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Regulation of Adaptive Immunity by Lipid Post-translational Modifications

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AMPK, AMP-activated protein kinase; BCR, B cell receptor; C, cysteine residues; DHHC, acid-histidine-histidine-cysteine; DSS, dextran sodium sulfate; ER, endoplasmic reticulum; FPP, farnesyl pyrophosphate; FTase, farnesyltransferase; GC, germinal center; GGPP, geranylgeranyl pyrophosphate;

ABSTRACT

The burgeoning field of immunometabolism highlights the interdependence between metabolic programs and efficacious immune responses. The current understanding that cellular metabolic remodeling is necessary for a competent adaptive immune response, along with acutely sensitive methodologies such as high-performance liquid chromatography/mass spectrometry and advanced proteomics, have ushered in a renaissance of lipid- and metabolic-based scientific inquiries. One facet of recent interest examines how lipids function as post-translational modifications (PTMs) and their resulting effects on adaptive immune responses. The goal of this review is to establish a fundamental understanding of these protein modifications and highlight recent findings that underscore the importance of continued investigation into lipids as PTMs.

Keywords: Prenylation; Myristoylation; Palmitoylation; Adaptive immunity

DE NOVO CHOLESTEROL AND FATTY ACID SYNTHESIS AND ADAPTIVE IMMUNE OUTCOMES

Upon B and T cell activation, the metabolic products and intermediates of the *de novo* cholesterol and fatty acid synthesis pathways become crucial for mounting an effective immune response. The enzymes involved in these metabolic pathways are regulated by the sterol regulatory element-binding protein (SREBP) family of transcription factors (1). In the mammalian genome, three SREBP protein isoforms are encoded by two genes. SREBP-1a and SREBP-1c are derived from an alternative splicing event in exon 1 of *Srebf1*, while SREBP-2 is encoded by *Srebf2*. The SREBP proteins undergo stringent regulation. Processing is initiated after SREBP cleavage-activating protein (SCAP) senses low levels of cholesterol through its sterol-sensing domain. SCAP then escorts the latent form of SREBP from the endoplasmic reticulum (ER) to the Golgi apparatus (Golgi). Within the Golgi, Site-1 and Site-2 proteases (S1P and S2P, respectively) cleave the SREBP molecule to liberate the NH_2-terminal domain and expose a nuclear localization sequence. The now mature form, termed nuclear SREBP (nSREBP), translocates to the nucleus and initiates transcription of multiple target genes by binding to sterol response elements (SREs) in promoter and enhancer regions. Whereas SREBP-1a is able to broadly activate all SREBP-responsive genes, including those mediating

GGTase-I, geranylgeranyltransferase type I; GGTase-II, geranylgeranyltransferase type II; Golgi, Golgi apparatus; GTP, guanosine triphosphate; HGAL, human germinal center-associated lymphoma; HLA, human leukocyte antigen; IDO, indoleamine-2,3-dioxygenase; LC-MS, liquid chromatography/mass spectrometry; nSREBP, nuclear sterol regulatory element-binding protein; PAT, palmitoyl acyl transferase; PLCγ2, phospholipase C gamma 2; PPT, palmitoyl-protein thioesterase; PTM, post-translational modification; RA, rheumatoid arthritis; S1P, Site-1 protease; S2P, Site-2 protease; SCAP, SREBP cleavage-activating protein; SRE, sterol response element; SREBP, sterol regulatory element-binding protein; Tfh, T follicular helper cell; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3.

Author Contributions

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the synthesis of cholesterol, fatty acids, triglycerides, and phospholipids, the roles of SREBP-1c and SREBP-2 are more limited. Canonically, SREBP-1c preferentially drives transcription of genes required for fatty acid synthesis, whereas SREBP-2 chiefly activates genes involved in the mevalonate pathway and cholesterol biosynthesis (**Fig. 1**) (1). The intermediates from both of these pathways can feed into the post-translational addition of lipids to proteins (**Fig. 1**). SREBP activation and subsequent transcriptional activity is critical for proper lymphocyte function, as deletion of SCAP in B and T cells dramatically restricts lymphocyte activation and effector functions. In both cell types, the addition of exogenous cholesterol rescued proliferative defects due to SCAP deficiency *in vitro*; however, it is unclear if other lipids or lipid post-translational modifications (PTMs) are required for productive effector functions *in vivo* (2-4).

