

Review

The effects of post-translational modifications on Th17/Treg cell differentiation

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ABSTRACT

Regulatory T (Treg) cells and Th17 cells are subsets of CD4⁺ T cells which play an essential role in immune homeostasis and infection. Dysregulation of the Th17/Treg cell balance was shown to be implicated in the development and progression of several disorders such as autoimmune disease, inflammatory disease, and cancer. Multiple factors, including T cell receptor (TCR) signals, cytokines, metabolic and epigenetic regulators can influence the differentiation of Th17 and Treg cells and affect their balance. Accumulating evidence indicates that the activity of key molecules such as forkhead box P3 (Foxp3), the retinoic acid-related orphan receptor gamma t (RORγt), and signal transducer and activator of transcription (STAT)s are modulated by the number of post-translational modifications (PTMs) such as phosphorylation, methylation, nitrosylation, acetylation, glycosylation, lipidation, ubiquitination, and SUMOylation. PTMs might affect the protein folding efficiency and protein conformational stability, and consequently determine protein structure, localization, and function. Here, we review the recent progress in our understanding of how PTMs modify the key molecules involved in the Th17/Treg cell differentiation, regulate the Th17/Treg balance, and initiate autoimmune diseases caused by dysregulation of the Th17/Treg balance. A better understanding of Th17/Treg regulation may help to develop novel potential therapeutics to treat immune-related diseases.

1. Introduction

CD4⁺ T cells coordinate adaptive immune responses and are involved in the induction of autoimmune and allergic diseases, and cancers. Naïve CD4⁺ T cells are activated after interaction of the T cell receptor (TCR) and co-stimulatory receptor (CD28) with the peptide – major histocompatibility complex (MHC) and co-stimulatory molecules; both of which are expressed on activated antigen-presenting cells (APCs) [1,2]. After activation, CD4⁺ T cells differentiate into several subtypes of effector cells with different functions. These subtypes of cells include T helper type 1 (Th1), Th2, Th9, Th17, or T follicular helper (Tfh) cells, as well as regulatory T (Treg) cells. The cytokine milieu in the local environment is crucial for the differentiation of naïve CD4⁺ T cells [1,2]. Stimulation of naïve CD4⁺ T cells with interleukin (IL)-12 and blockade of IL-4 signaling induces Th1 cells development, whereas activation of IL-4-mediated signaling and inhibition of interferon (IFN)-γ receptor leads to differentiation of Th2 cells. Interestingly, in the presence of IL-6 and/or IL-1β (together with transforming growth factor (TGF)-β), naïve

CD4⁺ T cells differentiate into IL-17 secreting Th17 cells; however, in the absence of proinflammatory cytokines, stimulation with TGF-β induces regulatory T cells development [3,4].

In brief, stimulation of naïve CD4⁺ T cells by IL-6 leads to the activation of the signal transducer and activator of transcription 3 (STAT3), that upon phosphorylation regulates the transcription of its target genes, such as transcription factors as a retinoic acid-related orphan receptor gamma t (RORγt) and RORα as well as signature cytokines IL-17A, IL-17F, IL-21, and IL-22, that drives cells toward Th17 cells development [2,5–7]. On the other hand, stimulation of naïve CD4⁺ T cells with TGF-β induces Smad- and Mad-related protein (SMAD)2 and SMAD3, which in turn activate transcription factor forkhead box P3 (Foxp3); that generates peripheral Treg cells development [8]. Additionally, naïve CD4⁺ T cells stimulation in the presence of IL-2 activates STAT5, which induces expression of Foxp3 and inhibits Th17 cell differentiation [9]. Interestingly, *in vitro* and *in vivo* studies revealed that transcription factor RORγt is activated by TGF-β, thus linking the differentiation of the Treg cells and Th17 cells [10–12]. In the absence of a second signal from pro-

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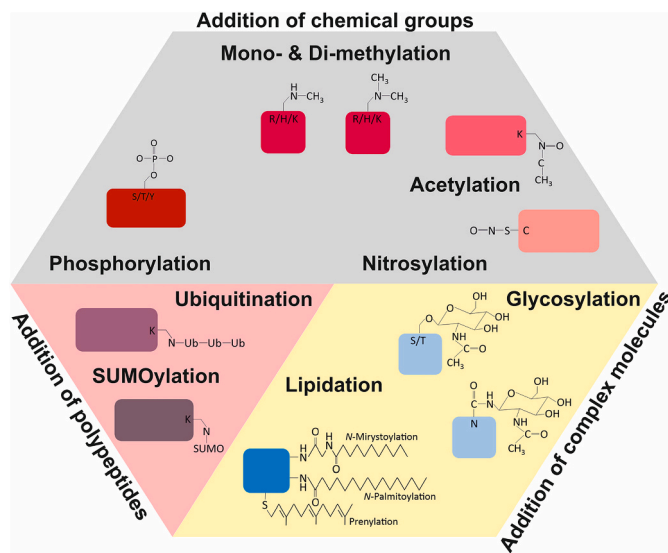


Fig. 1. General PTMs in proteins. Specific amino acids such as arginine (R), asparagine (N), cysteine (C), histidine (H), lysine (K), serine (S), threonine (T), and tyrosine (Y) are marked.

inflammatory cytokine in the milieu, Foxp3 can inhibit ROR γ t function and induce Treg cells development. On contrary, a second signal from a pro-inflammatory cytokine such as IL-6, IL-21, IL-23, and IL-1 β inhibits Foxp3 function and induces Th17 cell differentiation via the STAT3 signaling pathway [9,12,13]. Notably, reduction in STAT3 protein levels results in impaired Th17 differentiation [9]. Th17 cells secrete IL-17A, IL-17F, IL-21, and IL-22, recruit neutrophils to the site of infection or injury, are involved in tissue remodeling and repair, production of antimicrobial proteins as well as play an essential role in inflammation and autoimmunity. Treg cells secrete anti-inflammatory cytokines IL-10 and TGF- β , inhibit immune responses, and are essential for maintaining self-tolerance and homeostasis.

In recent years, studies have shown that Th17 and Treg cells play opposite roles during inflammatory and immune responses [3,14]. Th17 cells have been involved in the pathogenesis of multiple autoimmune-mediated inflammatory diseases, such as inflammatory bowel disease (IBD), multiple sclerosis (MS), psoriasis, rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE) [15]. Interestingly, the development of MS, RA, and SLE was associated with quantities and functional deficiencies of Treg cells and the breakdown of immunological tolerance in other studies [16–18]. Furthermore, experimental evidence shows that during CD4⁺ T cells differentiation, major regulatory transcription factors such as ROR γ t and Foxp3 are regulated at both the transcription [19] and protein level through post-translational modifications (PTMs), thereby releasing signature cytokines and promoting the immune-suppressive activity of Treg cells [20]. It was also found that metabolic reprogramming and epigenetic modification such as DNA methylation, histone post-translational modifications, and microRNA are critical for T cell activation, CD4⁺ T cells differentiation, and function [21,22].

PTMs are covalent modifications of proteins resulting from proteolytic cleavage of peptide bonds or from the addition of a modifying group, such as phosphoryl, methyl, glycosyl, and acetyl, to one or more amino acids [23] (Fig. 1). To date, more than 87,308 PTMs and 234,938 putative modifications on proteins have been identified by qualitative and quantitative analyses using tandem mass spectrometry combined with other techniques [24]. Among them, phosphorylation, acetylation, ubiquitination, methylation, glycosylation, and SUMOylation occur more frequently in regulatory proteins and are the most studied using the mass spectrometry-based approach [23]. Some PTMs, such as phosphorylation, ubiquitination are reversible and can be removed by

the action of specific deconjugating enzymes; while others, such as myristoylation are permanent and irreversible. PTMs occur in almost all proteins and play an essential role in various biological processes such as regulation of gene expression, signal transduction, DNA repair, cell-cell interaction, cell differentiation, and apoptosis [25,26]. It was also found that PTMs affect multiple aspects of ROR γ t and Foxp3 function as well as regulate Th17/Treg balance. PTMs occur in various cellular organelle including cytoplasm, endoplasmic reticulum, Golgi apparatus, and nucleus [27].

Here, we provide an overview of the post-translational modification of ROR γ t and Foxp3, as well as other proteins such as STAT3 and STAT5, summarize their potential impact on Th17/Treg balance, and provide future insight into the role of PTMs in various inflammatory and autoimmune diseases.

2. Addition of chemical groups

An important number of the small chemical group can be used as PTMs. The transfer of the chemical groups is catalyzed through enzymatic activity and the effect is reversible. Many chemical groups are known to be associated with PTMs; here we only focus on some of the most commonly known including phosphorylation, methylation, nitrosylation, and acetylation.

2.1. Phosphorylation

Protein phosphorylation catalyzed by certain kinases and phosphatases is one of the most common and important PTMs of proteins [28]. This reversible and transient modification consists of the addition of a covalently bound phosphate group (-PO₄) to the polar group R of various amino acids such as serine (Ser or S), threonine (Thr or T), or tyrosine (Tyr or Y) by a protein kinase. Phosphorylation of arginine (Arg or R), aspartic acid (Asp or D), cysteine (Cys or C), and histidine (His or H) residues are also reported [29], but this type of phosphorylation is less stable [30,31]. Protein phosphorylation is involved in many cellular processes such as cell division and cell growth, protein synthesis, protein-protein interactions, regulation of gene expression, signal transduction, and aging [32–34]. An essential role of phosphorylation in the regulation of Th17/Treg cells differentiation and many pathological processes has also been noticed.

It is well recognized that phosphorylation of Foxp3 is related to its stability, localization, and Tregs development, whereas little is known about the function of phosphorylation in the regulation of ROR γ t activity. A recent report revealed that in response to stimulation of TCR-mediated signaling in Treg cells, phosphorylation of Foxp3 can be regulated by a TGF- β activated kinase 1 (TAK1)-Nemo-like kinase (NLK) signaling pathway [35]. NLK interacts and phosphorylates Foxp3 which leads to reduced interaction of Foxp3 with the STIP1 homology and U-box containing protein 1 (STUB1), an E3 ubiquitin-protein ligase, in consequence its proteasome-dependent degradation is reduced [35,36]. Knockout of NLK in Treg cells results in an age-dependent increase in auto-inflammation and renders animals to develop more severe experimental autoimmune encephalomyelitis (EAE) [35]. In RA-derived Treg cells, phosphorylation of Foxp3 at the S418 residue in the C-terminal DNA-binding domain plays a positive role in the regulation of the suppressive function of Tregs [37]. *In vitro* studies revealed that tumor necrosis factor (TNF)- α upregulates the expression of protein phosphatase 1 (PP1), which dephosphorylates Foxp3 at the S418 residue, thereby inactivating Foxp3 and impairing synovial Treg cell's function. Moreover, impairment in Treg cell's function induced by TNF- α correlates with an increased number of IL-17⁺ and IFN- γ ⁺CD4⁺ T cells within the inflamed synovium in RA [37]. In human Treg cells, PIM1 protein kinase negatively regulates Foxp3 activity through phosphorylation of its C-terminal Fork-head domain (FHD) at the S422 residue [38]. Phosphorylated S422 leads to reduce Foxp3 DNA binding activity and down-regulate expression of Foxp3-induced target genes such as CD25,

cytotoxic T cell antigen 4 (*CTLA-4*), and glucocorticoid-induced tumor necrosis factor receptor (*GITR*) [38]. Knockdown of PIM1 in Treg cells enhances the immunosuppressive activity of Tregs. Interestingly, phosphorylation of Foxp3 at the S422 residue may be abolished by the PIM1-mediated phosphorylation of Foxp3 at the S418 residue [38]. Additionally, IL-6 could potentially regulate Treg cells activity by inducing PIM1 expression, whereas TCR signaling could effectively suppress the upregulation of PIM1 [38]. Natural flavonoid *Kaempferol*, found in vegetables and fruits, may reduce PIM1-mediated phosphorylation of Foxp3 at the S422 residue and enhance the suppressive function of Tregs [39]. Epidemiological studies have shown that *Kaempferol* intake could reduce the risk of many cancer types and may be used for the prevention and treatment of certain inflammatory diseases such as SLE, RA, and ankylosing spondylitis (AS) [39]. PIM2 kinase, a serine/threonine-protein kinase and human proto-oncogene, can phosphorylate multiple sites of the Foxp3 N-terminal domain, including S33 and S41, which decreases the suppressive function of Tregs [40]. Phosphorylation of the Foxp3 by PIM2 alters the expression of Treg's surface markers such as CD25 and GITR as well as affects Foxp3 protein binding activity with other cofactors. Treg cells specific PIM2-deficient mice appear resistant to dextran sodium sulfate (DSS)-induced colitis *in vivo* [40]. Intriguingly, lentiviral transduction of primary human CD4⁺CD25⁻ T cells with Foxp3 induced expression of PIM2 and conferred expansion of Treg cells in the presence of rapamycin [41]. *In vitro* experiments show that cyclin-dependent kinase 2 (CDK2) and lymphocyte-specific protein tyrosine kinase (LCK) promote the phosphorylation of Foxp3 [42,43]. CDK2 along with cyclin E binds to four CDK motifs (S/T–P) within the protein-rich N-terminal domain of Foxp3 and causes its phosphorylation at S19 and T175 residues [42]. Such a type of phosphorylation reduces Foxp3 protein stability and decreases the suppressive function of Treg cells. In contrast, replacement of serine or threonine with alanine (S/T → A) in each of CDK motifs results in enhanced Foxp3 protein stability and transcriptional activity, which manifests that CDK2 negatively regulates Treg cell's function [42]. It was found that Kenpaullone (ken), a potent CDKs (CDK1, CDK2, and CDK5) inhibitor, can promote the generation and differentiation of functional inducible Tregs (iTregs) by regulation of SMAD3-mediated TGF- β signaling pathway [44]. TGF- β can induce *Foxp3* expression by activating the SMAD pathway. In brief, phosphorylated SMAD2 and SMAD3 form a complex with SMAD4 and then translocate into the nucleus where bind to the enhancer and promoter of *Foxp3* to initiate and maintain its transcription [44]. In the human breast cancer cell line, MCF-7, phosphorylation of Foxp3 at the Y342 residue by LCK down-regulates expression of S-phase kinase-associated protein 2 (SKP2), vascular endothelial growth factor A (VEGF-A), and matrix metalloproteinase 9 (MMP9) [43]. However, mutation of Foxp3 *via* substitution of tyrosine 342 to phenylalanine (Y342F) abolishes the ability to suppress MMP9 expression and the invasive ability of cancer cells [43]. Taken together, these findings indicate that phosphorylation of Foxp3 by PIM1, PIM2, and CDK2 negatively regulates Foxp3 function, while NLK and PP1 are positive regulators of Foxp3 activity.

