PHLPP Signaling in Immune Cells

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Abstract Pleckstrin homology domain leucine-rich repeat protein phosphatases (PHLPP) belong to the protein phosphatase magnesium/manganese-dependent family of Ser/Thr phosphatases. Their general role as tumor suppressors has been documented for over a decade. In recent years, accumulating evidence suggests that PHLPP isozymes have key regulatory roles in both innate and adaptive immunity. In macrophages, PHLPP1 dampens signaling through TLR4 and the IFN-γ receptor

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by altering cytosolic signaling pathways. Additionally, nuclear-localized PHLPP1 inhibits STAT1-mediated inflammatory gene expression by direct dephosphorylation at Ser 727. PHLPP1 also regulates the migratory and inflammatory capacity of neutrophils in vivo. Furthermore, PHLPP1-mediated dephosphorylation of AKT on Ser 473 is required for both the suppressive function of regulatory T cells and for the pro-apoptotic effects of PHLPP1 in B cell chronic lymphocytic leukemia. In the context of immune homeostasis, PHLPP1 expression is modulated in multiple cell types by inflammatory signals, and the dynamics of its expression have varying effects on the pathogenesis of inflammatory bowel disease and septic shock. In this review, we summarize recent findings on the functions of PHLPP in inflammatory and regulatory signaling in the context of both innate and adaptive immunity.

Abbreviations

AGC	Related to protein kinase A, protein kinase G, protein kinase C
AP-1	Activator protein-1
BCR	B cell receptor
Cdk	Cyclin-dependent kinase
CLL	Chronic lymphocytic leukemia
CREB	CAMP-response element-binding protein
DAMPs	Damage-associated molecular patterns
DSS	Dextran sodium sulfate
EGFR	Epidermal growth factor receptor
Gbp5	Guanylate-binding protein 5
HDAC3	Histone deacetylase 3
IBD	Inflammatory bowel disease
IFN	Interferon
IFNGR	Interferon-γ receptor
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IRF3	Interferon regulatory factor 3
IκB	Inhibitor of nuclear factor κB
KLA	Kdo2-lipid A
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
MAPKs	Mitogen-activated protein kinases
NES	Nuclear export sequence
NF-κB	Nuclear factor-κB
NO	Nitric oxide
NTE	N-terminal extension
PAMPs	Pathogen-associated molecular patterns
PH	Pleckstrin homology
PHLPP	Pleckstrin homology domain leucine-rich repeat protein phosphatases

PHTS	PTEN hamartoma tumor syndrome
PI3K	Phosphoinositide-3-kinase
PKC	Protein kinase C
PPM	Protein Ser/Thr phosphatase magnesium/manganese-dependent
PRRs	Pattern recognition receptors
RTK	Receptor tyrosine kinase
SP1	Specificity protein 1
STAT	Signal transducer and activator of transcription
Tconvs	Conventional T cells
TCR	T cell receptor
TGF	Transforming growth factor
TLR4	Toll-like receptor 4
TNF	Tumor necrosis factor
Tregs	Regulatory T cells

1 Introduction

Phosphorylation plays a key role in the immune system, which comprises many biological processes an organism possesses to defend itself against foreign agents. The immune system consists of both innate and adaptive arms, with the former occurring in a rapid but non-specific manner, and the latter in a slower but more specific manner. Innate immune cells, such as macrophages, neutrophils, natural killer cells and dendritic cells, detect and eliminate invading pathogens or inappropriate damage by recognizing pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) via their pattern recognition receptors (PRRs) (Lu et al. 2008). Receptor engagement initiates signaling cascades that culminate in a variety of pathways related to host defense as well as the recruitment and activation of adaptive immune cells, namely B cells and T cells. The orchestration of an adaptive immune response relies on antigen recognition by B or T cell receptors (BCRs and TCRs) and has the potential to form immune memory. Engagement of BCRs and TCRs analogously triggers signaling cascades critically regulated by protein kinases and phosphatases.

Immune processes are tightly regulated at multiple levels, including epigenetic, transcriptional, post-transcriptional, translational, and post-translational (Liu et al. 2016). Among post-translational mechanisms, protein phosphorylation and dephosphorylation play important regulatory roles in both innate and adaptive immune responses (Liu et al. 2016). Aberrant signal transduction can lead to immune dysregulation and pathology. Understanding and modulating the basis of normal and pathological states of immune cells has thus become an area of particular interest. However, whereas much is known about kinases within these pathways, less is known about the phosphatases that regulate the immune response. An emerging player in the control of both innate and adaptive immune responses is the pleckstrin homology domain

leucine-rich repeat protein phosphatase (PHLPP) family, one of the most recently characterized members of the phosphatome (Gao et al. 2005; Brognard et al. 2007).