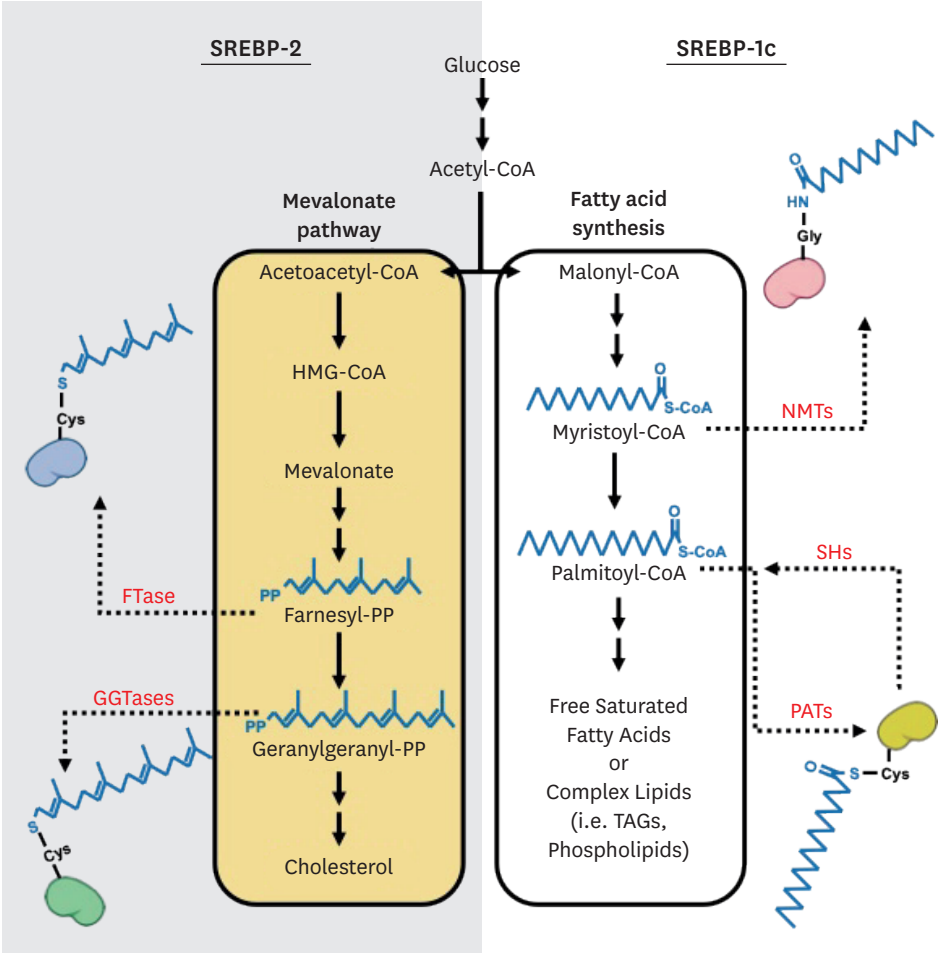

Figure 1. Simplified schematic of de novo cholesterol and fatty acid synthesis pathways. Canonically, SREBP-2 chiefly activates genes involved in cholesterol biosynthesis (left), whereas SREBP-1c preferentially drives transcription of genes required for fatty acid synthesis (right). The isoprenoid intermediates of the mevalonate pathway (farnesyl-PP and geranylgeranyl-PP) can be added to proteins as PTMs, known as prenylation. The fatty acid intermediates (myristoyl-CoA and palmitoyl-CoA) can be used as PTMs known as myristoylation and palmitoylation, respectively. Palmitoylation is the only known reversible lipid PTM.
NMT, N-myristoyltransferase; SH, serine hydrolyase; TAG, triacylglyceride.

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LIPIDS AS PTMs

The major classes of lipid PTMs are prenylation, myristoylation, and palmitoylation. Prenylation, which is further subdivided into farnesylation and geranylgeranylation, refers to the addition of a 15- or 20-carbon isoprenoid group onto C-terminal cysteine groups, respectively (**Fig. 1**, left). Isoprenoids required for prenylation are derived from intermediate metabolites produced during *de novo* cholesterol biosynthesis by the mevalonate pathway, which serve as the only source of isoprenoids in mammals (5). Fatty acids can also be utilized as PTMs. Myristoylation is the addition of the 14-carbon fatty acid myristate group to the N-terminal glycine of a target protein. Palmitoylation refers to the reversible, post-translational addition of the 16-carbon palmitate fatty acid group to cysteine residues. Cellular pools of myristate and palmitate can be generated by *de novo* fatty acid synthesis or imported from the extracellular environment (6,7). Exogenous myristate and palmitate can be obtained from dietary sources and taken up by cells via fatty acid transport proteins (8-11). Additionally, the breakdown of complex lipids like triglycerides may serve as another source of myristate and palmitate (12-14). The signals that dictate when a cell relies on synthesized, imported, or recycled fatty acids for PTMs are unclear; thus, further exploration is required to determine how specific cell types utilize particular metabolite pools for lipid PTMs. In the subsequent sections, this review will highlight recent findings pertaining to B and T cell biology.

PRENYLATION

Prenylation is the irreversible addition of an isoprenoid group onto the cysteine residue(s) at the C-terminal end of a target protein. This process is catalyzed by several prenyltransferases, including farnesyltransferase (FTase) and geranylgeranyltransferase type I (GGTase-I) (15,16). FTase catalyzes the transfer of the 15-carbon farnesyl group from farnesyl pyrophosphate (FPP) to a given protein, while GGTase-I transfers the 20-carbon geranylgeranyl group from geranylgeranyl pyrophosphate (GGPP) (**Fig. 1**, left). FTase and GGTase-I are heterodimeric enzymes, consisting of a common alpha subunit and unique beta subunits encoded by *Fntb* and *Pggt1b*, respectively. These enzymes recognize a "CaaX" motif, where "aa" are any two aliphatic residues and "X" determines substrate specificity. In general, FTase recognizes serine, methionine, or glutamine at the "X" position whereas GGTase-I recognizes leucine at this position. Following prenylation with the correct isoprenoid, the 'aaX' residues are commonly cleaved off by RAS-converting CAAX endopeptidase 1 and then the prenylcysteine residue is methylated by isoprenylcysteine carboxylmethyltransferase (15-17). Additional GGTases include geranylgeranyltransferase type II (GGTase-II), which recognizes CC- or CXC motif-containing proteins and catalyzes the addition of 2 geranylgeranyl groups onto both C-terminal cysteines, and GGTase-III, which was more recently discovered to catalyze the addition of a geranylgeranyl group to the leucine-rich repeat domain of F-box protein FBXL2 (18). FPP and GGPP are downstream products of the mevalonate pathway (**Fig. 1**, left); mevalonate is metabolized to isopentenyl pyrophosphate, followed by conversion into FPP and subsequently GGPP. Previous studies have delineated the relationship between mevalonate metabolism and T lymphocyte effector function (19-21).

Treg/Th17 CD4$^+$ T cells

Several studies have implicated prenylation as an integral factor in the plasticity of naïve T cell developmental fate. For instance, GGTase-I was shown to be essential in mediating signaling that induces differentiation of Tregs, with FTase playing a role in maintaining the

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resulting effector Treg population (22). Specifically, mice with Treg-specific deletions of *Fntb* or *Pggt1b* developed *scurfy*-like autoimmune disease and exhibited early lethality. Here, Su et al. (22) found that Treg specific deficiency of either *Fntb* or *Pggt1b* resulted in enhanced T cell activation, increased IFN-γ-producing $CD4^+$ and $CD8^+$ T cells, and increased IL-17-producing $CD4^+$ T cells. These phenotypes were observed earlier in mice with *Pggt1b*-deficient Tregs (as early as 7 days old), suggesting a stronger role for *Pggt1b* in establishing tolerance in early life. Autoimmune phenotypes were traced to a reduction in Treg proliferation and survival, suggesting that farnesylation- and geranylgeranylation-related defects in effector Treg function can disrupt T cell homeostasis and accumulation *in vivo*. Other studies have similarly linked Treg differentiation to GGTase-I activity (23,24). In a dextran sodium sulfate (DSS)-induced colitis model with C57BL/6 mice, GGPP treatment markedly increased Treg cell numbers, while Th1 and Th17 cell numbers were significantly reduced; however, these findings have not yet been confirmed in a T cell-specific model of colitis (24). In line with these data, another study found that naive $CD4^+$ T cells treated with GGPP under Th17 polarizing conditions preferentially differentiated into Tregs rather than Th17 cells. This phenotype was dependent on modulations in IL-2 production, where IL-2 neutralization blocked the ability of GGPP to alter Th17 differentiation. Notably, this study identified the guanosine triphosphate (GTP)-hydrolyzing protein, Ras, as an important target for prenylation in $CD4^+$ T cells, in which GGTase-I inhibition decreased Ras geranylgeranylation and reduced IL-2 expression. A separate study suggested that Treg/Th17 polarization is influenced by the concomitant action of microRNA-155 and protein prenylation, although the mechanism remains unclear (25). Combined, these studies highlight the importance for prenylation in maintaining the balance between Treg and Th17 polarization.