The inhibitor of nuclear factor κ B kinases (IKK) kinases are the only known family of protein enzymes with a confirmed role in the regulation of ROR γ t-mediated Th17 differentiation. Using mass spectrometry, twelve distinct phosphorylated serine residues of ROR γ t have been identified upon its immunoprecipitation from T cells polarized under Th17 conditions [45]. It was found that phosphorylation of ROR γ t at the S376 residue by the IKK α stimulates transcriptional activity of ROR γ t, whereas phosphorylation of ROR γ t at the S484 residue inhibits its transcriptional activity and blocks Th17 differentiation. Replacement of serine with alanine (S/A) or glutamic acid (S/E) at the S376 residue results in reduced or stimulated IL-17 reporter activity, respectively, while analogous alteration at the S484 residue provides reverse effect [45]. In addition, mutation of ROR γ t *via* substitution of serine 484 to alanine enhanced IKK α -independent Th17 differentiation, whereas substitution of serine 484 to glutamic acid, leading to decreased Th17

differentiation. Knockdown of IKK α decreases Th17 differentiation [45]. Recent data showed that the second member of the IKK complex, IKK β phosphorylates ROR γ t at the S489 residue and induces its interaction with aryl hydrocarbon receptor (AhR), which leads to ROR γ t nuclear translocation and stimulates IL-17A transcription [46]. In germinal center kinase-like kinase (GLK) transgenic T cells, phosphorylation of AhR at the S36 residue as well as its nuclear translocation is mediated by protein kinase C θ (PKC θ) [46].

Interestingly, recent work has shown that phloretin a natural phenol – dihydrochalcone found in apple tree leaves decreased Th17 cell generation and STAT3 phosphorylation as well as increased regulatory T cells generation and STAT5 phosphorylation in the process of inducing Th17 cells and Treg cells development [47]. An increase in the phosphorylation level of AMP-activated protein kinase (AMPK) and a decrease in the phosphorylation level of mammalian target of rapamycin (mTOR) has been observed in activated CD4⁺ T cells under phloretin treatment. Moreover, phloretin inhibited glucose uptake and attenuated the proliferation of activated CD4⁺ T cells that was arrested at the G0/G1 phase [47]. STAT3 phosphorylation was first reported to be associated with enhanced Th17 cells differentiation in a suppressor of cytokine signaling 3 (SOCS3) deficient T cells [6]. The authors found that SOCS3 regulates IL-23-mediated STAT3 phosphorylation and Th17 differentiation through STAT3 binding to the promoters of *IL-17A* and *IL-17F* genes [6].

The effects of STATs in regulating Th17/Treg cell differentiation and function have been well documented. Since STATs are phosphorylated by Janus kinases (JAKs), targeting JAKs has been associated with changes of Th17/Treg cells. Wu et al. (2016) found that SHRO302 (C₁₈H₂₂N₈O₂S•H₂SO₄), the Janus kinase 1 (JAK1) inhibitor, down-regulated IgG1, IgG2a and TNF- α , IL-1 β , IL-17 levels, down-regulated the percentage of CD4⁺IL-17⁺ Th17 cells as well as inhibited JAK1-STAT3 phosphorylation in adjuvant-induced arthritis rats [48]. It was found that AG490, the JAK2 inhibitor, inhibited phosphorylation of STAT3 at Y705 and S727 residues under Th17-polarizing conditions including TCR activation and TGF- β , and IL-6 and suppressed Th17 cells differentiation in mice with collagen-induced arthritis [49]. AG490 treatment also increased STAT5 phosphorylation, thereby increasing the number of Treg cells as well as the expression level of molecules associated with Treg cells development such as inducible T cell co-stimulator (ICOS), programmed death protein 1 (PD-1), intercellular adhesion molecule 1 (ICAM-1), and CD103 [49]. Importantly, BMS-911543, another JAK2 inhibitor, inhibited STAT5 phosphorylation at Y694 residue and reduced Foxp3⁺ cells in the environment of pancreatic cancer, while the level of STAT3 phosphorylation at Y705 residue was not changed [50]. Additionally, this inhibitor limited cytokine-driven expansion of T regulatory cells *in vitro* as well as had little *in vitro* effect on the viability of both murine and human pancreatic ductal adenocarcinoma-derived stellate cells [50]. CP-690,550 (tofacitinib), originally developed as JAK3 inhibitor, promoted Th17 differentiation at 10–50 nM concentration, while Th1 and Th2 cells development is inhibited [51]. CP-690,550 inhibited IFN-induced STAT1, IL-2-induced STAT5 and IL-4-induced STAT6 at 3–30 nM concentration, while suppression of IL-6-induced Y705 phosphorylation of STAT3 required a concentration greater than 100 nM [51]. Kenpaullone and Roscovitine, CDK inhibitors, enhanced STAT5 phosphorylation in the presence of TGF- β that suppresses Th17 and promotes iTreg differentiation in mice EAE model [52]. On the other hand, prostaglandin I₂ (PGI₂)-PGI₂ receptors (IP) interaction promoted the phosphorylation of STAT3 and suppressed the phosphorylation of STAT5, thus facilitating Th17 differentiation and attenuated Treg cells development [53]. Moreover, PGI₂ analog (Iloprost) decreased the proportion of Treg cells and Foxp3 transcript expression but increased the percentage of Th17 cells, ROR γ c mRNA, and IL-17A production in human CD4⁺ T cells [53]. An important role of midkine (MK), a heparin-binding growth factor, as a negative regulator of Treg cells differentiation, has been reported in another study [54]. The authors found that MK decreases the Treg cells

population *ex vivo* by suppressing the STAT5 phosphorylation and attenuates experimental EAE in mice. Administration of anti-MK RNA aptamers neutralizes expansion of the Treg cells population and alleviated EAE symptoms [54].

IL-1 receptor associated kinase 1 (IRAK1), is a NF- κ B signaling transducer, which plays a critical modulatory role in the Th17 and Treg cells differentiation. It was found that treatment of IRAK1^{-/-} CD4⁺ T cells with TGF- β and IL-6 decreased STAT3 phosphorylation at S727 residue and decreased expression of ROR γ t and IL-17A, whereas TGF- β treatment elevated nuclear factor of activated T-cells (NFATc) levels and increased expression of Foxp3, a key marker for Treg cells [55]. Stürner et al. (2014) revealed that IL-1 β signaling through IRAK1 phosphorylation decreasing STAT3 phosphorylation at S727 residue [56]. Acetyl-11-keto- β -boswellic acid (AKBA), a bioactive compound found in trees, decreased Th17 differentiation, reduced the secretion of IL-17A as well as ROR γ t mRNA expression in Th17 polarized human cells [56]. Recently, Zhou et al. (2018) revealed that CD4⁺ T cells derived from SLE patients exhibited more pronounced IRAK1 phosphorylation at T209 residue upon IL-1 β stimulation than those from control CD4⁺ T cells [57]. The authors showed that expression of IRAK1 was positively associated with the Th17/IL-17 in SLE patients. Inhibition of IRAK1 repressed production of IL-17A and the gene expression of Th17 cells markers such as RORc, IL-23 receptor, and IL-17 as well as attenuated Th17 differentiation [57].

Protein kinase CK2 is a constitutively active and conserved serine/threonine kinase that regulates many signaling pathways responsible for cellular processes. In mice and humans, the catalytic activity of CK2 is regulated by the inhibitor CX-4945 that inhibits Th17 cell differentiation, and promotes the generation of Treg cells [33]. CK2 suppresses phosphoinositide 3-kinase (PI3K)/Akt/mTOR activation and STAT3 phosphorylation through incubation of anti-CD3 and anti-CD28 antibodies with CX-4945. Additionally, CX-4945 treatment inhibits Th17 cells' maturation into inflammatory IFN- γ co-producing T effector cells and shifts the Th17/Treg axis throughout the disease [33]. It was found that resveratrol (3,5,4'-trihydroxystilbene, RSV), a plant-derived polyphenol, can inhibit the generation and function of tumor-evoked regulatory B cells (tBregs) by inactivating STAT3 (RSV blocks STAT3 phosphorylation and acetylation) [58]. RSV attenuates the production of TGF- β , thereby disabling TGF- β -induced conversion of Foxp3⁺ Tregs and inhibits lung metastasis in mice with metastatic 4 T1.2 cancer [58]. Interestingly, it was revealed that *Hirsutella sinensis* mycelium (HSM) treatment blocked the phosphorylation of the mTOR/protein S6 kinase (p70S6k) pathway and influenced Th1/Th2 and Th17/Treg imbalance *in vitro* [34]. HSM administration down-regulated the pro-fibrotic factors, such as α -smooth muscle actin, fibronectin, and vimentin, reduced the chemotaxis of alveolar macrophages, and potentially suppressed the expression of inflammatory cytokines as TGF- β [34].

2.2. Methylation

Protein methylation is a common type of PTM catalyzed by methyltransferases that are responsible for the addition of methyl (-CH₃) groups from S-adenosylmethionine (SAM) to target proteins. Methylation occurs mainly on the side chain of nitrogen atoms of arginine, histidine, and lysine residue, whereas methylation of the carboxyl groups of glutamic acid (Glu or E), leucine (Leu or L), and isoprenylated cysteine are less common. Protein methylation is involved in many different cellular processes, such as RNA metabolism, protein-protein interactions, signal transduction, regulation of gene expression as well as aging [59].

Arginine methylation of Foxp3 and ROR γ t, non-histone proteins, are implicated in the regulation of protein function and affect the Th17 and Treg cells differentiation. Asymmetric methylation of Foxp3 at the R48 and R51 residues by protein arginine methylase 1 (PRMT1) enhances Foxp3-mediated suppressive function of Treg cells [60]. Inhibition of arginine methylation at these two residues provides a reverse effect:

reduces the suppressive function of Tregs; and on the other hand, enhances the Th1-associated gene expression profiles in Foxp3⁺ T cells. Moreover, Foxp3⁺ Treg cells treated with MS023, a type 1 PRMT inhibitor, downregulated CD25 expression and increased IL-2 production, suggesting that its inhibition of PRMT attenuates the suppressive activity of Foxp3-transduced T cells [60]. Using mass spectrometry, Nagai et al. (2019) revealed that Foxp3 can be dimethylated at R27, R51, and R146 residues by PRMT5, a type II arginine methyltransferase [61]. Point mutation of Foxp3 via substitution of arginine 51 to lysine (R51K) leads to defective suppressive functions in human CD4⁺ T cells. Conditional PRMT5 deletion in mouse Treg cells results in Scurfy-like autoimmune disease, causes cell cycle abnormalities, and induces an inflammatory phenotype in Treg cells. In these mice, a reduced number of Tregs in the spleen and normal numbers of Tregs in the peripheral lymph nodes were observed in *in vitro* study [61]. Inhibition of PRMT5 by DS-437, an S-adenosylmethionine inhibitor, inhibits Foxp3 methylation by 293 systems with anti-sym10 antibody, which confirms that PRMT5 is essential for Treg function. Moreover, treatment of CD4⁺ T cells with DS-437 in combination with the anti-p185^{erbB2/neu} antibody 4D5 exerts a beneficial effect on inhibiting tumor growth by reducing Treg activity [61]. A relevant role of PRMT5 in cancer progression has been also observed in the second study [62]. Authors have shown that the inhibition of PRMT5 enhances tumor immunity by modulation of Foxp3 levels and Tip60 acetyltransferase activity as well as limits the inhibitory function of Treg cells [62]. Knockdown of PRMT5 decreases Foxp3 methylation during Treg cells activation and induces a better response of TGF- β in patients with ulcerative colitis (UC) [63]. PRMT5 enhances also trimethylation of histone H3 lysine 27 (H3K27me3) and increases DNA methyltransferase 1 (DNMT1) binding to Foxp3 promoter that results in restricted Tregs differentiation [63]. More recently, PRMT5 was shown to modulate Th17 differentiation via the symmetric dimethylation (SDM) of sterol regulatory element-binding protein 1 (SREBP1), which enhances SREBP activity after T cell induction and regulates T cell lipid metabolism [64]. PRMT5 deficiency in peripheral CD4⁺ T cells results in reduced Th17 differentiation and protects mice from developing EAE [64]. However, no other activity of PRMTs in the Th17 differentiation has been reported yet. Polansky et al. (2008) revealed that naturally occurring Foxp3⁺ Tregs display stable expression of Foxp3 that was associated with demethylation of Foxp3 Treg-specific demethylated region (TSDR) [65]. The authors showed that inhibition of DNA methylation by azacytidine, a chemical analog of the nucleoside cytidine which interferes with the function of DNMT1, promoted *de novo* induction of Foxp3 expression during priming and conferred stability of Foxp3 expression upon restimulation. It was also found that in the DNMT1-deficient T cells, TCR stimulation alone was sufficient to transcriptionally activate expression of Foxp3, suggesting a dominant role for TCR signaling in Foxp3 induction [66]. Additionally, *in vivo* study (mice model) revealed that DNMT1 deficiency and TCR stimulation resulted in augmented CD8⁺Foxp3⁺ T cells differentiation as well as indicated that DNMT1 may be, in part, responsible for restricting expression of Foxp3 to the CD4⁺ T cells [66]. It was reported that LKB1, a serine-threonine protein kinase, plays a positive role in the stabilization of Foxp3 expression by preventing methylation of the conserved non-coding sequence 2 (CNS2) of Foxp3 by STAT4 and enforcing Treg cell suppressor activity [67]. Moreover, LKB1 deletion in mouse Treg cells leads to the development of a fatal early-onset autoimmune disease, with no Foxp3 expression in most Tregs [67]. Interestingly, vitamin C treatment reduces Jmjd2 modulated trimethylation of histone H3 lysine 9 (H3K9me3) at the IL-17 promoter and enhancer CNS2 which enhances IL-17 expression [68]. The effect of vitamin C on IL-17 expression was independent of ROR γ t upregulation and induction of ten-eleven-translocation (Tet) DNA dioxygenase [68]. Therefore, these findings suggest that vitamin C seems to exert a positive effect on Th17 cell development. The epigenetic study revealed that Jmjd3 also functions as a positive regulator of Th17 differentiation as well as is a critical negative regulator of Th1 and Tregs differentiation [69]. Jmjd3

mediates histone H3K27 and/or H3K4 methylation levels in target genes e.g., *CD44*, *IFN- γ* , *Foxp3*, *GATA3*, *RORc*, and regulates target gene expression [69]. Liu et al. (2015) revealed that demethylation of histone H3 lysine 27 (H3K27) regulates Th17 cell differentiation both *in vitro* and *in vivo* [70]. Jmjd3 binds to the promoter of many Th17 associated genes such as *RORc*, *IL-17A*, *IL-17F*, and *IL-22*, and reduces the level of H3K27me3 trimethylation, thus resulting in the target genes activation and promoting Th17 cells differentiation. Moreover, inhibition of Jmjd3 activity suppresses Th17 cell differentiation, however, may be beneficial for patients with autoimmune diseases [70].

