized by a significant degree of instability; this is a highly heterogeneous subpopulation of effector cells whose protective role in inflammatory diseases remains to be studied. Treg cells, despite their isolated role in suppressing inflammatory reactions, also show features of plasticity and the possibility of transition to other subpopulations. In general, Treg cell plasticity is justified by the need to control different types of immune responses. Another important but poorly understood aspect of T cell plasticity is how different tissue microenvironments affect the differentiation and stability of human T cells. Determining the relative plasticity or stability of T cell subsets in different tissues is important for future therapeutic interventions in immune pathologies as diverse as chronic viral infections, cancer, and autoimmune diseases.

Conclusion

Decades of research on T-helper cells, using modern research tools such as ChiPseq, RNAseq, and scRNAseq, demonstrated the

diversity and heterogeneity of cell subpopulations, which provides the ability to respond rapidly and successfully to various challenges. T-helper cells are characterized by susceptibility to many exogenous and endogenous signals that can alter existing transcriptional programs, resulting in changes in the identity of T-helper cell subpopulations. It became evident that basic transcriptional networks could be cross-regulated and cross-expressed, creating unique subpopulations of T lymphocytes required for specific patterns of stimulation. Maintenance of tissue homeostasis and intracellular metabolism is closely linked to the stabilization of T cell subpopulations. Unraveling these complex mechanisms of plasticity and flexibility of T-helper cells and the conditions of their maintenance will allow for the generation of specific subpopulations of antigen-specific T-helper cells, regulating and controlling the type of immune response under a specific antigen. In the future, this could be a powerful tool for discovering new targets and optimizing existing therapies for specific patients and/or specific diseases.

Author contributions

JK: Investigation, Writing – original draft. SS: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glossary

AP-1	activating protein-1
BATF	Basic leucine zipper transcription factor
Bcl2/Bcl6	B-cell CLL/lymphoma 2/6
Blimp1	B-lymphocyte-induced maturation protein 1
CCR	CC chemokines receptor
CXCR	CXC chemokines receptor
DGK	Diacylglycerol kinase
DNA	Deoxyribonucleic acid
DUSP2	Dual Specificity Phosphatase 2
EOMES	eomesodermin
ERK	extracellular signal-regulated kinase
FOXO	forkhead box O
FOXP3	forkhead box P3
GATA-3	GATA Binding Protein 3
GFI1	growth-factor independent 1
HIF1α	hypoxia-inducible factor 1α
IFN	interferon
IKK	IκB kinase
IL	interleukin
IP_3	inositol 1, 4, 5-trisphosphate
IRF4	interferon-regulatory factor 4
ITAM	immunoreceptor tyrosine-based activation motif
Itk	interleukin-2-inducible T-cell kinase
iTreg	inducible regulatory T-cell
LAT	linker for activation of T cells
LMP7	low molecular weight polypeptide 7
MAF	macrophage-activating factor
MHC	major histocompatibility complex
mTOR	mammalian target of rapamycin
NFAT	Nuclear factor of activated T-cells
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NR4A	nuclear receptor 4A
PKCθ	Protein kinase C theta
PP2A	Protein phosphatase 2A
RAR	retinoic acid receptor;
Ras	small GTFase, from **Ra**t **s**arcoma virus
ROR	retinoic acid receptor-related orphan receptor;
RUNX3	runt-related transcription factor 3
SHP	**S**rc-homology 2 domain-containing **p**rotein tyrosine **p**hosphatases
STAT	Signal transducer and activator of transcription
Stub1	STIP1 Homology And U-Box Containing Protein 1
T-bet	T-Cell-Specific T-Box Transcription Factor (or TBX21)
TCR	T cell receptor
Tfh	follicular helper T cell
TGF-β	transforming growth factor
Th	T-helper cell
TNF	tumor necrosis factor
Tregs	regulatory T-cells
TSC	tuberous sclerosis complex
Zap70	Zeta-chain-associated protein kinase 70