Th1/Th2 $CD4^+$ T cells

In addition to the Treg/Th17 axis, growing evidence supports the idea that mevalonate metabolism controls the balance of Th1 and Th2 $CD4^+$ T cell phenotypes (recently reviewed) (21). One study found that treatment of naive $CD4^+$ T cells with an FTase inhibitor significantly blunted differentiation into IL-5- and IL-13-producing Th2 cells (26). Protein farnesylation levels and Th2 differentiation were also reduced following treatment of $CD4^+$ T cells with a pyruvate dehydrogenase kinase (PDHK) inhibitor.

B cells

Protein geranylgeranylation also plays an important role in the ability of B cells to function as antigen presenting cells (27). Primary human B cells treated with statins–a class of drug that inhibits the rate-limiting enzyme of the mevalonate pathway, 3-hydroxy-3-methylglutaryl-CoA reductase–exhibited reduced surface expression of the co-stimulatory molecules CD80 and CD86 as well as human leukocyte antigen (HLA)-DR. Treatment of human B cells with geranylgeranyl transferase inhibitor fully recapitulated the reduction in surface expression of CD80, CD86, and HLA-DR observed with statin treatment, and was only rescued to wild-type levels following addition of GGPP or a precursory metabolite, mevalonate. These experiments suggest that the inhibitory activity of statins on B cell antigen presentation is due to a disruption in geranylgeranylation; however, the specific targets and mechanisms remain to be elucidated.

N-MYRISTOYLATION

N-glycine myristoylation (hereafter referred to as myristoylation) is a post-translational modification whereby a 14-carbon acyl chain is irreversibly linked to the N-terminal glycine of

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proteins via an amide bond (**Fig. 1**, right). This process is catalyzed by N-myristoyltransferases, of which humans have 2 isoforms: NMT1 and NMT2. These enzymes share partially overlapping functions and substrate selectivity and are principally localized to the cytosol (28). NMT1/2 recognize a consensus sequence for attaching the myristic molecule: Met-Gly-X-X-X-Ser/Thr (where "X" denotes any amino acid residue) (29). Myristoylation potentiates membrane localization of modified proteins, but is insufficient on its own (30). Other post-translational modifications like palmitoylation (described below), or the presence of positively charged amino acid residues, allow for proper membrane integration. This unique regulation of membrane localization based on additional, often reversible modifications is referred to as a "myristoyl switch." Three main switch mechanisms have been proposed: myristoyl-electrostatic, myristoyl-ligand, and myristoyl-palmitoyl (31). In the myristoyl-electrostatic switch model, affecting a protein's surface charge enhances or prevents binding of the myristoylated protein to the plasma membrane. Binding of a ligand such as calcium ions or GTP can induce a conformational change in the modified protein, exposing the myristoyl group and enhancing membrane retention. Lastly, addition of a 16-carbon saturated palmitoyl chain can enhance recruitment of the modified protein to membrane structures, and may be required for stable integration. For additional information on this particular post-translational modification, please refer to other excellent reviews (29,31,32).

T cells

Several myristoylation targets have been identified in adaptive immune cells. The most prominent of these are the Src family of tyrosine kinases, including the adaptor proteins Lck, Fyn, Blk, and Lyn, which are involved in T and B cell development and activation (33,34). These proteins also share a motif of three alternating lysine residues that, in conjunction with myristoylation, promote membrane localization and potentiate competent early and late signaling events (29). Notably, Nmt1, Nmt2, and Nmt1/2 knockout in T cells exhibited respective 30%, 25%, and 83% reductions in thymocytes, with Nmt1/2 mutant mice experiencing increased apoptosis during all stages of T cell development (35). Peripheral T cells within Nmt1 and Nmt1/2 knockout mice were significantly decreased in the blood, lymph nodes, and spleen, with a bias for activated/memory phenotype. NMT2 knockout T cells largely presented a phenotype similar to wild-type mice, suggesting that Nmt1 is the dominant myristoylation enzyme in T cells. Nmt1 and Nmt1/2 mutant thymocytes displayed defective TCR signaling cascades as evidenced by their inability to phosphorylate CD3ζ or Zap70 after TCR stimulation. Another recent study found that the energy sensing AMP-activated protein kinase (AMPK) relies on myristoylation by NMT1 for proper functionality in T cells (36). Upon activation, AMPK initiates metabolic programs to support effector T cell bioenergetics and viability (37,38). Human $CD4^+$ T cells from patients with rheumatoid arthritis (RA) displayed reduced protein levels of NMT1 and increased differentiation into pro-inflammatory Th1 and Th17 T cells when activated under non-polarizing conditions. The anti-inflammatory effects of NMT1 were fully recapitulated in a humanized mouse model of synovitis with enforced overexpression of NMT1 resulting in significant decreases in T cell infiltration, IFN-γ-producing T cells, and inflammatory markers. These studies offer a glimpse into potential targets for therapeutic intervention and highlight the importance of myristoylation in T cell development, activation, and differentiation.

B cells

The full characterization of myristoylation events in B cells remains understudied. One identified target of myristoylation in B cells is human germinal center-associated lymphoma (HGAL, also named GCET2). HGAL is a protein found on the surface of germinal center

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(GC) B cells and T follicular helper cells (Tfhs); it serves as a useful prognostic marker for GC B cell-derived malignancies, such as diffuse large B-cell lymphomas. HGAL has been implicated in the development of lymphoid hyperplasia due to its inhibition of cell migration in response to IL-6 signaling as well as its adaptor protein function in B cell receptor (BCR) signaling (39,40). Within GC B cells, HGAL is constitutively localized to the plasma membrane despite the lack of a transmembrane domain. Through expression and directed mutagenesis in DHL16 cells, HGAL was shown to contain myristoylation and palmitoylation sites that are required for proper localization to the plasma membrane (40). Recent studies found that myristoylation and palmitoylation events localize HGAL protein to lipid rafts in the non-Hodgkin lymphoma cell lines RCK8 and U2932, facilitating HGAL's interaction with and activation of the protein-tyrosine kinase Syk following BCR stimulation (41,42). An additional BCR regulator is the myristoylated alanine-rich C kinase substrate, a protein that undergoes a myristoyl-electrostatic switch mechanism and was found to regulate tonic and chronic BCR signaling (43,44). To date, B cell specific knockouts of NMT1 or NMT2 have not been described; thus, a comprehensive understanding of the immunobiological targets and outcomes of myristoylation within B cell and organismal biology require further inquiry.