Shimazu et al. (2016) showed that demethylation of the TSDR within the *Foxp3* gene locus stabilizes *Foxp3* expression, while *Foxp3* TSDR hypomethylation exerts increased suppressive function in adult T-cell leukemia (ATL) cells and was associated with poor outcomes in ATL [71]. Interestingly, it was found that hydrogen sulfide (H_2S) promotes the expression of methylcytosine dehydrogenases Tet1 and Tet2, which are recruited to *Foxp3* by TGF- β and IL-2 to maintain *Foxp3* demethylation and Treg cell-associated immune homeostasis. H_2S deficiency results in reduced Tet1 and Tet2 expression, which leads to *Foxp3* hypermethylation, impairment of Treg cells generation and function, and autoimmune disease [72]. As a conclusion, H_2S was required for *Foxp3*⁺ Treg cell differentiation and function [72].

2.3. Nitrosylation

Protein nitrosylation is a ubiquitous PTM in which the nitric oxide “nitrosyl” (-NO) group is attached to a transition metal or cysteine thiol (-SH) on a target protein (S-nitrosylation). Nitrosylation of proteins is a key mechanism by which nitric oxide regulates cell signaling as well as allows cells to respond to their environmental changes in a specific and flexible manner [73].

It is known that increasing the level of NO by an NO donor, S-nitrosoglutathione (GSNO), results in reduced expression of *Foxp3* and less number of Treg cells [74]. It has been reported that myelin basic protein (MBP) priming reduces the number of *Foxp3*⁺*CD25*⁺ T cells in the presence of NO; whereas, the addition of pharmacological inhibitors of NO such as gemfibrozil or pravastatin prevents the attenuation of *Foxp3* expression in MBP-primed T cells [74]. NO might also suppress the *Foxp3* expression by interfering with the glucocorticoid (GR)/estrogen (ER)-dependent transcriptional activation of *Foxp3* promoter. Incubation of *Foxp3*-expressing human melanoma and breast cancer cells in the presence of NO donor DETA/NONOate results in suppression of *Foxp3* transcript expression [75]. The authors suggest that NO can modify GR and ER through S-nitrosylation of cysteine residue that coordinates zinc within the two DNA-binding zinc-finger domains, which results in inhibition of DNA-binding at the specific GR and ER elements [75]. It was also found that NO in combination with IL-6 suppressed Treg development induced by TGF- β and retinoic acid as well as antagonized IL-6 to block TGF- β -mediated differentiation of Th17 cells [76]. Besides the determination of Th17/Treg cells balance, NO modulate TGF- β activity away from Treg cells that result in the Th1 development [76]. Suppression activity of Treg cells by inhibition of TGF- β production is promoted also by inducible nitric oxide synthase (iNOS) [77]. Inhibition or knockout of iNOS in host *CD4*⁺ T cells enhanced induction of Treg cells from purified human or mouse *CD4*⁺ T cells or activated mouse splenocytes in the presence of TGF- β . Moreover, inhibition of iNOS attenuates intratumoral infiltration of myeloid-derived suppressor cells (MDSCs) [77]. On the other hand, iNOS deficient mice displayed enhanced Th17 cells differentiation but without effect on Th1 and Th2 cells [78]. The addition of iNOS-selective inhibitor, N6-(1-iminoethyl)-L-lysine dihydrochloride (L-NIL), to *CD4*⁺ T cells enhanced Th17 differentiation, whereas the addition of NO donor, S-nitroso-N-acetylpenicillamine (SNAP) reduced the number of Th17 cells [78]. Simultaneously, no significant effect on cell viability and proliferation was observed for both compounds, L-NIL and SNAP. Additionally, NO mediates nitration of a tyrosine residue in ROR γ t that results in ROR γ t-

induced activation of IL-17 promoter [78]. Obermajer et al. (2013) observed that expression of nitric oxide synthase 2 (NOS/iNOS) positively correlates with Th17 cells responses in women with ovarian cancer (OvCa) [79]. The physiological concentration of NO produced by patients' MDSCs promotes the development of ROR γ t(RORc)⁺IL-23R⁺IL-17⁺ Th17 cells. Inhibition of NOS2 abolishes the *de novo* Th17 cells activation and suppresses IL-17 production by establishing Th17 cells isolated from women with OvCa [79]. Interestingly, NO-induced Treg cells (NO-Tregs; *CD4*⁺*CD25*⁺*Foxp3*⁻) suppress Th17 but not Th1 cell differentiation in mice model of EAE [80]. The authors reported that NO-Tregs attenuated the expression of ROR γ t but not T-bet in mice spleen cells, whereas natural Tregs suppressed expression of T-bet, but not ROR γ t. Suppression of the Th17 activity by NO-Tregs was cell-contact dependent and was associated with the presence of IL-10. It was also found that GSNO attenuates EAE disease by reducing the production of IL-17 and the infiltration of *CD4*⁺ T cells into the central nervous system (CNS), while the Th1 and Th2 immune response was not affected [81]. Mice treatment with GSNO reduced phosphorylation of STAT3 and expression of ROR γ which indicates that GSNO targets Th17 cells [81]. Furthermore, N6022, a GSNO reductase (GSNOR) inhibitor attenuates pro-inflammatory subsets of *CD4*⁺ T cells Th1, Th17 and upregulates anti-inflammatory subsets of *CD4*⁺ T cells Th2, Treg in NO metabolizing cells in a mouse model of EAE [82]. Moreover, N6022 is effective in the induction of protein S-nitrosylation in the spleen, and treatment of the mice with N6022 increases the number of Tregs as well as upregulates their expression of IL-10.

2.4. Acetylation

Protein acetylation occurs by the transfer of an acetyl group from the co-factor Acetyl-CoA onto a lysine residue of a target protein. The reaction is catalyzed by lysines acetyltransferases enzymes (KATs) and, is reversible by the action of deacetylase enzymes (KDACs) such as histone deacetylases (HDACs) and sirtuins (SIRTs) [83].

Acetylation has been proven to play a role in the differentiation of Th17/Treg and regulation of their balance by directly targeting *Foxp3* and ROR γ t transcription factors. ROR γ t is acetylated at K69, K81, and K99 residues by the acetyltransferase p300 in Th17 cells and deacetylated by HDAC1 and SIRT1 [84,85]. SIRT1 is required for IL-17 production and inhibition of IL-2 production, consequently promoting Th17 differentiation [84]. Deletion of SIRT1 in T cells or chemical inhibition of SIRT1 protects mice from EAE [84]. SUMOylation of ROR γ t facilitates the recruitment of HDAC2 inhibiting IL-17 mRNA and protein expression in *CD4*⁺ cells. Deletion of HDAC2 in *CD4*⁺ cells enhances T cell-induced colitis and EAE development [86].

P300, through binding with steroid receptor coactivator 3 (SRC-3) were found to play a role in Th17 development. The deficiency of SRC-3 impairs the generation of pathogenic Th17 cells through the regulation of IL-17 expression. SRC-3 bound to IL-17 loci in a ROR γ t dependent manner led to the recruitment of p300 and chromatin activation [87]. Also, the inhibition of ROR γ t acetylation in human *CD4*⁺ cells using JQ1, an inhibitor of p300 shows a decrease in IL-17 mRNA expression and cytokine production [88]. p300 is essential in the development of inflammatory disease with a decrease in EAE development and amelioration of liver fibrosis symptoms in a schistosomiasis liver fibrosis mouse model in response to SRC-3 deficiency and JQ1 treatment respectively [87,88].

In addition to ROR γ t, STAT3 is also known to be subject to acetylation by the action of p300 on K685 residue, activating its transcriptional function [89]. In human lymphocytes, STAT3 has been shown to be constitutively acetylated in patients suffering from chronic lymphocytic leukemia (CLL) with enhanced CLL cells survival [90]. In mouse T cells as well as in healthy human samples, the deacetylase SIRT1 was shown to regulate IL-17A and ROR γ t expression through STAT3 deacetylation leading to reduced STAT3 binding to RORc promoter. STAT3 deacetylation and decrease IL-17 production could also be observed in human

metastatic colon cancer samples treated with SIRT1 activator. A delay in the progression of tumor growth in two different models of cancer in mice, B16F10 and CT26 treated with SIRT1 activators was observed [91].

SIRT2 and glycolysis-related proteins expression, as well as the percentage of Th17 cells, are increased in peripheral blood mononuclear cells (PBMCs) of patients suffering from UC [92]. Pharmacological inhibition of SIRT2 with thiomristoyl (TM) in mice and human T cells leads to a decrease in Th17 cell differentiation and IL-17 production. Also, splenocytes isolated from SIRT2 KO mice were transfected with SIRT2 plasmid and SIRT2 mutant plasmid shows a decrease in the proportion of Th17 cells as well as ROR γ t and IL-17 protein expression when transfected with the latter [92]. TM treatment in UC animal models has anti-inflammatory effects that improve DSS-induced colitis symptoms [92]. Opposite to a direct action of SIRT2 on ROR γ t or STAT3, this paper shows that SIRT2 acts on Th17 differentiation indirectly by modulation of the metabolism of the cells. SIRT2 inhibition shows increased acetylation of lactate dehydrogenase A, and inhibition aerobic glycolysis necessary to Th17 differentiation [92].

Treg differentiation is enhanced by Foxp3 acetylation (K31, K262, and K267 residues) that increases Treg stability, enhances Treg suppressive function as well as prevents Foxp3 degradation [25,93]. SIRT1 deacetylated Foxp3, leading to a decrease in its expression. Human PBMCs and mouse splenocytes treated *in vitro* with resveratrol (SIRT activator) or NAM (SIRT inhibitor), respectively results in a decrease and increase of Foxp3⁺ cells. Furthermore, mice T cells treated with NAM show enhance Treg suppressive function [93].

Administration of HDAC inhibitor (HDACi) in mice leads to an increase of Foxp3 gene expression and is associated with an increased amount of natural Foxp3⁺ Treg cells and suppressive function of Tregs cells [94]. Numerous KDACs have been studied in Treg cells such as deletion of HDAC6, HDAC9, and Sirtuin-1 that have been shown to augment Foxp3 acetylation and Treg function leading to protection against inflammatory responses such as DSS-induced colitis, T cell-induced colitis, and allograft rejection [94–97]. Furthermore, the combined deletion or inhibition of these three deacetylases has a synergetic effect on the increased Treg suppressive function [98]. HDAC9 is highly expressed in Treg cells among other HDACs and its expression was shown to be regulated by Glycoprotein A repetitions predominant (GARP) protein [94,99]. Mutation of GARP in a human was found in primary immunodeficiency patients and was associated with Treg dysfunction. Loss of GARP didn't influence Treg cell frequency or *in vitro* suppressive function but impaired *in vivo* Treg suppressive function and showed increased susceptibility to inflammatory diseases such as EAE and DSS-induced colitis. GARP deficiency led to a decrease in the availability of TGF- β that causes an increase in HDAC9 expression and deacetylation of Foxp3 [99].

Also, a recent study showed that deletion of HDAC10 enhances Foxp3⁺ Treg cell function and resistance against T cell-induced colitis outcomes and allograft survival [100]. On the other hand, deletion of other KDACs, HDAC3, HDAC5, or Sirtuin-3 shown opposite results with impaired Foxp3⁺ Treg function [101–103]. Deletion of these deacetylases is then associated with a decreased allograft transplant survival [101–103], the development of lethal autoimmunity as well as enhanced susceptibility to T cell-induced colitis [102].

Opposite to a direct acetylation/deacetylation of Foxp3, PTM of histones can also interfere with T cells differentiation. The CoREST complex which includes HDAC1/2, Rcor1, and Lsd1 was shown to be associated with Foxp3 protein in Treg cells through co-immunoprecipitation. In the absence of Rcor1 in Treg cells, the frequency of Foxp3⁺ cells in the spleen, as well as their suppressive function, are decreased. Rcor1 deletion led to the disruption of the CoREST complex in Treg cells and its ability to regulate histone acetylation leading to an increase in IFN- γ and T-bet expression in Treg cells [104].

3. Addition of complex molecules

The addition of more complex molecules can also be used as PTMs. Protein function, cellular localization, and cell signaling can then be regulated through the attachment of glucids, in the form of carbohydrates, and numerous lipids respectively belonging to PTMs called glycosylation and lipidation.

3.1. Glycosylation

Protein glycosylation is the PTM of protein in which carbohydrate (glycan) is attached to hydroxyl (-OH) group of serine and threonine (O-linked glycosylation) or at the carboxamide side chain (-NH₂) of asparagine (Asn or N) or glutamines (N-linked glycosylation). Glycosylation of proteins occurs mainly in the Golgi apparatus and the endoplasmic reticulum and results in the modulation of protein structure and function as well as is involved in the cell-cell recognition and signaling.

Cabral et al. (2017) reported that surface glycosylation of Treg cells is important for Treg development and suppressive functions [105]. Surface levels of tri/tetra-antennary N-glycans in Treg cells correlated with higher expression of CD39, CD73, ICOS, GITR, PD-1, PD-L1, and CTLA-4, which play important role in determining Treg cells phenotype and suppressive functions. Treatment of Tregs with PGNase F decreases surface N-glycan expression and impairs splenic Treg cell suppression [105]. Recently, it was found that O-GlcNAc glycosylation deficiency can impair Treg cells' differentiation [106]. *In vitro* experiments showed that glycosylation deficiency suppresses the Notch signaling that prevents Treg cell differentiation. Inhibition of the Notch signaling leads to CD4⁺ T lymphocyte infiltration and results in the aggravation of hepatic function in concanavalin-A induced autoimmune hepatitis [106]. More recently, using liquid chromatography with tandem mass spectrometry (LC-MS/MS), Liu et al. (2019) revealed that Foxp3 can be modified at T38, S57, S58, S270, and S273 residues by O-GlcNAc transferase (OGT) [107]. In mature Treg cells, the protein O-GlcNAc acetylation stabilizes Foxp3 and activates STAT5 as well as is essential for lineage stability and effector function in Treg cells. In the presence of ubiquitin specific peptidase (USP)7, a protein deubiquitinase that controls Foxp3 poly-ubiquitination and degradation, OGT could not further increase a Foxp3 protein level which indicates that O-GlcNAc acetylation may counteract ubiquitination of Foxp3 protein. Moreover, deficiency in protein O-GlcNAc acetylation attenuates IL-2/STAT5 activity in Treg cells and decreases their suppressive function [107]. Interestingly, galectin-1 (Gal1), a member of the highly conserved glycan-binding protein family, negatively regulates Th1- and Th17-mediated response based on differential glycosylation of T cell subsets [108]. It was found that Th1 and Th17 differentiated cells expressed a distinct set of cell surface glycans *e.g.*, galactose- β 1-4-N-acetylglucosamine that is essential for galectin-1 signaling and cell death [108].