1.1 PHLPP Phosphatases: Structure and Function

PHLPP isozymes belong to the protein phosphatase magnesium/manganese-dependent (PPM) family of Ser/Thr phosphatases. Their catalytic activity depends on Mn^{2+}/Mg^{2+}, and they are insensitive to common phosphatase inhibitors such as microcystin and okadaic acid (Gao et al. 2005; Grzechnik and Newton 2016). Presently, the PHLPP family consists of two members, PHLPP1 and PHLPP2, each encoded by separate genes. PHLPP1 was first identified in 2005 following a rational search for an AKT-directed phosphatase (Gao et al. 2005); two years later, a second member of the PHLPP family was identified and named PHLPP2 (Brognard et al. 2007). Both isozymes of PHLPP are evolutionarily conserved from yeast to humans (Newton and Trotman 2014).

Unlike most other Ser/Thr phosphatases, PHLPP isozymes are multi-domain enzymes whose regulatory regions are located on the same polypeptide (Grzechnik and Newton 2016). Both PHLPP family members share a similar structure with several conserved domains including a pleckstrin homology (PH) domain, a hydrophobic leucine-rich repeat (LRR) region, a catalytic PP2C phosphatase domain, and a PDZ (post-synaptic density protein PSD95, Drosophila disk large tumor suppressor DLG1, and zonula occludens-1 protein zo-1)-binding motif (Gao et al. 2005; Grzechnik and Newton 2016). These regulatory domains are essential for appropriate intracellular targeting of PHLPP to access specific downstream substrates, crucial for its biological functions. The PH domain is required to dephosphorylate cellular protein kinase C (PKC) (Gao et al. 2008), the LRR region enables regulation of receptor tyrosine kinase (RTK) transcription (Reyes et al. 2014), and the C-terminal tail and PDZ-binding motif are important for scaffold binding and AKT dephosphorylation (Brognard et al. 2007) (Fig. 1). Because the catalytic efficiency of the phosphatase domain is relatively low (Sierecki and Newton 2014), coordination of this domain in close proximity to its substrates, mediated by the regulatory domains, is essential for PHLPP biology.

PHLPP1 differs from PHLPP2 in several aspects. Strikingly, PHLPP1 possesses a lengthy N-terminal extension (NTE) of approximately 50 kDa that is phosphorylated by cyclin-dependent kinase (Cdk) 1 during mitosis, regulating the protein interaction network of PHLPP1 (Cohen Katsenelson et al. 2019; Kawashima et al. 2021). This NTE contains a nuclear localization sequence (NLS) required for PHLPP1 nuclear translocation (Cohen Katsenelson et al. 2019). Although poorly conserved through evolution, the NTE is necessary for dephosphorylation of a recently identified substrate of PHLPP1, the signal transducer and activator of transcription (STAT) 1 (see Sect. 2.2). However, it is not required for dephosphorylation of other known substrates, including AKT (Gao et al. 2005; Brognard et al. 2007), PKC (Gao et al. 2008), and S6K1 (Liu et al. 2011). PHLPP1 also contains a nuclear export sequence

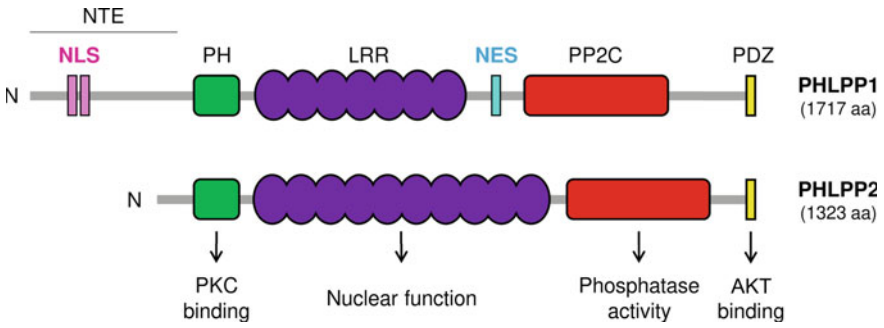

Fig. 1 PHLPP1 and PHLPP2 protein structure. The PHLPP family of phosphatases comprises two isozymes: PHLPP1 and PHLPP2. Both isozymes have similar domain structures, including a pleckstrin homology (PH) domain required for targeting to PKC, a leucine-rich repeat (LRR) required for nuclear PHLPP function, a PP2C phosphatase domain, and a PDZ-binding motif required for targeting AKT. Unlike PHLPP2, PHLPP1 also has a large, 50-kDa N-terminal extension (NTE) containing a nuclear localization signal (NLS), as well as a nuclear export sequence (NES) between the LRR and the PP2C phosphatase domains. Adapted from (Grzechnik and Newton 2016)

(NES) between the LRR region and PP2C domain that is not present in PHLPP2 (Cohen Katsenelson et al. 2019) (Fig. 1). Finally, both proteins diverge in their C-terminal sequences, which include the PDZ-binding motifs.