Therapeutic interventions

Markedly, exploration of the membrane-targeting characteristics of myristoylation for therapeutic purposes has begun. Researchers have successfully delivered mRNA containing the myristoylation site of Src fused to a human indoleamine-2,3-dioxygenase (IDO) 1 transcript, which resulted in anchoring of the Src-IDO1 protein to the inner face of the plasma membrane (45). This encoded lipid PTM extended the lifespan of IDO1 *in vitro* and led to the expression of functional IDO1 in the liver and spleen of mice. In a semi-allogenic model of graft versus host disease, donor T cells expand and host B cells are reduced. However, delivery of this modified IDO1 mRNA significantly minimized host B cell depletion and increased apoptosis of effector T cells. Notably, there was an increase in the percentage of both host and donor Tregs, which may account for some of the observed immunosuppressive phenotypes following IDO1 treatment. In this study, similar immunosuppressive characteristics were observed in two additional models of autoimmunity, suggesting promise in myristate-fused proteins to therapeutically impact disease phenotypes.

S-ACYLATION (PALMITOYLATION)

Cysteine (S)-acylation, more commonly referred to as S-palmitoylation, is the reversible PTM whereby a 16-carbon palmitate molecule is covalently added onto a cysteine residue of a target protein (**Fig. 1**, right). The addition of palmitate is mediated by palmitoyl acyl transferases (PATs), which harbor an aspartic acid-histidine-histidine-cysteine (DHHC) catalytic domain. Humans encode 23 of these PATs, which are designated DHHC1-24 (skipping DHHC10) and are localized to various compartments within the cell, including the Golgi, ER, plasma membrane, and cytosol (28). Palmitoylation events can be subsequently removed by serine hydrolases such as the acyl protein thioesterases (APT1/2, also called LYPLA1 and LYPLA2), the α/β-hydrolase domain 17 (ABHD17) family of proteins (ABHD17a/b/c), as well as the palmitoyl-protein thioesterases (PPT1/2) (28,46,47). APT1 and APT2 have been shown to undergo palmitoylation at Cys-2 in HEK293T cells and astrocyte cultures, which is thought to enhance recruitment to the plasma membrane and promote depalmitoylation of target proteins (48). APT1 was found to enhance cytoplasmic localization of both APT1 and APT2 through catalyzing the depalmitoylation of both

enzymes. However, another report using Madin-Darby canine kidney cells suggested that the soluble, depalmitoylated forms of APT1 and APT2 carry out depalmitoylation on all membranes in the cell (49). This study also reported that palmitoylation of APT1 and APT2 promoted localization to the Golgi, thus highlighting the need for further study to determine the mechanism of APT1/2 regulation in specific cell types. The serine hydrolase ABHD17a was shown to undergo palmitoylation in COS-7 cells, which enhanced its recruitment to the plasma membrane as well as early and recycling endosomes (50). Similarly, PPT1 has been shown to localize to endosomes and lysosomes (46,51). Due to its reversible nature and exquisite spatial regulation, palmitoylation has become an intense topic of exploration within adaptive immunity in recent years.

T cells

Both CD4 and CD8 T cell coreceptors were initially reported to be palmitoylated over 2 decades ago, yet much controversy still exists over whether palmitoylation events promote localization to lipid rafts and/or enhance interaction with downstream signaling adaptor molecules (52-55). In a more recent study, CD80 was found to be palmitoylated on multiple residues by DHHC20, which was shown to protect CD80 from ubiquitination degradation (56). Within the Jurkat human acute T cell lymphoma line, palmitoylation-deficient CD80 displayed reduced early activation markers (CD69), reduced IL-2 mRNA levels, and diminished Erk phosphorylation activation. Altogether, these results suggest that palmitoylation is required for the recruitment of CD80 to the surface of T cells where it can function as a costimulatory molecule. Palmitoylation of the apoptotic mediator Fas (CD95) is necessary to induce receptor clustering and apoptosis in primary mouse T cells, B cells, and dendritic cells (57). However, ablating the ability to palmitoylate Fas did not result in the typical lymphoaccumulation or autoantibody production associated with Fas deficiency, suggesting a non-classical mechanism of action.

$CD8^+$ T cell therapeutic interventions

Identification of palmitoylated proteins has resulted in promising therapeutic interventions and enhanced vaccination strategies for cancer. Notably, two proteins involved in T cell exhaustion, PD-L1 and T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), have been identified as palmitoylation targets (58,59). Specific competitive inhibitors for the DHHC enzyme involved in each case resulted in reduced expression of the T cell exhaustion marker and significantly reduced tumor volumes. Intriguingly, both publications implicated the respective palmitoylation events in preventing proteasomal and lysosomal processes, suggesting that palmitoylation plays an essential role in stabilizing membrane integration and preventing degradation/recycling of modified proteins. In line with these data, the marine-derived molecule benzosceptrin C was also identified to inhibit DHHC3-mediated palmitoylation of PD-L1, resulting in suppressed murine tumor growth (60). In cancer vaccine strategies, researchers have begun to utilize palmitoylation to direct cellular localization of antigens. Stolk et al. (61) demonstrate that mono-palmitic acid-modified (C16:0) antigenic peptides enhanced $CD8^+$ T cell responses, resulting in improved tumor suppression.

$CD4^+$ T cell differentiation

Palmitoylation has also been implicated in the differentiation programs of Th cell subsets. A new study found that Foxp3, the master transcription factor for Tregs, undergoes palmitoylation at multiple cysteine residues mainly catalyzed by DHHC2 (62). CD4-specific deletion of DHHC2 reduced nuclear levels of Foxp3 and resulted in a concomitant reduction in

the prevalence of Tregs in the spleen and peripheral lymph nodes. Moreover, when mice with a T-cell-specific loss of DHHC2 were subcutaneously challenged with YUMM3.3 melanoma cells, they exhibited suppressed tumor growth coupled with increased levels of IFN-γ-expressing $CD8^+$ T cells. In another study, STAT3 was found to undergo a palmitoylation cycle mediated by DHHC7 and APT2, promoting nuclear localization of phosphorylated STAT3 and initiation of pathogenic Th17 differentiation programs (63). Further exploration is required to determine if palmitoylation cycles are ubiquitous during all types of $CD4^+$ T cell activation and polarization (i.e. Th1 and Th2 $CD4^+$ T cells) or only function in specific contexts.