3.2. Lipidation

Compared to other PTMs, there are very few reported studies concerning the role of lipidation in T cell biology. Lipidation defines the binding of a lipid molecule onto a target protein. As essential components of cells, lipids can be used for cellular membranes, energy storage, metabolic substrate, and secondary messenger. Lipidation is implicated in the regulation of membranes-protein interactions such as membrane trafficking, protein secretion, signal transduction, and apoptosis [109]. Depending on the type of lipid used, the process can be reversible (palmitoylation) or irreversible (prenylation), and attach to different residues. There are five main types of lipidations: 1) Glycine-Myristoylation, 2) Cysteine-Palmitoylation, 3) Serine or Lysine-fatty acylation, 4) Cysteine-Prenylation, and 5) Cholesterol esterification.

The role of lipidation in Th17/Treg differentiation is still largely unknown but seems to have gotten more attention in the last years.

3.2.1. Myristoylation

Myristoylation refers to the addition of a myristic acid to proteins. Myristoylation plays an important role in immune response and TCR signaling with an association between myristoylation of LCK and TCR activation [110]. T cell-specific deletion of myristoyl-CoA:protein N-myristoyltransferase (NMT) results in an aberrant T cell development with a decrease in double-positive (DP), CD4, and CD8 single-positive cells suggesting a developmental block in the transition from double-negative to DP T cell populations. Consequently, a strong reduction of T cells was observed in the spleen and lymph nodes. Upon TCR stimulation, lower CD69 expression and decreased phosphorylation of CD3 ζ , Zap70, and ERK respectively indicated a defect in T cell activation and TCR signaling in the absence of myristoylation in T cells. Interestingly, the development of $\gamma\delta$ T cells, a minor subset of T cells (5–10%), was not affected by the absence of myristoylation in the thymus. Compare to $\alpha\beta$ T cells that differentiate into distinct subsets, $\gamma\delta$ T cells are more plastic and an important source of IL-17 production as Th17 cells [111,112]. A study from Rampoldi et al. shows that in the absence of myristoylation, ROR γ t mRNA level in the thymocytes was significantly reduced and T-bet a key transcription factor of Th1 subset as well as IFN- γ production were increased in $\gamma\delta$ T cells [113]. In humans, a patient suffering from RA exhibited an NMT deficiency. Rescue of myristoylation in RA T cells with transfection of an NMT1 overexpression construct reduced T-bet and ROR γ t expression and as well as IFN- γ ⁺ and IL-17⁺ frequencies. Knockdown of NMT1 in CD4⁺ T cells of healthy donors as the opposite effects by forcing Th1 and Th17 cell differentiation. Further experiments then indicate that NMT defect leads to an inactivation of AMPK due to a defect in lysosomal recruitment. Consecutively, the metabolic status in T cells is altered in favor of mTORC1 activation and pro-inflammatory response [114].

3.2.2. Prenylation

Prenylation is the attachment of mevalonate-derived, short-chain isoprenoids to specific proteins such as the isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) [115]. In addition to protein prenylation, the mevalonate is the precursor of the biosynthesis of cholesterol.

Inhibition of prenylation in T cells causes T cell development defect leading to lymphopenia as well as increased cell death [116–118]. Following HMG-CoA reductase (HMGCR) deletion in T cell, cell death can be rescued by the addition of mevalonate or GGPP [116]. Prenylation is also essential for chemokine receptor signaling and plays an important role in the migration and localization of T cells [117,118]. Prenylation can also regulate the balance between Th17 and Treg differentiation.

Inhibitors of HMGCR (simvastatin), geranylgeranyltransferase (GGTase) (GGTI-298), farnesyltransferase (FTase) (FTI-277) respectively catalyzing the conversion of HMG-CoA to mevalonate, protein geranylgeranylation, and protein farnesylation were used to analyze the role of prenylation on Th17 and Treg differentiation. Interestingly, the latter didn't show any effect on Th17/Treg differentiation while the others inhibit Th17 and promote Treg differentiation. Inhibition of IL-6-induced STAT3 phosphorylation was found to be implicated in the decrease of Th17 generation leading to a resistance to T cell-induced colitis with GGTI-298 treatment [119]. Specific deletion of β -subunit of GGTase-I (PGGT1b) in CD4 cells shows the same results associated with resistance to EAE development [118]. While López-Posadas et al. (2019) reported that T cell-specific PGGT1b deficient mice developed spontaneous colitis-associated to an increased ROR γ ⁺ CD4 cells and IL-17 mRNA expression in the intestine. Reduced expression of PGGT1b in T cells isolated from intestinal tissues in IBD patients was also found [117].

On the other hand, mediators of the activation of the mevalonate pathway including HMGCR, PGGT1b, FNTB, and LKB1 were shown to play a role in Treg cells [116,120,121]. Increased *Hmgcr* and *Pggt1a* but not *Fntb* mRNA expression were reported in Treg cells upon activation

[120]. Specific deletion of HMGCR in Treg cells provoked a reduced percentage of Foxp3⁺ cells in the spleen and mesenteric lymph nodes [116] and induces inflammatory cytokine production in Treg cells [121]. Specific deletion of PGGT1b or FNTB in Treg cells led to an increase in the total cell number in the spleen and lymph nodes of mutant mice resulting from a lower percentage of Foxp3⁺ cells and an increased percentage of effector cells such as IFN- γ ⁺, IL-4⁺, IL-17⁺ CD4⁺ cells, and IFN- γ ⁺ CD8⁺ cells [120]. Further analysis has revealed that protein farnesylation was implicated in the maintenance of mTORC1 and ICOS signaling in Treg cells, while protein geranylgeranylation plays a role in the TCR signaling inducing Treg differentiation [120]. Treg suppressive function was shown to be inhibited with statins treatment, an inhibitor of HMGCR, and rescued with the addition of FPP or GGPP to statins treated cells [120].

T cell LKB1 deletion in mice shows a reduction of Treg cell percentage in the spleen, a decrease in Treg suppressive function, and an increase susceptibility to the T cell transfer colitis model. Specific deletion of LKB1 in Treg cells revealed the production of inflammatory cytokines such as IFN- γ , IL-4, IL-13, and IL-17 by Treg cells [121]. Deletion of LKB1 in Treg cells provokes impairment of the mevalonate pathway. Addition of mevalonate or GGPP restores Foxp3 expression as well as suppressive function in Treg cells. Polarized Treg cells from LKB1 deficient mice treated with GGPP show an induction of the phosphorylation and activation of STAT5 leading to Treg lineage stability [121]. Altogether, prenylation is required for Foxp3 expression, as well as the stability and suppressive function of Treg cells.

3.2.3. Palmitoylation

Palmitoylation, covalent attachment of palmitate onto proteins, is undergone in T cells and regulates different functions of T cells such as TCR signaling, calcium flux, apoptosis, or anti-tumours activity [122]. Concerning the role of palmitoylation on Th17/Treg cell differentiation, several proteins of the IL-6 signaling pathway and STAT3 were found to be regulated by palmitoylation [123,124].

STAT3 has been described as a target of a palmitoylation/depalmitoylation cycle associated with its activation state. Palmitoylation of STAT3 by the palmitoyltransferases DHHC7 favours its recruitment to the membrane and activation (p-STAT3). Once activated, the palmitic acid is removed by the action of the acyl protein thioesterases APT2 and p-STAT3 enter the nucleus and activate the transcription of many important genes required for the Th17 differentiation. Co-expression of STAT3 and DHHC7 (control or inactivated form) in mouse splenocytes shows that Th17 differentiation was promoted in the presence of palmitoylated STAT3. On the other hand, inhibition of the depalmitoylation of STAT3 provokes a decrease in STAT3 target genes expression and Th17 differentiation suggesting the importance of the STAT3 palmitoylation/depalmitoylation cycle. Upregulation of genes coding for DHHC7 and APT2 has been observed in PBMCs of patients suffering from IBD and is coherent with amelioration of DSS-induced colitis symptoms in mice following the invalidation or deletion of these two proteins [124].

4. Addition of polypeptides

In this category of PTM, target proteins are regulated through the covalent attachment of other proteins or peptides. In most cases, this type of PTMs is associated with protein degradation. The most common PTM with the addition of polypeptides are ubiquitination and SUMOylation.

4.1. Ubiquitination

Ubiquitination is a PTM in which proteins are marked by a ubiquitin protein. This process includes three steps: 1) Ubiquitin activation by the ubiquitin-activating enzyme (E1), 2) Ubiquitin conjugation by the ubiquitin-conjugating enzyme (E2), 3) Ubiquitin transfer onto the target

protein on a lysine residue by ubiquitin-protein ligase (E3). Ubiquitination regulates protein degradation through activation of the proteasome pathway but can also serve to alter protein-protein interaction. Ubiquitin contains seven lysine residues, and it is established that ubiquitination function is associated with the lysine residue linked to the target protein. K48-linked chains are commonly associated with degradative ubiquitination while K63-linked chains are associated with non-degradative ubiquitination [125]. Also, deubiquitinating enzymes (DUBs) catalyze the removal of ubiquitin from the target protein.

Ubiquitination is a commonly studied PTM that has often been investigated in T cells and Th17/Treg differentiation. In 2021, two reviews have been extensively reported while focussing their attention on the ubiquitination/deubiquitylation of the transcription factors Foxp3 and ROR γ t [20,126]. Altogether, deubiquitylation of Foxp3 by USP family such as USP7, USP21, and USP44 as well as Foxp3 ubiquitination by mouse double minute 2 homolog (MDM2), TNF receptor-associated factor 6 (TRAF6), and RING finger protein 31 (RNF31) are required for maintaining Treg stability and suppressive function. Whereas, STUB1 and hypoxia-inducible factor 1 subunit alpha (HIF1 α) promote ubiquitination of Foxp3 and its degradation [36,127]. In response to LPS exposure, STUB1 interacts with Foxp3 and mediates its K48-linked polyubiquitination leading to Foxp3 degradation by the proteasome. Overexpression of STUB1 in primary mouse T cells impairs Treg suppressive function *in vitro* and *in vivo* which contributes to aggravated symptoms in T cell transfer colitis model [36]. For Th17 cells, K63 ubiquitination of ROR γ t by TRAF5 as well as deubiquitylation by USP4, USP15, and USP17 contribute to its stabilization while E3 ubiquitin ligase Itch inhibits Th17 differentiation through K48-linked polyubiquitination of ROR γ t [20,126].

Another USP family member, USP18 has been shown to be involved in the generation of Th17 cells through regulation of TGF- β activated kinase 1 (MAP3K7) binding protein 1 (TAB1)-TAK1 activity [128] and Treg differentiation and function [129]. Naïve CD4⁺ cells from USP18 deficient mice show a decrease in IL-17⁺ cells percentage and IL-17 mRNA expression and increase of Foxp3⁺ cells and Foxp3 mRNA expression upon Th17 and Treg *in vitro* polarization respectively. The defect in Th17 differentiation was associated with resistance to EAE induction. Through the deubiquitination of TAB1/TAK1, USP18 negatively regulates TCR-induced activation of nuclear factor- κ B (NF- κ B) and NFAT, leading to an inhibition of IL-2 production [128]. In USP18 knock-out mice, Treg differentiation is enhanced with an increase in the percentage of Foxp3⁺ cells in the spleen and lymph nodes but their suppressive function is downregulated. Other effector T cells showed a reduction in cytokine production including IFN- γ ⁺, IL-17⁺, and IL-4⁺ cells [129].

Regulation of Th17 generation can also be associated with STAT3 ubiquitination by PDZ and LIM domain protein 2 (PDLIM2) and HECT E3 ubiquitin ligase (HECTD3). PDLIM2 is a ubiquitin E3 ligase known to promote ubiquitination and proteasomal degradation of STAT4. PDLIM2 suppresses Th17 and Th1 cell differentiation through degradation of poly-ubiquitinated STAT3 then preventing the development of EAE [130]. In mice, HECTD3 promotes non-degradative poly-ubiquitination of MALT1 and STAT3 in which ubiquitination of MALT1 at K648 and STAT3 at K180 is essential for the generation of Th17 cells (IL-17⁺ROR γ t⁺ cells) [131]. However, Th17 cells have reduced pathogenicity in absence of HECTD3 leading to a resistance in EAE development [131]. Brauner et al. (2018) reported that E3 ubiquitinase ligase tripartite motif-containing protein 21 (TRIM21) expression was found inversely correlated with Th17 lineage crucial cytokine IL-17, IL-23, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and regulator of Th17 differentiation such as interferon regulatory factor 4 (IRF4) and RORc in patients with atherosclerotic plaques [132]. Therefore, TRIM21 is a negative regulator of Th17 cell differentiation. Another E3 ubiquitin ligase, Von Hippel-Lindau (VHL), was shown to

have the opposite effect in which, VHL-deficient mice exhibit a reduced capacity for Th17 cell differentiation and resistance to EAE development. In these mice, VHL deficiency was paired with an increase in glycolysis and glycolytic capacity in Th17 cells [133].

4.2. Sumoylation

Protein SUMOylation corresponds to the attachment of a Small Ubiquitin-like Modifier (SUMO) to protein onto a lysine residue. The SUMO family is composed of four members, SUMO-1, -2, -3, and -4 that are synthesized as pro-peptide. Similar to ubiquitination, SUMOs are sequentially matured by cleaved through sentrin-specific protease (SENPs), activated by heterodimer E1 activating enzyme, and conjugated to the ubiquitin-conjugating enzyme 9 (UBC9)/E3 ligating enzyme prior to SUMOylation to the target protein. SUMOylation is a reversible PTM that is catalyzed by the action of SENPs. SUMOylation functions include the regulation of protein subcellular localization, protein-DNA and protein-protein interactions, and transcriptional regulation.

During the last ten years, the roles of SUMOylation in T cells have been investigated even it is still not as well studied as some other PTMs. However, it seems more clear now that SUMOylation is required in the regulation of Th17/Treg cells.

In Th17 cells, direct SUMOylation of ROR γ t at K187 through UBC9 facilitates the binding of HDAC2 to the IL-17 promoter and the inhibition of IL-17 expression [86]. Mutation of K187 to an R residue led to a defect in SUMOylation of ROR γ t resulting in increased severity of T cell-induced colitis [86]. PIAS4, E3 ligase SUMOylation of SUMO3 on K31 residue of ROR γ t recruits KAT2A, leading to the stabilization of the binding of SRC1 to enhance ROR γ t transcription factor activity [134]. Defect in ROR γ t SUMOylation through mutation of K31 to R residue, induces a defect in Th17 differentiation associated with resistance to EAE development [134]. Under febrile temperature, SUMOylation of SMAD4 by UBC9 was required for Th17 differentiation and implicated in aggravation of autoimmune EAE disease [135]. Upon SUMOylation, the Nuclear factor of activated T cells (NFAT)c1 repressed IL-2 production leading to an increase in IL-17 and IFN- γ cytokines production and elevated Th17 and Th1 cell differentiation to the detriment of Treg differentiation. Abolition of NFATc1 SUMOylation through mutation of K702 and K914 SUMOylated site to arginine residues protected mice from the development of autoimmune and alloimmune diseases such as EAE and acute graft-versus-host disease [136].

The role of de/SUMOylation in Treg cell is still largely unknown although no direct SUMOylation of Treg was found, Treg differentiation is regulated by this PTM. PIAS1, through regulation of the binding of DNMTs to the Foxp3 promoter, maintains a repressive chromatin state at Foxp3 promoter that inhibits Treg cell generation [137]. In absence of PIAS1, Treg differentiation is enhanced, and mice are protected from EAE development. However, SUMOylation through UBC9 was shown to be required for Treg cell homeostasis, proliferation, and suppressive function and UBC9 deletion led to early and lethal autoimmune disorder development. Ubc9 effects come from the regulation of TCR signaling pathways such as NFAT and IRF4. SUMOylation of IRF4 promotes its stability necessary for Treg cells' function [138]. SENP3 specific deletion in CD4⁺ cells and Treg⁺ cells induces a decrease in Foxp3⁺ cells frequency in all the lymphoid organs as well as an increase in IFN- γ ⁺ and IL-17⁺ cells in the spleen of the latter transgenic mice [139]. In absence of SENP3 in Treg cells, Treg suppressive function was decreased *in vitro* and *in vivo*, and tumor growth from B16-F10 melanoma and MC38 colon cancer cells injection was reduced. Further experiments showed that SENP3 promotes Treg stability through deSUMOylation of BACH2 that suppresses effector programs and maintains Treg homeostasis and function [139].

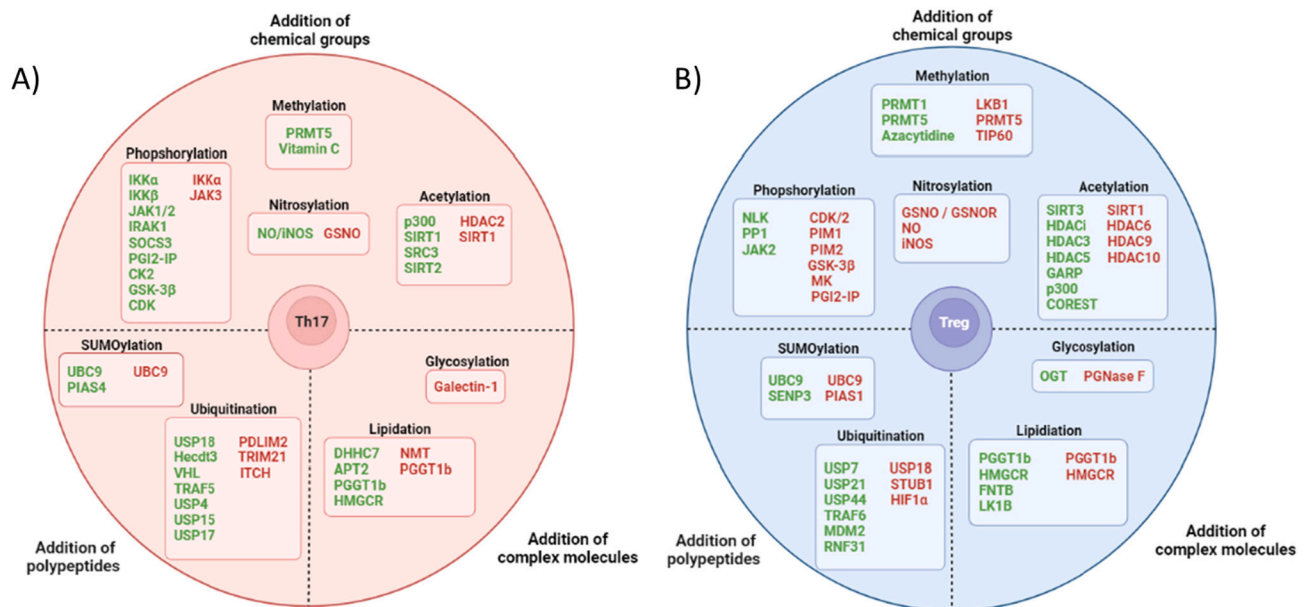


Fig. 2. Effects of PTMs associated molecules on Th17/Treg cell differentiation and functions.

Summary of the effects of molecules related to PTMs on Th17 and Treg cell differentiation and functions are presented in A) and B) respectively. Promotion or/and inhibitory effects of the molecules on Th17 (A) and/or Treg (B) cell differentiation and functions are indicated in green and red colour respectively.

5. Conclusion

In this review, we focus on summarizing the role of the different types of PTMs on the regulation of Th17 and Treg cell differentiation and function (Fig. 2, Table S1). In addition to the PTMs directly associated with transcription factors Foxp3 and ROR γ t, we have described here a lot of other proteins involved in these processes. Control of the Th17/Treg balance by PTMs is then linked to multiple molecular mechanisms such as regulation of proteins involved in T cell metabolism, regulation of protein stability, localization and function, or regulation of gene transcription through epigenetic modifications. We also notice interactions, either cooperation or inhibition between different PTMs affecting the Th17 and Treg differentiation. For example, STAT3 palmitoylation enhances its recruitment to the membrane that will contribute to STAT3 phosphorylation and activation or O-GlcNAcylation that seems to promote Foxp3 stability by counteracting with ubiquitination. In this review, we have reviewed the role of some well-known PTMs including phosphorylation and ubiquitination but also some less studied PTMs such as nitrosylation, glycosylation, and lipidation in T cells. However, there are more than 200 different types of PTMs identified so far for which their functions have yet to be investigated in T cells or other cells, such as neddylation, citrullination, crotonylation, and so on.

Th17/Treg balance is known to be dysregulated in a lot of autoimmune diseases and cancers. Given the important role of PTMs in the regulation of the Th17/Treg balance, targeting PTMs seems to be promising in the treatment of these diseases. A better understanding of the role of PTMs and their interaction with each other in the regulation of Th17/Treg differentiation and function will then allow the development of more effective treatment targeting PTMs regulation in T cells.

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Declaration of competing interest

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References

- [1] R.V. Luckheeram, R. Zhou, A.D. Verma, B. Xia, CD4⁺ T cells: differentiation and functions, *Clin. Dev. Immunol.* 2012 (2012) 1–12, <https://doi.org/10.1155/2012/925135>.
- [2] J. Zhu, W.E. Paul, Peripheral CD4⁺ T-cell differentiation regulated by networks of cytokines and transcription factors: transcription factor network in Th cells, *Immunol. Rev.* 238 (2010) 247–262, <https://doi.org/10.1111/j.1600-065X.2010.00951.x>.
- [3] G. Lee, The balance of Th17 versus treg cells in autoimmunity, *Int. J. Mol. Sci.* 19 (2018) 730, <https://doi.org/10.3390/ijms19030730>.
- [4] M. Ruterbusch, K.B. Pruner, L. Shehata, M. Pepper, In vivo CD4⁺ T cell differentiation and function: revisiting the Th1/Th2 paradigm, *Annu. Rev. Immunol.* 38 (2020) 705–725, <https://doi.org/10.1146/annurev-immunol-103019-085803>.
- [5] Z. Chen, J.J. O'Shea, Th17 cells: a new fate for differentiating helper T cells, *Immunol. Res.* 41 (2008) 87–102, <https://doi.org/10.1007/s12026-007-8014-9>.
- [6] Z. Chen, A. Laurence, Y. Kanno, M. Pachter-Zavisin, B.-M. Zhu, C. Tato, A. Yoshimura, L. Hennighausen, J.J. O'Shea, Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells, *Proc. Natl. Acad. Sci.* 103 (2006) 8137–8142, <https://doi.org/10.1073/pnas.0600666103>.
- [7] J.J. O'Shea, W.E. Paul, Mechanisms underlying lineage commitment and plasticity of helper CD4⁺ T cells, *Science* 327 (2010) 1098–1102, <https://doi.org/10.1126/science.1178334>.
- [8] M. Kanamori, H. Nakatsukasa, M. Okada, Q. Lu, A. Yoshimura, Induced regulatory T cells: their development, stability, and applications, *Trends Immunol.* 37 (2016) 803–811, <https://doi.org/10.1016/j.it.2016.08.012>.
- [9] A. Laurence, C.M. Tato, T.S. Davidson, Y. Kanno, Z. Chen, Z. Yao, R.B. Blank, F. Meylan, R. Siegel, L. Hennighausen, E.M. Shevach, J.J. O'Shea, Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation, *Immunity* 26 (2007) 371–381, <https://doi.org/10.1016/j.immuni.2007.02.009>.
- [10] I.I. Ivanov, B.S. McKenzie, L. Zhou, C.E. Tadokoro, A. Lepelletier, J.J. Lafaille, D. J. Cua, D.R. Littman, The orphan nuclear receptor ROR γ t directs the differentiation program of proinflammatory IL-17+ T helper cells, *Cell* 126 (2006) 1121–1133, <https://doi.org/10.1016/j.cell.2006.07.035>.
- [11] M. Veldhoen, R.J. Hocking, R.A. Flavell, B. Stockinger, Signals mediated by transforming growth factor- β initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease, *Nat. Immunol.* 7 (2006) 1151–1156, <https://doi.org/10.1038/ni1391>.
- [12] F. Zhang, L.J. Fuss, Z. Yang, W. Strober, Transcription of ROR γ t in developing Th17 cells is regulated by E-proteins, *Mucosal Immunol.* 7 (2014) 521–532, <https://doi.org/10.1038/mi.2013.69>.