B cells

Exploration into palmitoylation events and their immunobiological repercussions in B cells remains understudied. CD81 is a tetraspanin essential for the colocalization of the BCR with the CD19/CD21/CD81 coreceptor complex in lipid rafts (64). Early studies into palmitoylation in the Daudi human B cell line revealed that CD81 is reversibly palmitoylated upon co-ligation of the BCR and the CD19/CD21/CD81 coreceptor complex (65). Palmitoylation of CD81 was detected within 1 h of BCR and coreceptor complex ligation and was shown to wane over a period of 3 h. Inhibition of CD81 palmitoylation using the non-specific inhibitor 2-bromopalmitate abrogated downstream phosphorylation of the integral B cell signaling intermediates Vav and phospholipase C gamma 2 (PLCγ2) (66). As referenced earlier, HGAL was shown to be palmitoylated in HEK293T cells at cysteine residues (C)43 and $C45^{42}$. Concomitant myristoylation and palmitoylation events were required for HGAL localization within lipid rafts in the non-Hodgkin lymphoma cell lines RCK8 and U2932 transfected with green fluorescent protein-tagged HGAL. In accordance with earlier reports, BCR stimulation with α-IgM in U2932 cells expressing the HGAL point mutations C43A and C45A ablated interaction with the protein-tyrosine kinase Syk, leading to decreased activation (41,42). These authors also found that palmitoylation/myristoylation-deficient HGAL mutants led to decreased cell motility in the RCK8 cell line, suggesting a role for palmitoylation in regulating B cell trafficking. Only one proteomic analysis on S-acylated proteins in primary human B cells has been completed (67). This inquiry found CD20 and CD23 to be palmitoylated; however, the immunobiological repercussions of these modifications, or other postulated targets, were not assessed. The specific DHHC and serine hydrolase associated with the aforementioned palmitoylation targets in B cells have not yet been elucidated. Furthermore, much of these investigations took place in cell lines where the immunobiological outcomes of these palmitoylation events cannot be thoroughly studied at the organismal level.

CONCLUDING REMARKS

Lipid post-translational modifications are essential facilitators in B and T cell biology. Recent studies on the roles of prenylation, myristoylation, and palmitoylation have demonstrated the importance of lipid PTMs in a variety of contexts, including Treg/Th17 plasticity, Th1/Th2 differentiation, BCR signaling, T cell exhaustion, and lymphocyte migration (summarized in **Fig. 2**). Excitingly, interference with or manipulation of these lipid post-translational modifications have already begun to show promise in therapeutic contexts. The studies discussed herein have mainly focused on identifying a lipidated protein and then determining its immunobiological consequences. While these studies are vital to advance our knowledge of the underlying mechanisms involved in specific modification events, comprehensive investigations aimed at enumerating the full breadth of lipid-modified proteins in adaptive immune cells are especially needed. Additionally, further study is