- [13] L. Durant, W.T. Watford, H.L. Ramos, A. Laurence, G. Vahedi, L. Wei, H. Takahashi, H.-W. Sun, Y. Kanno, F. Powrie, J.J. O'Shea, Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis, *Immunity* 32 (2010) 605–615, <https://doi.org/10.1016/j.immuni.2010.05.003>.
- [14] C. Scheinecker, L. Göschl, M. Bonelli, Treg cells in health and autoimmune diseases: new insights from single cell analysis, *J. Autoimmun.* 110 (2020), 102376, <https://doi.org/10.1016/j.jaut.2019.102376>.
- [15] L. Han, J. Yang, X. Wang, D. Li, L. Lv, B. Li, Th17 cells in autoimmune diseases, *Front. Med.* 9 (2015) 10–19, <https://doi.org/10.1007/s11684-015-0388-9>.
- [16] M. Bonelli, A. Savitskaya, K. von Dalwigk, C.W. Steiner, D. Aletaha, J.S. Smolen, C. Scheinecker, Quantitative and qualitative deficiencies of regulatory T cells in patients with systemic lupus erythematosus (SLE), *Int. Immunol.* 20 (2008) 861–868, <https://doi.org/10.1093/intimm/dxn044>.
- [17] T. Kinnunen, N. Chamberlain, H. Morbach, T. Cantaert, M. Lynch, P. Preston-Hurlburt, K.C. Herold, D.A. Hafler, K.C. O'Connor, E. Meffre, Specific peripheral B cell tolerance defects in patients with multiple sclerosis, *J. Clin. Invest.* 123 (2013) 2737–2741, <https://doi.org/10.1172/JCI68775>.
- [18] L. Sun, J. Fu, Y. Zhou, Metabolism controls the balance of Th17/T-regulatory cells, *Front. Immunol.* 8 (2017) 1632, <https://doi.org/10.3389/fimmu.2017.01632>.
- [19] F. Yu, S. Sharma, J. Edwards, L. Feigenbaum, J. Zhu, Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance, *Nat. Immunol.* 16 (2015) 197–206, <https://doi.org/10.1038/ni.3053>.
- [20] H.K. Kim, M.G. Jeong, E.S. Hwang, Post-translational modifications in transcription factors that determine T helper cell differentiation, *Mol. Cells* 44 (2021) 318–327, <https://doi.org/10.14348/molcells.2021.0057>.
- [21] C.H. Patel, J.D. Powell, Targeting T cell metabolism to regulate T cell activation, differentiation and function in disease, *Curr. Opin. Immunol.* 46 (2017) 82–88, <https://doi.org/10.1016/j.coi.2017.04.006>.
- [22] Z. Wang, Q. Lu, Z. Wang, Epigenetic alterations in cellular immunity: new insights into autoimmune diseases, *Cell. Physiol. Biochem.* 41 (2017) 645–660, <https://doi.org/10.1159/000457944>.
- [23] S. Ramazi, J. Zahiri, Post-translational modifications in proteins: resources, tools and prediction methods, in: Database. 2021, 2021, p. baab012, <https://doi.org/10.1093/database/baab012>.
- [24] G.A. Khoury, R.C. Baliban, C.A. Floudas, Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database, *Sci. Rep.* 1 (2011) 90, <https://doi.org/10.1038/srep00090>.
- [25] G. Deng, X. Song, S. Fujimoto, C.A. Piccirillo, Y. Nagai, M.I. Greene, Foxp3 post-translational modifications and Treg suppressive activity, *Front. Immunol.* 10 (2019) 2486, <https://doi.org/10.3389/fimmu.2019.02486>.
- [26] I.-R. Tak, F. Ali, J.S. Dar, A.R. Magray, B.A. Ganai, M.Z. Chishti, Posttranslational modifications of proteins and their role in biological processes and associated diseases, in: *Protein Modif*, Elsevier, 2019, pp. 1–35, <https://doi.org/10.1016/B978-0-12-811913-6.00001-1>.
- [27] N. Blom, T. Sicheritz-Pontén, R. Gupta, S. Gammeltoft, S. Brunak, Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence, *Proteomics* 4 (2004) 1633–1649, <https://doi.org/10.1002/pmic.200300771>.
- [28] F. Ardito, M. Giuliani, D. Perrone, G. Troiano, L.L. Muzio, The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (review), *Int. J. Mol. Med.* 40 (2017) 271–280, <https://doi.org/10.3892/ijmm.2017.3036>.
- [29] Y. Shi, Y. Zhang, S. Lin, C. Wang, J. Zhou, D. Peng, Y. Xue, dbPSP 2.0, an updated database of protein phosphorylation sites in prokaryotes, *Sci. Data.* 7 (2020) 164, <https://doi.org/10.1038/s41597-020-0506-7>.
- [30] A. Hauser, M. Penkert, C.P.R. Hackenberger, Chemical approaches to investigate labile peptide and protein phosphorylation, *Acc. Chem. Res.* 50 (2017) 1883–1893, <https://doi.org/10.1021/acs.accounts.7b00170>.
- [31] A. Sickmann, H.E. Meyer, Histidine phosphorylation site identification by ESI-MS, in: *Cold Spring Harb. Protoc.* 2007, 2007, <https://doi.org/10.1101/pdb.prot4628>.
- [32] H.-C. Chuang, T.-H. Tan, MAP4K3/GLK in autoimmune disease, cancer and aging, *J. Biomed. Sci.* 26 (2019) 82, <https://doi.org/10.1186/s12929-019-0570-5>.
- [33] S.A. Gibson, W. Yang, Z. Yan, Y. Liu, A.L. Rowse, A.S. Weinmann, H. Qin, E. N. Benveniste, Protein kinase CK2 controls the fate between Th17 cell and regulatory T cell differentiation, *J. Immunol.* 198 (2017) 4244–4254, <https://doi.org/10.4049/jimmunol.1601912>.
- [34] H. Yue, Y. Zhao, H. Wang, F. Ma, F. Liu, S. Shen, Y. Hou, H. Dou, Anti-fibrosis effect for *Hirsutiella sinensis* mycelium based on inhibition of mTOR p70S6K phosphorylation, *Innate Immun.* 23 (2017) 615–624, <https://doi.org/10.1177/1753425917726361>.
- [35] V. Fleskens, C.M. Minutti, X. Wu, P. Wei, C.E.G.M. Pals, J. McCrae, S. Hemmers, V. Groenewold, H.-J. Vos, A. Rudensky, F. Pan, H. Li, D.M. Zaiss, P.J. Coffer, Nemo-like kinase drives Foxp3 stability and is critical for maintenance of immune tolerance by regulatory T cells, *Cell Rep.* 26 (2019) 3600–3612.e6, <https://doi.org/10.1016/j.celrep.2019.02.087>.
- [36] Z. Chen, J. Barbi, S. Bu, H.-Y. Yang, Z. Li, Y. Gao, D. Jinasena, J. Fu, F. Lin, C. Chen, J. Zhang, N. Yu, X. Li, Z. Shan, J. Nie, Z. Gao, H. Tian, Y. Li, Z. Yao, Y. Zheng, B.V. Park, Z. Pan, J. Zhang, E. Dang, Z. Li, H. Wang, W. Luo, L. Li, G. L. Semenza, S.-G. Zheng, K. Loser, A. Tsun, M.I. Greene, D.M. Pardoll, F. Pan, B. Li, The ubiquitin ligase Stub1 negatively modulates regulatory T cell suppressive activity by promoting degradation of the transcription factor Foxp3, *Immunity* 39 (2013) 272–285, <https://doi.org/10.1016/j.immuni.2013.08.006>.
- [37] H. Nie, Y. Zheng, R. Li, T.B. Guo, D. He, L. Fang, X. Liu, L. Xiao, X. Chen, B. Wan, Y.E. Chin, J.Z. Zhang, Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF- α in rheumatoid arthritis, *Nat. Med.* 19 (2013) 322–328, <https://doi.org/10.1038/nm.3085>.
- [38] Z. Li, F. Lin, C. Zhuo, G. Deng, Z. Chen, S. Yin, Z. Gao, M. Piccioni, A. Tsun, S. Cai, S.G. Zheng, Y. Zhang, B. Li, PIM1 kinase phosphorylates the human transcription factor FOXP3 at serine 422 to negatively regulate its activity under inflammation, *J. Biol. Chem.* 289 (2014) 26872–26881, <https://doi.org/10.1074/jbc.M114.586651>.
- [39] F. Lin, X. Luo, A. Tsun, Z. Li, D. Li, B. Li, Kaempferol enhances the suppressive function of Treg cells by inhibiting FOXP3 phosphorylation, *Int. Immunopharmacol.* 28 (2015) 859–865, <https://doi.org/10.1016/j.intimp.2015.03.044>.
- [40] G. Deng, Y. Nagai, Y. Xiao, Z. Li, S. Dai, T. Ohtani, A. Banham, B. Li, S.-L. Wu, W. Hancock, A. Samanta, H. Zhang, M.I. Greene, Pim-2 kinase influences regulatory T cell function and stability by mediating Foxp3 protein N-terminal phosphorylation, *J. Biol. Chem.* 290 (2015) 20211–20220, <https://doi.org/10.1074/jbc.M115.638221>.
- [41] S. Basu, T. Golovina, T. Mikheeva, C.H. June, J.L. Riley, Cutting edge: Foxp3-mediated induction of Pim 2 allows human T regulatory cells to preferentially expand in rapamycin, *J. Immunol.* 180 (2008) 5794–5798, <https://doi.org/10.4049/jimmunol.180.9.5794>.
- [42] P.A. Morawski, P. Mehra, C. Chen, T. Bhatti, A.D. Wells, Foxp3 protein stability is regulated by cyclin-dependent kinase 2*, *J. Biol. Chem.* 288 (2013) 24494–24502, <https://doi.org/10.1074/jbc.M113.467704>.
- [43] K. Nakahira, A. Morita, N.-S. Kim, I. Yanagihara, Phosphorylation of FOXP3 by LCK downregulates MMP9 expression and represses cell invasion, *PLoS One* 8 (2013), e77099, <https://doi.org/10.1371/journal.pone.0077099>.
- [44] H. Gu, L. Ding, S. Xiong, X. Gao, B. Zheng, Inhibition of CDK2 promotes inducible regulatory T-cell differentiation through TGF β -Smad3 signaling pathway, *Cell. Immunol.* 290 (2014) 138–144, <https://doi.org/10.1016/j.cellimm.2014.05.004>.
- [45] Z. He, F. Wang, J. Zhang, S. Sen, Q. Pang, S. Luo, Y. Gwack, Z. Sun, Regulation of Th17 differentiation by IKK α -dependent and -independent phosphorylation of ROR γ t, *J. Immunol.* 199 (2017) 955–964, <https://doi.org/10.4049/jimmunol.1700457>.
- [46] H.-C. Chuang, C.-Y. Tsai, C.-H. Hsueh, T.-H. Tan, GLK-IKK β signaling induces dimerization and translocation of the AhR-ROR γ t complex in IL-17A induction and autoimmune disease, *Sci. Adv.* 4 (2018) eaat5401, <https://doi.org/10.1126/sciadv.aat5401>.
- [47] A. Jiao, Z. Yang, X. Fu, X. Hua, Phloretin modulates human Th17/Treg cell differentiation in vitro via AMPK signaling, *Biomed. Res. Int.* 2020 (2020) 1–12, <https://doi.org/10.1155/2020/6267924>.
- [48] H. Wu, S. Yan, J. Chen, X. Luo, P. Li, X. Jia, X. Dai, C. Wang, Q. Huang, L. Liu, Y. Zhang, A. Zhou, Y. Chang, L. Zhang, W. Wei, JAK1-STAT3 blockade by JAK inhibitor SHR0302 attenuates inflammatory responses of adjuvant-induced arthritis rats and decreases Th17 and total B cells, *Joint Bone Spine* 83 (2016) 525–532, <https://doi.org/10.1016/j.jbspin.2015.09.002>.
- [49] J.-S. Park, J. Lee, M.-A. Lim, E.-K. Kim, S.-M. Kim, J.-G. Ryu, J.H. Lee, S.-K. Kwok, K.-S. Park, H.-Y. Kim, S.-H. Park, M.-L. Cho, JAK2-STAT3 blockade by AG490 suppresses autoimmune arthritis in mice via reciprocal regulation of regulatory T cells and Th17 cells, *J. Immunol.* 192 (2014) 4417–4424, <https://doi.org/10.4049/jimmunol.1300514>.
- [50] T.A. Mace, R. Shakya, O. Elnaggar, K. Wilson, H.M. Komar, J. Yang, J.R. Pitarresi, G.S. Young, M.C. Ostrowski, T. Ludwig, T. Bekaii-Saab, M. Bloomston, G. B. Lesinski, Single agent BMS-911543 Jak2 inhibitor has distinct inhibitory effects on STAT5 signaling in genetically engineered mice with pancreatic cancer, *Oncotarget.* 6 (2015) 44509–44522, <https://doi.org/10.18632/oncotarget.6332>.
- [51] H. Yoshida, A. Kimura, T. Fukaya, T. Sekiya, R. Morita, T. Shichita, H. Inoue, A. Yoshimura, Low dose CP-690,550 (tofacitinib), a pan-JAK inhibitor, accelerates the onset of experimental autoimmune encephalomyelitis by potentiating Th17 differentiation, *Biochem. Biophys. Res. Commun.* 418 (2012) 234–240, <https://doi.org/10.1016/j.bbrc.2011.12.156>.
- [52] H. Yoshida, H. Kotani, T. Kondo, I. Tani, X. Wei, S. Tsuruta, A. Kimura, M. Asakawa, M. Ito, S. Nagai, A. Yoshimura, CDK inhibitors suppress Th17 and promote iTreg differentiation, and ameliorate experimental autoimmune encephalomyelitis in mice, *Biochem. Biophys. Res. Commun.* 435 (2013) 378–384, <https://doi.org/10.1016/j.bbrc.2013.04.096>.
- [53] W. Liu, H. Li, X. Zhang, D. Wen, F. Yu, S. Yang, X. Jia, B. Cong, C. Ma, Prostaglandin I 2 -IP signalling regulates human Th17 and Treg cell differentiation, *Prostaglandins Leukot. Essent. Fatty Acids.* 89 (2013) 335–344, <https://doi.org/10.1016/j.plefa.2013.08.006>.
- [54] J. Wang, H. Takeuchi, Y. Sonobe, S. Jin, T. Mizuno, S. Miyakawa, M. Fujiwara, Y. Nakamura, T. Kato, H. Muramatsu, T. Muramatsu, A. Suzumura, Inhibition of midline alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population, *Proc. Natl. Acad. Sci.* 105 (2008) 3915–3920, <https://doi.org/10.1073/pnas.0709592105>.
- [55] U. Maitra, S. Davis, C.M. Reilly, L. Li, Differential regulation of Foxp3 and IL-17 expression in CD4 T helper cells by IRAK-1, *J. Immunol.* 182 (2009) 5763–5769, <https://doi.org/10.4049/jimmunol.0900124>.
- [56] K.H. Stürner, N. Verse, S. Yousef, R. Martin, M. Sospedra, Boswellic acids reduce Th17 differentiation via blockade of IL-1 β -mediated IRAK1 signaling: clinical immunology, *Eur. J. Immunol.* 44 (2014) 1200–1212, <https://doi.org/10.1002/eji.201343629>.
- [57] Z. Zhou, Z. Tian, M. Zhang, Y. Zhang, B. Ni, F. Hao, Upregulated IL-1 receptor-associated kinase 1 (IRAK1) in systemic lupus erythematosus: IRAK1 inhibition represses Th17 differentiation with therapeutic potential, *Immunol. Investig.* 47 (2018) 468–483, <https://doi.org/10.1080/08820139.2018.1458105>.

- [58] C. Lee-Chang, M. Bodogai, A. Martin-Montalvo, K. Wejsza, M. Sanghvi, R. Moaddel, R. de Cabo, A. Biragyn, Inhibition of breast cancer metastasis by resveratrol-mediated inactivation of tumor-evoked regulatory T cells, *J. Immunol.* 191 (2013) 4141–4151, <https://doi.org/10.4049/jimmunol.1300606>.
- [59] S. Clarke, Protein methylation, *Curr. Opin. Cell Biol.* 5 (1993) 977–983, [https://doi.org/10.1016/0955-0674\(93\)90080-A](https://doi.org/10.1016/0955-0674(93)90080-A).
- [60] Y. Kagoya, H. Saijo, Y. Matsunaga, T. Guo, K. Saso, M. Anczurowski, C.-H. Wang, K. Sugata, K. Murata, M.O. Butler, C.H. Arrowsmith, N. Hirano, Arginine methylation of FOXP3 is crucial for the suppressive function of regulatory T cells, *J. Autoimmun.* 97 (2019) 10–21, <https://doi.org/10.1016/j.jaut.2018.09.011>.
- [61] Y. Nagai, M.Q. Ji, F. Zhu, Y. Xiao, Y. Tanaka, T. Kambayashi, S. Fujimoto, M. M. Goldberg, H. Zhang, B. Li, T. Ohtani, M.I. Greene, PRMT5 associates with the FOXP3 homomer and when disabled enhances targeted p185erbB2/neu tumor immunotherapy, *Front. Immunol.* 10 (2019) 174, <https://doi.org/10.3389/fimmu.2019.00174>.
- [62] P.N. Goel, P. Grover, M.I. Greene, PRMT5 and Tip60 modify FOXP3 function in tumor immunity, *Crit. Rev. Immunol.* 40 (2020) 283–295, <https://doi.org/10.1615/CritRevImmunol.2020034789>.
- [63] Y. Zheng, L. Huang, W. Ge, M. Yang, Y. Ma, G. Xie, W. Wang, B. Bian, L. Li, H. Nie, L. Shen, Protein arginine methyltransferase 5 inhibition upregulates Foxp3+ regulatory T cells frequency and function during the ulcerative colitis, *Front. Immunol.* 8 (2017) 596, <https://doi.org/10.3389/fimmu.2017.00596>.
- [64] L.M. Webb, S. Sengupta, C. Edell, Z.L. Piedra-Quintero, S.A. Amici, J.N. Miranda, M. Bevins, A. Kennemer, G. Laliotis, P.N. Tschlis, M. Guerau-de-Arellano, Protein arginine methyltransferase 5 promotes cholesterol biosynthesis-mediated Th17 responses and autoimmunity, *J. Clin. Invest.* 130 (2020) 1683–1698, <https://doi.org/10.1172/JCI131254>.
- [65] J.K. Polansky, K. Kretschmer, J. Freyer, S. Floess, A. Garbe, U. Baron, S. Olek, A. Hamann, H. von Boehmer, J. Huehn, DNA methylation controls Foxp3 gene expression, *Eur. J. Immunol.* 38 (2008) 1654–1663, <https://doi.org/10.1002/eji.200838105>.
- [66] S.Z. Josefowicz, C.B. Wilson, A.Y. Rudensky, Cutting edge: TCR stimulation is sufficient for induction of Foxp3 expression in the absence of DNA methyltransferase 1, *J. Immunol.* 182 (2009) 6648–6652, <https://doi.org/10.4049/jimmunol.0803320>.
- [67] D. Wu, Y. Luo, W. Guo, Q. Niu, T. Xue, F. Yang, X. Sun, S. Chen, Y. Liu, J. Liu, Z. Sun, C. Zhao, H. Huang, F. Liao, Z. Han, D. Zhou, Y. Yang, G. Xu, T. Cheng, X. Feng, Lkb1 maintains Treg cell lineage identity, *Nat. Commun.* 8 (2017) 15876, <https://doi.org/10.1038/ncomms15876>.
- [68] M.H. Song, V.S. Nair, K.I. Oh, Vitamin C enhances the expression of IL17 in a Jmjd2-dependent manner, *BMB Rep.* 50 (2017) 49–54, <https://doi.org/10.5483/BMBRep.2017.50.1.193>.
- [69] Q. Li, J. Zou, M. Wang, X. Ding, I. Chepelev, X. Zhou, W. Zhao, G. Wei, J. Cui, K. Zhao, H.Y. Wang, R.-F. Wang, Critical role of histone demethylase Jmjd3 in the regulation of CD4+ T-cell differentiation, *Nat. Commun.* 5 (2014) 5780, <https://doi.org/10.1038/ncomms5780>.
- [70] Z. Liu, W. Cao, L. Xu, X. Chen, Y. Zhan, Q. Yang, S. Liu, P. Chen, Y. Jiang, X. Sun, Y. Tao, Y. Hu, C. Li, Q. Wang, Y. Wang, C.D. Chen, Y. Shi, X. Zhang, The histone H3 lysine-27 demethylase Jmjd3 plays a critical role in specific regulation of Th17 cell differentiation, *J. Mol. Cell Biol.* 7 (2015) 505–516, <https://doi.org/10.1093/jmcb/mjv022>.
- [71] Y. Shimazu, Y. Shimazu, M. Hisishizawa, M. Hamaguchi, Y. Nagai, N. Sugino, S. Fujii, M. Kawahara, N. Kadowaki, H. Nishikawa, S. Sakaguchi, A. Takaoi-Kondo, Hypomethylation of the Treg-specific demethylated region in FOXP3 is a Hallmark of the regulatory T-cell subtype in adult T-cell leukemia, *Cancer Immunol. Res.* 4 (2016) 136–145, <https://doi.org/10.1158/2326-6066.CCR-15-0148>.
- [72] R. Yang, C. Qu, Y. Zhou, J.E. Konkel, S. Shi, Y. Liu, C. Chen, S. Liu, D. Liu, Y. Chen, E. Zandi, W. Chen, Y. Zhou, S. Shi, Hydrogen sulfide promotes Tet1- and Tet2-mediated Foxp3 demethylation to drive regulatory T cell differentiation and maintain immune homeostasis, *Immunity* 43 (2015) 251–263, <https://doi.org/10.1016/j.immuni.2015.07.017>.
- [73] J.B. Mannick, C.M. Schonhoff, ReviewNO means no and yes: regulation of cell signaling by protein nitrosylation, *Free Radic. Res.* 38 (2004) 1–7, <https://doi.org/10.1080/10715760310001629065>.
- [74] S. Brahmachari, K. Pahan, Myelin basic protein priming reduces the expression of Foxp3 in T cells via nitric oxide, *J. Immunol.* 184 (2010) 1799–1809, <https://doi.org/10.4049/jimmunol.0804394>.
- [75] S. Olson, L. Ignarro, J. Economou, H. Garban, Nitric Oxide Suppresses the Expression of the Tolerogenic Transcription Factor FOXP3: Role in Tumor Immunoregulation, 2007.
- [76] S.-W. Lee, H. Choi, S.-Y. Eun, S. Fukuyama, M. Croft, Nitric oxide modulates TGF- β -directed signals to suppress Foxp3+ regulatory T cell differentiation and potentiate Th1 development, *J. Immunol.* 186 (2011) 6972–6980, <https://doi.org/10.4049/jimmunol.1100485>.
- [77] P. Jayaraman, M.G. Alfarano, P.F. Svider, F. Parikh, G. Lu, S. Kidwai, H. Xiong, A. G. Sikora, iNOS expression in CD4+ T cells limits Treg induction by repressing TGF β 1: combined iNOS inhibition and Treg depletion unmask endogenous antitumor immunity, *Clin. Cancer Res.* 20 (2014) 6439–6451, <https://doi.org/10.1158/1078-0432.CCR-13-3409>.
- [78] J. Yang, R. Zhang, G. Lu, Y. Shen, L. Peng, C. Zhu, M. Cui, W. Wang, P. Arnaboldi, M. Tang, M. Gupta, C.-F. Qi, P. Jayaraman, H. Zhu, B. Jiang, S. Chen, J.C. He, A. T. Ting, M.-M. Zhou, V.K. Kuchroo, H.C. Morse, K. Ozato, A.G. Sikora, H. Xiong, T cell-derived inducible nitric oxide synthase switches off TH17 cell differentiation, *J. Exp. Med.* 210 (2013) 1447–1462, <https://doi.org/10.1084/jem.20122494>.
- [79] N. Obermajer, J.L. Wong, R.P. Edwards, K. Chen, M. Scott, S. Khader, J.K. Kolls, K. Dunsen, T.R. Billiar, P. Kalinski, Induction and stability of human Th17 cells require endogenous NOS2 and cGMP-dependent NO signaling, *J. Exp. Med.* 210 (2013) 1433–1445, <https://doi.org/10.1084/jem.20121277>.
- [80] W. Niedbala, A.-G. Besnard, H.R. Jiang, J.C. Alves-Filho, S.Y. Fukada, D. Nascimento, A. Mitani, P. Pushparaj, M.H. Alqahtani, F.Y. Liew, Nitric oxide-induced regulatory T cells inhibit Th17 but not Th1 cell differentiation and function, *J. Immunol.* 191 (2013) 164–170, <https://doi.org/10.4049/jimmunol.1202580>.
- [81] N. Nath, O. Morinaga, I. Singh, S-nitrosoglutathione a physiologic nitric oxide carrier attenuates experimental autoimmune encephalomyelitis, *J. Neuroimmune Pharmacol.* 5 (2010) 240–251, <https://doi.org/10.1007/s11481-009-9187-x>.
- [82] N. Saxena, J. Won, S. Choi, A.K. Singh, I. Singh, S-nitrosoglutathione reductase (GSNOR) inhibitor as an immune modulator in experimental autoimmune encephalomyelitis, *Free Radic. Biol. Med.* 121 (2018) 57–68, <https://doi.org/10.1016/j.freeradbiomed.2018.04.558>.
- [83] T. Narita, B.T. Weinert, C. Choudhary, Functions and mechanisms of non-histone protein acetylation, *Nat. Rev. Mol. Cell Biol.* 20 (2019) 156–174, <https://doi.org/10.1038/s41580-018-0081-3>.
- [84] H.W. Lim, S.G. Kang, J.K. Ryu, B. Schilling, M. Fei, I.S. Lee, A. Kehasse, K. Shirakawa, M. Yokoyama, M. Schnölzer, H.G. Kasler, H.-S. Kwon, B.W. Gibson, H. Sato, K. Akassoglou, C. Xiao, D.R. Littman, M. Ott, E. Verdin, SIRT1 deacetylates ROR γ t and enhances Th17 cell generation, *J. Exp. Med.* 212 (2015) 607–617, <https://doi.org/10.1084/jem.20132378>.
- [85] Q. Wu, J. Nie, Y. Gao, P. Xu, Q. Sun, J. Yang, L. Han, Z. Chen, X. Wang, L. Lv, A. Tsun, J. Shen, B. Li, Reciprocal regulation of ROR γ t acetylation and function by p300 and HDAC1, *Sci. Rep.* 5 (2015) 16355, <https://doi.org/10.1038/srep16355>.
- [86] A.K. Singh, P. Khare, A. Obaid, K.P. Conlon, V. Basrur, R.A. DePinho, K. Venuprasad, SUMOylation of ROR γ t inhibits IL-17 expression and inflammation via HDAC2, *Nat. Commun.* 9 (2018) 4515, <https://doi.org/10.1038/s41467-018-06924-5>.
- [87] K. Tanaka, G.J. Martinez, X. Yan, W. Long, K. Ichiyama, X. Chi, B.-S. Kim, J. M. Reynolds, Y. Chung, S. Tanaka, L. Liao, Y. Nakanishi, A. Yoshimura, P. Zheng, X. Wang, Q. Tian, J. Xu, B.W. O'Malley, C. Dong, Regulation of pathogenic T helper 17 cell differentiation by steroid receptor coactivator-3, *Cell Rep.* 23 (2018) 2318–2329, <https://doi.org/10.1016/j.celrep.2018.04.088>.
- [88] X. Wang, Y. Yang, D. Ren, Y. Xia, W. He, Q. Wu, J. Zhang, M. Liu, Y. Du, C. Ren, B. Li, J. Shen, Y. Zhang, JQ1, a bromodomain inhibitor, suppresses Th17 effectors by blocking p300-mediated acetylation of ROR γ t, *Br. J. Pharmacol.* 177 (2020) 2959–2973, <https://doi.org/10.1111/bph.15023>.
- [89] S. Zhuang, Regulation of STAT signaling by acetylation, *Cell. Signal.* 25 (2013) 1924–1931, <https://doi.org/10.1016/j.cellsig.2013.05.007>.
- [90] U. Rozovski, D.M. Harris, P. Li, Z. Liu, P. Jain, A. Ferrajoli, J. Burger, P. Thompson, N. Jain, W. Wierda, M.J. Keating, Z. Estrov, STAT3 is constitutively acetylated on lysine 685 residues in chronic lymphocytic leukemia cells, *Oncotarget* 9 (2018) 33710–33718, <https://doi.org/10.18632/oncotarget.26110>.
- [91] E. Limagne, M. Thibaudin, R. Euvrard, H. Berger, P. Chalons, F. Végan, E. Humblin, R. Boidot, C. Rébé, V. Derangère, S. Ladoire, L. Apetoh, D. Delmas, F. Ghiringhelli, Sirtuin-1 activation controls tumor growth by impeding Th17 differentiation via STAT3 deacetylation, *Cell Rep.* 19 (2017) 746–759, <https://doi.org/10.1016/j.celrep.2017.04.004>.
- [92] Y. Xu, R. Cai, Z. Zhao, L. Zhou, Q. Zhou, S. Hassan, S. Huang, M. Zhang, G. Xu, X. Zou, Thiomyristoyl ameliorates colitis by blocking the differentiation of Th17 cells and inhibiting SIRT2-induced metabolic reprogramming, *Int. Immunopharmacol.* 90 (2021), 107212, <https://doi.org/10.1016/j.intimp.2020.107212>.
- [93] J. van Loosdregt, Y. Vercoulen, T. Guichelaar, Y.Y.J. Gent, J.M. Beekman, O. van Beekum, A.B. Brenkman, D.-J. Hijnen, T. Mutis, E. Kalkhoven, B.J. Prakken, P. J. Coffey, Regulation of Treg functionality by acetylation-mediated Foxp3 protein stabilization, *Blood* 115 (2010) 965–974, <https://doi.org/10.1182/blood-2009-02-207118>.
- [94] R. Tao, E.F. de Zoeten, E. Özkaynak, C. Chen, L. Wang, P.M. Porrett, B. Li, L. A. Turka, E.N. Olson, M.I. Greene, A.D. Wells, W.W. Hancock, Deacetylase inhibition promotes the generation and function of regulatory T cells, *Nat. Med.* 13 (2007) 1299–1307, <https://doi.org/10.1038/nm1652>.
- [95] U.H. Beier, L. Wang, T.R. Bhatti, Y. Liu, R. Han, G. Ge, W.W. Hancock, Sirtuin-1 targeting promotes Foxp3+ T-regulatory cell function and prolongs allograft survival, *Mol. Cell Biol.* 31 (2011) 1022–1029, <https://doi.org/10.1128/MCB.01206-10>.
- [96] E.F. de Zoeten, L. Wang, H. Sai, W.H. Dillmann, W.W. Hancock, Inhibition of HDAC9 increases T regulatory cell function and prevents colitis in mice, *Gastroenterology* 138 (2010) 583–594, <https://doi.org/10.1053/j.gastro.2009.10.037>.
- [97] E.F. de Zoeten, L. Wang, K. Butler, U.H. Beier, T. Akimova, H. Sai, J.E. Bradner, R. Mazitschek, A.P. Kozikowski, P. Matthias, W.W. Hancock, Histone deacetylase 6 and heat shock protein 90 control the functions of Foxp3+ T-regulatory cells, *Mol. Cell Biol.* 31 (2011) 2066–2078, <https://doi.org/10.1128/MCB.05155-11>.
- [98] U.H. Beier, L. Wang, R. Han, T. Akimova, Y. Liu, W.W. Hancock, Histone deacetylases 6 and 9 and Sirtuin-1 control Foxp3+ regulatory T cell function through shared and isoform-specific mechanisms, *Sci. Signal.* 5 (2012), <https://doi.org/10.1126/scisignal.2002873> (ra45–ra45).
- [99] P. Lehmkühl, M. Gentz, A.C. Garcia de Oteyza, B. Grimbacher, H. Schulze-Koops, A. Skapenko, Dysregulated immunity in PID patients with low GARP expression