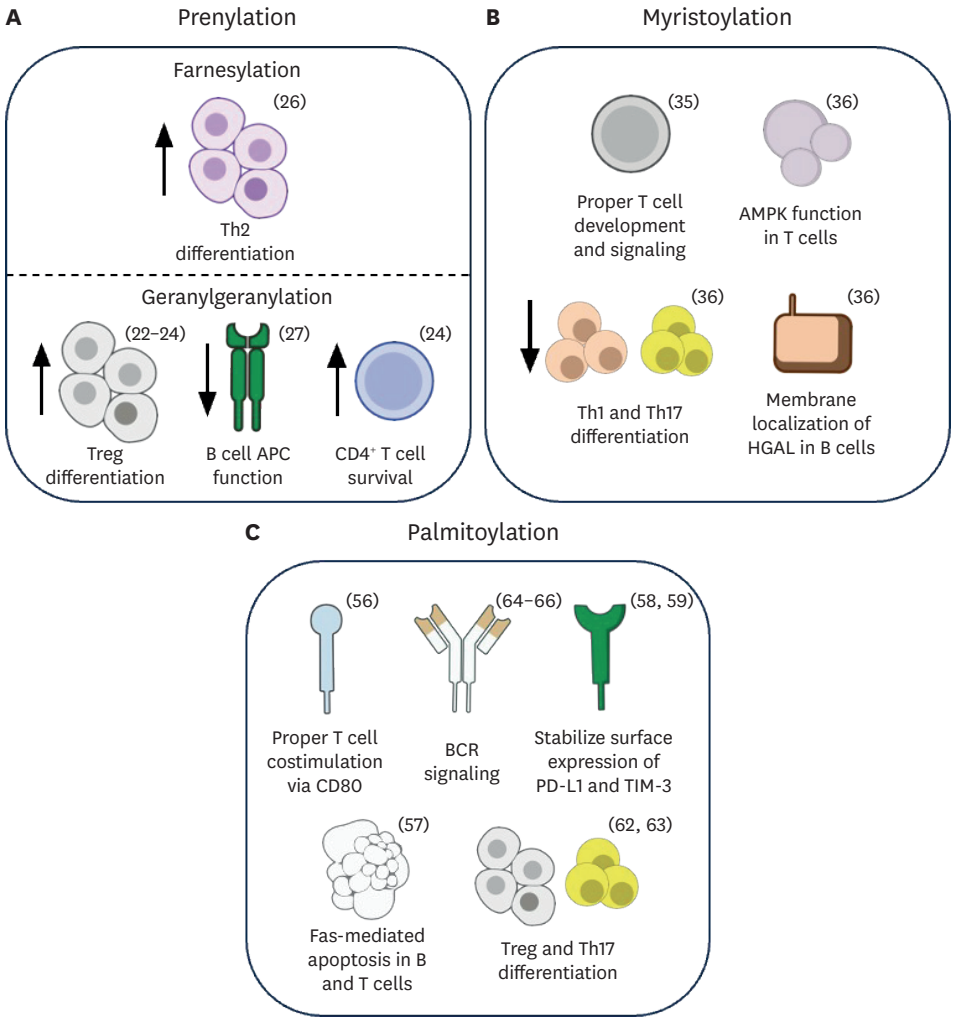

Figure 2. Adaptive immune outcomes resulting from lipid PTMs.
(A) Inhibition studies have implicated farnesylation in the differentiation programs of Th2 cells. Geranylgeranylation is essential in mediating signaling that induces differentiation of Tregs. Inhibition of geranylgeranylation has been shown to decrease the antigen presentation capacity of B cells through the downregulation of CD80, CD86, and HLA-DR. Additionally, geranylgeranylation of Ras is vital to support the survival of $CD4^+$ T cells through the production of IL-2. (B) Myristoylation events are essential for the proper development of T cells, phosphorylation of TCR signaling molecules, and functioning of AMPK. Myristoylation defects in $CD4^+$ T cells display increased differentiation into pro-inflammatory Th1 and Th17 cells. Myristoylation of HGAL (also named GCET2) is required for proper membrane localization in B cells. (C) Palmitoylation of CD80 stabilizes its membrane integration allowing for proper T cell costimulation. The T cell exhaustion markers PD-L1 and TIM-3 are palmitoylated to stabilize membrane integration and prevent degradation/recycling. Palmitoylation of CD81 potentiates proper downstream phosphorylation of the integral B cell signaling intermediates Vav and PLCγ2. Palmitoylation of the apoptotic mediator Fas (CD95) is necessary to induce receptor clustering and apoptosis in primary mouse B and T cells. Foxp3 and STAT3 undergo palmitoylation to drive differentiation of Tregs and Th17 cells, respectively.
TIM-3, T cell Ig and mucin domain-containing protein 3; PLCγ2, phospholipase C gamma 2; APC, Ag-presenting cell.

required to understand the cellular lipid pools from which fatty acids are being drawn to modify proteins and if those pools are dependent upon adaptive immune cell activation and inflammatory status of the surrounding tissue. Thus, while recent work has begun to reveal exciting connections between lipid metabolism and the adaptive immune response, the outstanding questions, undiscovered targets, and ill-defined mechanisms support continued efforts to understand the immunobiological outcomes and therapeutic potential of lipid post-translational modifications.

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REFERENCES

1. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002;109:1125-1131. PUBMED | CROSSREF
2. Chen HW, Heiniger HJ, Kandutsch AA. Relationship between sterol synthesis and DNA synthesis in phytohemagglutinin-stimulated mouse lymphocytes. *Proc Natl Acad Sci U S A* 1975;72:1950-1954. PUBMED | CROSSREF
3. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, Marbois BN, Komisopoulou E, Wilson EB, Osborne TF, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat Immunol* 2013;14:489-499. PUBMED | CROSSREF
4. Luo W, Adamska JZ, Li C, Verma R, Liu Q, Hagan T, Wimmers F, Gupta S, Feng Y, Jiang W, et al. SREBP signaling is essential for effective B cell responses. *Nat Immunol* 2023;24:337-348. PUBMED | CROSSREF
5. Lange BM, Rujan T, Martin W, Croteau R. Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. *Proc Natl Acad Sci U S A* 2000;97:13172-13177. PUBMED | CROSSREF
6. Howie D, Ten Bokum A, Necula AS, Cobbold SP, Waldmann H. The role of lipid metabolism in T lymphocyte differentiation and survival. *Front Immunol* 2018;8:1949. PUBMED | CROSSREF
7. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol* 2015;36:81-91. PUBMED | CROSSREF
8. Garcia C, Andersen CJ, Blesso CN. The role of lipids in the regulation of immune responses. *Nutrients* 2023;15:3899. PUBMED | CROSSREF
9. Hubler MJ, Kennedy AJ. Role of lipids in the metabolism and activation of immune cells. *J Nutr Biochem* 2016;34:1-7. PUBMED | CROSSREF
10. Carta G, Murru E, Banni S, Manca C. Palmitic acid: physiological role, metabolism and nutritional implications. *Front Physiol* 2017;8:902. PUBMED | CROSSREF
11. Abumrad N, Harmon C, Ibrahimi A. Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J Lipid Res* 1998;39:2309-2318. PUBMED | CROSSREF
12. Jarc E, Petan T. A twist of FATe: lipid droplets and inflammatory lipid mediators. *Biochimie* 2020;169:69-87. PUBMED | CROSSREF
13. Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. *Nat Rev Mol Cell Biol* 2019;20:137-155. PUBMED | CROSSREF
14. Kim YC, Lee SE, Kim SK, Jang HD, Hwang I, Jin S, Hong EB, Jang KS, Kim HS. Toll-like receptor mediated inflammation requires FASN-dependent MYD88 palmitoylation. *Nat Chem Biol* 2019;15:907-916. PUBMED | CROSSREF
15. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 1996;65:241-269. PUBMED | CROSSREF
16. Wang M, Casey PJ. Protein prenylation: unique fats make their mark on biology. *Nat Rev Mol Cell Biol* 2016;17:110-122. PUBMED | CROSSREF
17. Palsuledesai CC, Distefano MD. Protein prenylation: enzymes, therapeutics, and biotechnology applications. *ACS Chem Biol* 2015;10:51-62. PUBMED | CROSSREF
18. Kuchay S, Wang H, Marzio A, Jain K, Homer H, Fehrenbacher N, Philips MR, Zheng N, Pagano M. GGTase3 is a newly identified geranylgeranyltransferase targeting a ubiquitin ligase. *Nat Struct Mol Biol* 2019;26:628-636. PUBMED | CROSSREF
19. Thurnher M, Gruenbacher G. T lymphocyte regulation by mevalonate metabolism. *Sci Signal* 2015;8:re4. PUBMED | CROSSREF
20. Gruenbacher G, Thurnher M. Mevalonate metabolism governs cancer immune surveillance. *OncoImmunology* 2017;6:e1342917. PUBMED | CROSSREF
21. Kennewick KT, Bensinger SJ. Decoding the crosstalk between mevalonate metabolism and T cell function. *Immunol Rev* 2023;317:71-94. PUBMED | CROSSREF
22. Su W, Chapman NM, Wei J, Zeng H, Dhungana Y, Shi H, Saravia J, Zhou P, Long L, Rankin S, et al. Protein prenylation drives discrete signaling programs for the differentiation and maintenance of effector Treg cells. *Cell Metab* 2020;32:996-1011.e7. PUBMED | CROSSREF
23. Swan G, Geng J, Park E, Ding Q, Zhou J, Walcott C, Zhang JJ, Huang HI, Hammer GE, Wang D. A requirement of protein geranylgeranylation for chemokine receptor signaling and Th17 cell function in an animal model of multiple sclerosis. *Front Immunol* 2021;12:641188. PUBMED | CROSSREF
24. Pandit M, Acharya S, Gu Y, Seo SU, Kweon MN, Kang B, Chang JH. Geranylgeranyl pyrophosphate amplifies T_{reg} differentiation via increased IL-2 expression to ameliorate DSS-induced colitis. *Eur J Immunol* 2021;51:1461-1472. PUBMED | CROSSREF