- on Tregs due to mutations in LRRC32, *Cell. Mol. Immunol.* 18 (2021) 1677–1691, <https://doi.org/10.1038/s41423-021-00701-z>.
- [100] S. Dahiya, U.H. Beier, L. Wang, R. Han, J. Jiao, T. Akimova, A. Angelin, D. C. Wallace, W.W. Hancock, HDAC10 deletion promotes Foxp3+ T-regulatory cell function, *Sci. Rep.* 10 (2020) 424, <https://doi.org/10.1038/s41598-019-57294-x>.
- [101] U.H. Beier, A. Angelin, T. Akimova, L. Wang, Y. Liu, H. Xiao, M.A. Koike, S. A. Hancock, T.R. Bhatti, R. Han, J. Jiao, S.C. Veasey, C.A. Sims, J.A. Baur, D. C. Wallace, W.W. Hancock, Essential role of mitochondrial energy metabolism in Foxp3+ T-regulatory cell function and allograft survival, *FASEB J.* 29 (2015) 2315–2326, <https://doi.org/10.1096/fj.14-268409>.
- [102] L. Wang, Y. Liu, R. Han, U.H. Beier, T.R. Bhatti, T. Akimova, M.I. Greene, S. W. Hiebert, W.W. Hancock, FOXP3+ regulatory T cell development and function require histone/protein deacetylase 3, *J. Clin. Invest.* 125 (2015) 1111–1123, <https://doi.org/10.1172/JCI77088>.
- [103] H. Xiao, J. Jiao, L. Wang, S. O'Brien, K. Newick, L.-C.S. Wang, E. Falkensammer, Y. Liu, R. Han, V. Kapoor, F.K. Hansen, T. Kurz, W.W. Hancock, U.H. Beier, HDAC5 controls the functions of Foxp3+ T-regulatory and CD8+ T cells: HDAC5 controls TREG and CD8 T cell function, *Int. J. Cancer* 138 (2016) 2477–2486, <https://doi.org/10.1002/ijc.29979>.
- [104] Y. Xiong, L. Wang, E. Di Giorgio, T. Akimova, U.H. Beier, R. Han, M. Trevisanut, J.H. Kalin, P.A. Cole, W.W. Hancock, Inhibiting the coregulator CoREST impairs Foxp3+ Treg function and promotes antitumor immunity, *J. Clin. Invest.* 130 (2020) 1830–1842, <https://doi.org/10.1172/JCI131375>.
- [105] J. Cabral, S.A. Hanley, J.Q. Gerlach, N. O'Leary, S. Cunningham, T. Ritter, R. Ceredig, L. Joshi, M.D. Griffin, Distinctive surface glycosylation patterns associated with mouse and human CD4+ regulatory T cells and their suppressive function, *Front. Immunol.* 8 (2017) 987, <https://doi.org/10.3389/fimmu.2017.00987>.
- [106] X. Hao, Y. Li, J. Wang, J. Ma, S. Zhao, X. Ye, L. He, J. Yang, M. Gao, F. Xiao, H. Wei, Deficient O-GlcNAc glycosylation impairs regulatory T cell differentiation and notch signaling in autoimmune hepatitis, *Front. Immunol.* 9 (2018) 2089, <https://doi.org/10.3389/fimmu.2018.02089>.
- [107] B. Liu, O.C. Salgado, S. Singh, K.L. Hippen, J.C. Maynard, A.L. Burlingame, L. E. Ball, B.R. Blazar, M.A. Farrar, K.A. Hogquist, H.-B. Ruan, The lineage stability and suppressive program of regulatory T cells require protein O-GlcNAcylation, *Nat. Commun.* 10 (2019) 354, <https://doi.org/10.1038/s41467-019-08300-3>.
- [108] M.A. Toscano, G.A. Bianco, J.M. Ilarregui, D.O. Croci, J. Correale, J. D. Hernandez, N.W. Zwirner, F. Poirier, E.M. Riley, L.G. Baum, G.A. Rabinovich, Differential glycosylation of TH1, TH2 and TH-17 effector cells selectively regulates susceptibility to cell death, *Nat. Immunol.* 8 (2007) 825–834, <https://doi.org/10.1038/ni1482>.
- [109] H. Jiang, X. Zhang, X. Chen, P. Aramsangtienchai, Z. Tong, H. Lin, Protein lipidation: occurrence, mechanisms, biological functions, and enabling technologies, *Chem. Rev.* 118 (2018) 919–988, <https://doi.org/10.1021/acs.chemrev.6b00750>.
- [110] F. Rampoldi, M. Bonrouhi, M.E. Boehm, W.D. Lehmann, Z.V. Popovic, S. Kaden, G. Federico, F. Brunk, H.-J. Gröne, S. Porubsky, Immunosuppression and aberrant T cell development in the absence of N-Myristoylation, *J. Immunol.* 195 (2015) 4228–4243, <https://doi.org/10.4049/jimmunol.1500622>.
- [111] R.L. O'Brien, C.L. Roark, W.K. Born, IL-17-producing $\gamma\delta$ T cells: FORUM, *Eur. J. Immunol.* 39 (2009) 662–666, <https://doi.org/10.1002/eji.200839120>.
- [112] S. Paul, A.K. Singh, G. Lal Shilpi, Phenotypic and functional plasticity of gamma-delta ($\gamma\delta$) T cells in inflammation and tolerance, *Int. Rev. Immunol.* 33 (2014) 537–558, <https://doi.org/10.3109/08830185.2013.863306>.
- [113] F. Rampoldi, F. Brunk, M. Bonrouhi, G. Federico, D. Kronic, S. Porubsky, H.-J. Gröne, Z.V. Popovic, Deficiency of N-myristoylation reveals calcineurin activity as regulator of IFN- γ -producing $\gamma\delta$ T cells, *J. Leukoc. Biol.* 101 (2017) 1005–1014, <https://doi.org/10.1189/jlb.1A0616-264R>.
- [114] Z. Wen, K. Jin, Y. Shen, Z. Yang, Y. Li, B. Wu, L. Tian, S. Shoor, N.E. Roche, J. J. Goronzy, C.M. Weyand, N-myristoylation transferase deficiency impairs activation of kinase AMPK and promotes synovial tissue inflammation, *Nat. Immunol.* 20 (2019) 313–325, <https://doi.org/10.1038/s41590-018-0296-7>.
- [115] M. Wang, P.J. Casey, Protein prenylation: unique fats make their mark on biology, *Nat. Rev. Mol. Cell Biol.* 17 (2016) 110–122, <https://doi.org/10.1038/nrm.2015.11>.
- [116] S.M. Lacher, J. Bruttger, B. Kalt, J. Berthelet, K. Rajalingam, S. Wörtge, A. Waisman, HMG-CoA reductase promotes protein prenylation and therefore is indispensable for T-cell survival, *Cell Death Dis.* 8 (2017) e2824, <https://doi.org/10.1038/cddis.2017.221>.
- [117] R. López-Posadas, P. Fastancz, L. del C. Martínez-Sánchez, J. Panteleev-Ivlev, V. Thonn, T. Kisseleva, L.S. Becker, A. Schulz-Kuhnt, S. Zundler, S. Wirtz, R. Atreya, B. Carlé, O. Friedrich, S. Schürmann, M.J. Waldner, C. Neufert, C. H. Brakebusch, M.O. Bergö, M.F. Neurath, I. Atreya, Inhibiting PGGT1B disrupts function of RHOA, resulting in T-cell expression of integrin $\alpha 4\beta 7$ and development of colitis in mice, *Gastroenterology* 157 (2019) 1293–1309, <https://doi.org/10.1053/j.gastro.2019.07.007>.
- [118] G. Swan, J. Geng, E. Park, Q. Ding, J. Zhou, C. Walcott, J.J. Zhang, H.-I. Huang, G. E. Hammer, D. Wang, A requirement of protein geranylgeranylation for chemokine receptor signaling and Th17 cell function in an animal model of multiple sclerosis, *Front. Immunol.* 12 (2021), 641188, <https://doi.org/10.3389/fimmu.2021.641188>.
- [119] S.-i. Kagami, T. Owada, H. Kanari, Y. Saito, A. Suto, K. Ikeda, K. Hirose, N. Watanabe, I. Iwamoto, H. Nakajima, Protein geranylgeranylation regulates the balance between Th17 cells and Foxp3+ regulatory T cells, *Int. Immunol.* 21 (2009) 679–689, <https://doi.org/10.1093/intimm/dxp037>.
- [120] W. Su, N.M. Chapman, J. Wei, H. Zeng, Y. Dhungana, H. Shi, J. Saravia, P. Zhou, L. Long, S. Rankin, A. Kc, P. Vogel, H. Chi, Protein prenylation drives discrete signaling programs for the differentiation and maintenance of effector Treg cells, *Cell Metab.* 32 (2020) 996–1011.e7, <https://doi.org/10.1016/j.cmet.2020.10.022>.
- [121] M. Timilshina, Z. You, S.M. Lacher, S. Acharya, L. Jiang, Y. Kang, J.-A. Kim, H. W. Chang, K.-J. Kim, B. Park, J.-H. Song, H.-J. Ko, Y.-Y. Park, M.-J. Ma, M. R. Nepal, T.C. Jeong, Y. Chung, A. Waisman, J.-H. Chang, Activation of mevalonate pathway via LKB1 is essential for stability of Treg cells, *Cell Rep.* 27 (2019) 2948–2961.e7, <https://doi.org/10.1016/j.celrep.2019.05.020>.
- [122] X. Yang, V. Chatterjee, Y. Ma, E. Zheng, S.Y. Yuan, Protein Palmitoylation in leukocyte signaling and function, *Front. Cell Dev. Biol.* 8 (2020), 600368, <https://doi.org/10.3389/fcell.2020.600368>.
- [123] K.M. Collura, J. Niu, S.S. Sanders, A. Montersino, S.M. Holland, G.M. Thomas, The palmitoyl acyltransferases ZDHHC5 and ZDHHC8 are uniquely present in DRG axons and control retrograde signaling via the Gp130/JAK/STAT3 pathway, *J. Biol. Chem.* 295 (2020) 15427–15437, <https://doi.org/10.1074/jbc.RA120.013815>.
- [124] M. Zhang, L. Zhou, Y. Xu, M. Yang, Y. Xu, G.P. Komaniecki, T. Kosciuk, X. Chen, X. Lu, X. Zou, M.E. Linder, H. Lin, A STAT3 palmitoylation cycle promotes TH17 differentiation and colitis, *Nature* 586 (2020) 434–439, <https://doi.org/10.1038/s41586-020-2799-2>.
- [125] K.N. Swatek, D. Komander, Ubiquitin modifications, *Cell Res.* 26 (2016) 399–422, <https://doi.org/10.1038/cr.2016.39>.
- [126] W. Zhang, X. Liu, Y. Zhu, X. Liu, Y. Gu, X. Dai, B. Li, Transcriptional and posttranslational regulation of Th17/Treg balance in health and disease, *Eur. J. Immunol.* 51 (2021) 2137–2150, <https://doi.org/10.1002/eji.202048794>.
- [127] L.Z. Shi, R. Wang, G. Huang, P. Vogel, G. Neale, D.R. Green, H. Chi, HIF1 α -dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells, *J. Exp. Med.* 208 (2011) 1367–1376, <https://doi.org/10.1084/jem.20110278>.
- [128] X. Liu, H. Li, B. Zhong, M. Blonska, S. Gorjestani, M. Yan, Q. Tian, D.-E. Zhang, X. Lin, C. Dong, USP18 inhibits NF- κ B and NFAT activation during Th17 differentiation by deubiquitinating the TAK1-TAB1 complex, *J. Exp. Med.* 210 (2013) 1575–1590, <https://doi.org/10.1084/jem.20122327>.
- [129] L. Yang, Y. Jing, D. Kang, P. Jiang, N. Li, X. Zhou, Y. Chen, L.S. Westerberg, C. Liu, Ubiquitin-specific peptidase 18 regulates the differentiation and function of Treg cells, *Genes Dis.* 8 (2021) 344–352, <https://doi.org/10.1016/j.gendis.2020.03.004>.
- [130] Z. Qu, J. Fu, H. Ma, J. Zhou, M. Jin, M.Y. Mapara, M.J. Grusby, G. Xiao, PDLIM2 restricts Th1 and Th17 differentiation and prevents autoimmune disease, *Cell Biosci.* 2 (2012) 23, <https://doi.org/10.1186/2045-3701-2-23>.
- [131] J.J. Cho, Z. Xu, U. Parthasarathy, T.T. Drashansky, E.Y. Helm, A.N. Zuniga, K. J. Lorentsen, S. Mansouri, J.Y. Cho, M.J. Edelmann, D.M. Duong, T. Gehring, T. Seeholzer, D. Krappmann, M.N. Uddin, D. Califano, R.L. Wang, L. Jin, H. Li, D. Lv, D. Zhou, L. Zhou, D. Avram, Hectd3 promotes pathogenic Th17 lineage through Stat3 activation and Malt1 signaling in neuroinflammation, *Nat. Commun.* 10 (2019) 701, <https://doi.org/10.1038/s41467-019-08605-3>.
- [132] S. Brauner, X. Jiang, G.E. Thorlacius, A.M. Lundberg, T. Östberg, Z.-Q. Yan, V. K. Kuchroo, G.K. Hansson, M. Wahren-Herlenius, Augmented Th17 differentiation in Trim21 deficiency promotes a stable phenotype of atherosclerotic plaques with high collagen content, *Cardiovasc. Res.* 114 (2018) 158–167, <https://doi.org/10.1093/cvr/cvx181>.
- [133] A. Chitrakar, S.A. Budda, J.G. Henderson, R.C. Axtell, L.A. Zenewicz, E3 ubiquitin ligase Von Hippel-Lindau protein promotes Th17 differentiation, *J. Immunol.* 205 (2020) 1009–1023, <https://doi.org/10.4049/jimmunol.2000243>.
- [134] Z. He, J. Zhang, Z. Huang, Q. Du, N. Li, Q. Zhang, Y. Chen, Z. Sun, SUMoylation of ROR γ regulates Th17 differentiation and thymocyte development, *Nat. Commun.* 9 (2018) 4870, <https://doi.org/10.1038/s41467-018-07203-z>.
- [135] X. Wang, L. Ni, S. Wan, X. Zhao, X. Ding, A. Dejean, C. Dong, Febrile temperature critically controls the differentiation and pathogenicity of T helper 17 cells, *Immunity* 52 (2020) 328–341.e5, <https://doi.org/10.1016/j.immuni.2020.01.006>.
- [136] Y. Xiao, M. Qureshchi, L. Dietz, M. Vaeth, S.D. Vallabhapurapu, S. Klein-Hessling, M. Klein, C. Liang, A. König, E. Serfling, A. Mottok, T. Bopp, A. Rosenwald, M. Buttmann, I. Berberich, A. Beilhack, F. Berberich-Siebelt, Lack of NFATc1 SUMOylation prevents autoimmunity and alloreactivity, *J. Exp. Med.* 218 (2021), e20181853, <https://doi.org/10.1084/jem.20181853>.
- [137] B. Liu, S. Tahk, K.M. Yee, G. Fan, K. Shuai, The ligase PIAS1 restricts natural regulatory T cell differentiation by phenotypic repression, *Science*. 330 (2010) 521–525, <https://doi.org/10.1126/science.1193787>.
- [138] X. Ding, A. Wang, X. Ma, M. Demarq, W. Jin, H. Xin, A. Dejean, C. Dong, Protein SUMOylation is required for regulatory T cell expansion and function, *Cell Rep.* 16 (2016) 1055–1066, <https://doi.org/10.1016/j.celrep.2016.06.056>.
- [139] X. Yu, Y. Lao, X.-L. Teng, S. Li, Y. Zhou, F. Wang, X. Guo, S. Deng, Y. Chang, X. Wu, Z. Liu, L. Chen, L.-M. Lu, J. Cheng, B. Li, B. Su, J. Jiang, H.-B. Li, C. Huang, J. Yi, Q. Zou, SENP3 maintains the stability and function of regulatory T cells via BACH2 deSUMOylation, *Nat. Commun.* 9 (2018) 3157, <https://doi.org/10.1038/s41467-018-05676-6>.