25. Wang X, Zhang R, Huang Z, Zang S, Wu Q, Xia L. Inhibition of the miR-155 and protein prenylation feedback loop alleviated acute graft-versus-host disease through regulating the balance between T helper 17 and Treg cells. *Transpl Immunol* 2021;69:101461. PUBMED | CROSSREF
26. Yagi Y, Kuwahara M, Suzuki J, Imai Y, Yamashita M. Glycolysis and subsequent mevalonate biosynthesis play an important role in Th2 cell differentiation. *Biochem Biophys Res Commun* 2020;530:355-361. PUBMED | CROSSREF
27. Shimabukuro-Vornhagen A, Zoghi S, Liebig TM, Wennhold K, Chemitz J, Draube A, Kochanek M, Blaschke F, Pallasch C, Holtick U, et al. Inhibition of protein geranylgeranylation specifically interferes with CD40-dependent B cell activation, resulting in a reduced capacity to induce T cell immunity. *J Immunol* 2014;193:5294-5305. PUBMED | CROSSREF
28. Jiang H, Zhang X, Chen X, Aramsangtienchai P, Tong Z, Lin H. Protein lipidation: occurrence, mechanisms, biological functions, and enabling technologies. *Chem Rev* 2018;118:919-988. PUBMED | CROSSREF
29. Resh MD. Myristylation and palmitylation of Src family members: the fats of the matter. *Cell* 1994;76:411-413. PUBMED | CROSSREF
30. Resh MD. Covalent lipid modifications of proteins. *Curr Biol* 2013;23:R431-R435. PUBMED | CROSSREF
31. Wang B, Dai T, Sun W, Wei Y, Ren J, Zhang L, Zhang M, Zhou F. Protein N-myristoylation: functions and mechanisms in control of innate immunity. *Cell Mol Immunol* 2021;18:878-888. PUBMED | CROSSREF
32. Wright MH, Heal WP, Mann DJ, Tate EW. Protein myristoylation in health and disease. *J Chem Biol* 2010;3:19-35. PUBMED | CROSSREF
33. Palacios EH, Weiss A. Function of the Src-family kinases, Lck and Fyn, in T-cell development and activation. *Oncogene* 2004;23:7990-8000. PUBMED | CROSSREF
34. Saijo K, Schmedt C, Su IH, Karasuyama H, Lowell CA, Reth M, Adachi T, Patke A, Santana A, Tarakhovsky A. Essential role of Src-family protein tyrosine kinases in NF-kappaB activation during B cell development. *Nat Immunol* 2003;4:274-279. PUBMED | CROSSREF
35. Rampoldi F, Bonrouhi M, Boehm ME, Lehmann WD, Popovic ZV, Kaden S, Federico G, Brunk F, Gröne HJ, Porubsky S. Immunosuppression and aberrant T cell development in the absence of N-myristoylation. *J Immunol* 2015;195:4228-4243. PUBMED | CROSSREF
36. Wen Z, Jin K, Shen Y, Yang Z, Li Y, Wu B, Tian L, Shoor S, Roche NE, Goronzy JJ, et al. N-myristoyltransferase deficiency impairs activation of kinase AMPK and promotes synovial tissue inflammation. *Nat Immunol* 2019;20:313-325. PUBMED | CROSSREF
37. Ma EH, Poffenberger MC, Wong AH, Jones RG. The role of AMPK in T cell metabolism and function. *Curr Opin Immunol* 2017;46:45-52. PUBMED | CROSSREF
38. Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vázquez G, Yurchenko E, Raissi TC, van der Windt GJ, Viollet B, Pearce EL, et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses *in vivo*. *Immunity* 2015;42:41-54. PUBMED | CROSSREF
39. Lu X, Chen J, Malumbres R, Cubedo Gil E, Helfman DM, Lossos IS. HGAL, a lymphoma prognostic biomarker, interacts with the cytoskeleton and mediates the effects of IL-6 on cell migration. *Blood* 2007;110:4268-4277. PUBMED | CROSSREF
40. Pan Z, Shen Y, Ge B, Du C, McKeithan T, Chan WC. Studies of a germinal centre B-cell expressed gene, GCET2, suggest its role as a membrane associated adapter protein. *Br J Haematol* 2007;137:578-590. PUBMED | CROSSREF
41. Romero-Camarero I, Jiang X, Natkunam Y, Lu X, Vicente-Dueñas C, Gonzalez-Herrero I, Flores T, Garcia JL, McNamara G, Kunder C, et al. Germinal centre protein HGAL promotes lymphoid hyperplasia and amyloidosis via BCR-mediated Syk activation. *Nat Commun* 2013;4:1338. PUBMED | CROSSREF
42. Lu X, Sicard R, Jiang X, Stockus JN, McNamara G, Abdulreda M, Moy VT, Landgraf R, Lossos IS. HGAL localization to cell membrane regulates B-cell receptor signaling. *Blood* 2015;125:649-657. PUBMED | CROSSREF
43. Chen Z, Zhang W, Selmi C, Ridgway WM, Leung PSC, Zhang F, Gershwin ME. The myristoylated alanine-rich C-kinase substrates (MARCKS): a membrane-anchored mediator of the cell function. *Autoimmun Rev* 2021;20:102942. PUBMED | CROSSREF
44. Xu C, Fang Y, Yang Z, Jing Y, Zhang Y, Liu C, Liu W. MARCKS regulates tonic and chronic active B cell receptor signaling. *Leukemia* 2019;33:710-729. PUBMED | CROSSREF
45. Kenney LL, Chiu RS, Dutra MN, Wactor A, Honan C, Shelerud L, Corrigan JJ, Yu K, Ferrari JD, Jeffrey KL, et al. mRNA-delivery of IDO1 suppresses T cell-mediated autoimmunity. *Cell Rep Med* 2024;5:101717. PUBMED | CROSSREF
46. Verkruyse LA, Hofmann SL. Lysosomal targeting of palmitoyl-protein thioesterase. *J Biol Chem* 1996;271:15831-15836. PUBMED | CROSSREF

47. Remsberg JR, Suciu RM, Zambetti NA, Hanigan TW, Firestone AJ, Inguva A, Long A, Ngo N, Lum KM, Henry CL, et al. ABHD17 regulation of plasma membrane palmitoylation and N-Ras-dependent cancer growth. *Nat Chem Biol* 2021;17:856-864. PUBMED | CROSSREF

48. Kong E, Peng S, Chandra G, Sarkar C, Zhang Z, Bagh MB, Mukherjee AB. Dynamic palmitoylation links cytosol-membrane shuttling of acyl-protein thioesterase-1 and acyl-protein thioesterase-2 with that of proto-oncogene H-ras product and growth-associated protein-43. *J Biol Chem* 2013;288:9112-9125. PUBMED | CROSSREF

49. Vartak N, Papke B, Grecco HE, Rossmannek L, Waldmann H, Hedberg C, Bastiaens PI. The autodepalmitoylating activity of APT maintains the spatial organization of palmitoylated membrane proteins. *Biophys J* 2014;106:93-105. PUBMED | CROSSREF

50. Lin DT, Conibear E. ABHD17 proteins are novel protein depalmitoylases that regulate N-Ras palmitate turnover and subcellular localization. *eLife* 2015;4:e11306. PUBMED | CROSSREF

51. Bagh MB, Peng S, Chandra G, Zhang Z, Singh SP, Pattabiraman N, Liu A, Mukherjee AB. Misrouting of v-ATPase subunit V0a1 dysregulates lysosomal acidification in a neurodegenerative lysosomal storage disease model. *Nat Commun* 2017;8:14612. PUBMED | CROSSREF

52. Crise B, Rose JK. Identification of palmitoylation sites on CD4, the human immunodeficiency virus receptor. *J Biol Chem* 1992;267:13593-13597. PUBMED | CROSSREF

53. Fragoso R, Ren D, Zhang X, Su MW, Burakoff SJ, Jin YJ. Lipid raft distribution of CD4 depends on its palmitoylation and association with Lck, and evidence for CD4-induced lipid raft aggregation as an additional mechanism to enhance CD3 signaling. *J Immunol* 2003;170:913-921. PUBMED | CROSSREF

54. West SJ, Boehning D, Akimzhanov AM. Regulation of T cell function by protein S-acylation. *Front Physiol* 2022;13:1040968. PUBMED | CROSSREF

55. Arcaro A, Grégoire C, Boucheron N, Stotz S, Palmer E, Malissen B, Luescher IF. Essential role of CD8 palmitoylation in CD8 coreceptor function. *J Immunol* 2000;165:2068-2076. PUBMED | CROSSREF

56. Lu B, Sun YY, Chen BY, Yang B, He QJ, Li J, Cao J. zDHHC20-driven S-palmitoylation of CD80 is required for its costimulatory function. *Acta Pharmacol Sin* 2024;45:1214-1223. PUBMED | CROSSREF

57. Cruz AC, Ramaswamy M, Ouyang C, Klebanoff CA, Sengupta P, Yamamoto TN, Meylan F, Thomas SK, Richoz N, Eil R, et al. Fas/CD95 prevents autoimmunity independently of lipid raft localization and efficient apoptosis induction. *Nat Commun* 2016;7:13895. PUBMED | CROSSREF

58. Yao H, Lan J, Li C, Shi H, Brosseau JP, Wang H, Lu H, Fang C, Zhang Y, Liang L, et al. Inhibiting PD-L1 palmitoylation enhances T-cell immune responses against tumours. *Nat Biomed Eng* 2019;3:306-317. PUBMED | CROSSREF

59. Zhang Z, Ren C, Xiao R, Ma S, Liu H, Dou Y, Fan Y, Wang S, Zhan P, Gao C, et al. Palmitoylation of TIM-3 promotes immune exhaustion and restrains antitumor immunity. *Sci Immunol* 2024;9:eadp7302. PUBMED | CROSSREF

60. Wang Q, Wang J, Yu D, Zhang Q, Hu H, Xu M, Zhang H, Tian S, Zheng G, Lu D, et al. Benzosceptrin C induces lysosomal degradation of PD-L1 and promotes antitumor immunity by targeting DHHC3. *Cell Rep Med* 2024;5:101357. PUBMED | CROSSREF

61. Stolk DA, Horrevorts SK, Schetters STT, Kruijssen LJW, Duinkerken S, Keuning E, Ambrosini M, Kalay H, van de Ven R, Garcia-Vallejo JJ, et al. Palmitoylated antigens for the induction of anti-tumor $CD8^+$ T cells and enhanced tumor recognition. *Mol Ther Oncolytics* 2021;21:315-328. PUBMED | CROSSREF

62. Zhou B, Zhang M, Ma H, Wang Y, Qiu J, Liu Y, Lu L, Li T, Zhang L, Huang R, et al. Distinct palmitoylation of Foxp3 regulates the function of regulatory T cells via palmitoyltransferases. *Cell Mol Immunol* 2024;21:787-789. PUBMED | CROSSREF

63. Zhang M, Zhou L, Xu Y, Yang M, Xu Y, Komaniecki GP, Kosciuk T, Chen X, Lu X, Zou X, et al. A STAT3 palmitoylation cycle promotes T_H17 differentiation and colitis. *Nature* 2020;586:434-439. PUBMED | CROSSREF

64. Cherukuri A, Shoham T, Sohn HW, Levy S, Brooks S, Carter R, Pierce SK. The tetraspanin CD81 is necessary for partitioning of coligated CD19/CD21-B cell antigen receptor complexes into signaling-active lipid rafts. *J Immunol* 2004;172:370-380. PUBMED | CROSSREF

65. Cherukuri A, Carter RH, Brooks S, Bornmann W, Finn R, Dowd CS, Pierce SK. B cell signaling is regulated by induced palmitoylation of CD81. *J Biol Chem* 2004;279:31973-31982. PUBMED | CROSSREF

66. Weber M, Treanor B, Depoil D, Shinohara H, Harwood NE, Hikida M, Kurosaki T, Batista FD. Phospholipase C-gamma2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. *J Exp Med* 2008;205:853-868. PUBMED | CROSSREF

67. Ivaldi C, Martin BR, Kieffer-Jaquinod S, Chapel A, Levade T, Garin J, Journet A. Proteomic analysis of S-acylated proteins in human B cells reveals palmitoylation of the immune regulators CD20 and CD23. *PLoS One* 2012;7:e37187. PUBMED | CROSSREF