1.2 PHLPP Substrates

The first identified substrate of PHLPP is AKT, a member of the AGC (related to protein kinase A, protein kinase G, and protein kinase C) family of protein kinases, suggesting a role for PHLPP in opposing growth factor signaling (Gao et al. 2005). PHLPP directly and selectively dephosphorylates the hydrophobic motif of AKT (Ser 473 on AKT1), a key regulatory site, without affecting the activation loop (Thr 308 on AKT1). Although PHLPP1 and PHLPP2 both dephosphorylate the same residue on AKT, PHLPP isozymes differentially regulate AKT isozymes in cells: PHLPP1 dephosphorylates AKT2, whereas PHLPP2 dephosphorylates AKT1. Both isozymes dephosphorylate AKT3 (Brognard et al. 2007). Because full activation of AKT requires phosphorylation at both Ser 473 and Thr 308, PHLPP-mediated dephosphorylation of the AKT hydrophobic motif results in inactivation of the kinase. Thus, as terminators of phosphoinositide-3-kinase (PI3K)-AKT signaling, PHLPP proteins are considered tumor suppressors, limiting cell proliferation and tumor growth and promoting apoptosis (Fig. 2) (Gao et al. 2005; Brognard et al. 2007).

PHLPP is now known to dephosphorylate several other AGC kinases on their hydrophobic motifs, as well as other kinases on unrelated phosphorylation switches (Fig. 2) (Grzechnik and Newton 2016). Both PHLPP isozymes dephosphorylate

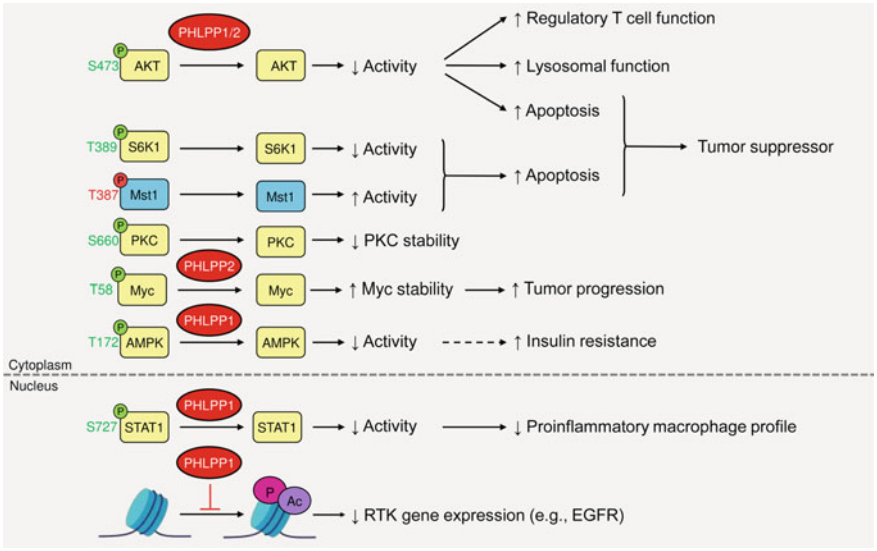

Fig. 2 PHLPP1 and PHLPP2 substrates and function. Both PHLPP1 and PHLPP2 can regulate several cellular pathways through different substrates. PHLPP1/2 directly dephosphorylate AGC kinases such as AKT, S6K1, and PKC at their hydrophobic motifs (green), resulting in kinase inactivation (AKT and S6K) or increased degradation and hence decreased kinase activity (PKC). PHLPP1/2 also dephosphorylate non-AGC kinases such as the pro-apoptotic kinase Mst1 at an inhibitory site (red). In addition, PHLPP2 dephosphorylates Myc (Thr 58) to promote its stability, and PHLPP1 dephosphorylates AMPK at its activation site (Thr 172). Finally, in the nucleus, PHLPP1 directly dephosphorylates STAT1 at Ser 727, and both PHLPP1 and PHLPP2 inhibit receptor tyrosine kinase (RTK) expression via suppression of histone acetylation and phosphorylation

the hydrophobic motif of PKC (Ser 660 on PKCβII) to promote degradation of the kinase (Gao et al. 2008). This dephosphorylation has recently been shown to provide a quality control mechanism to ensure that only functional PKC accumulates in the cell (Baffi et al. 2019). PHLPP also regulates cell cycle progression via activation or inhibition of several kinase targets. PHLPP dephosphorylates the hydrophobic motif of S6K1 (Thr 389) to inhibit protein translation and cell growth (Liu et al. 2011) and Mst1 at an inhibitory site (Thr 387) to promote apoptosis (Qiao et al. 2010). In addition, PHLPP1 dephosphorylates SGT1 at four conserved residues (Ser 17, Ser 249, Ser 289, and Thr 233) to facilitate kinetochore assembly during mitosis (Gangula and Maddika 2017). Finally, PHLPP1 dephosphorylates AMPK at an activating site (Thr 172) in myoblasts to induce ER stress (Behera et al. 2018), a phenomenon frequently accompanied by insulin resistance and diabetes (Hotamisligil 2010). This suggests a potential role for this phosphatase in the impairment of insulin signaling pathways.

The repertoire of PHLPP substrates is expanding to include non-kinase substrates. Nuclear-localized PHLPP1 restrains RTK signaling epigenetically by inhibiting histone phosphorylation and acetylation, thereby dampening transcription of RTKs

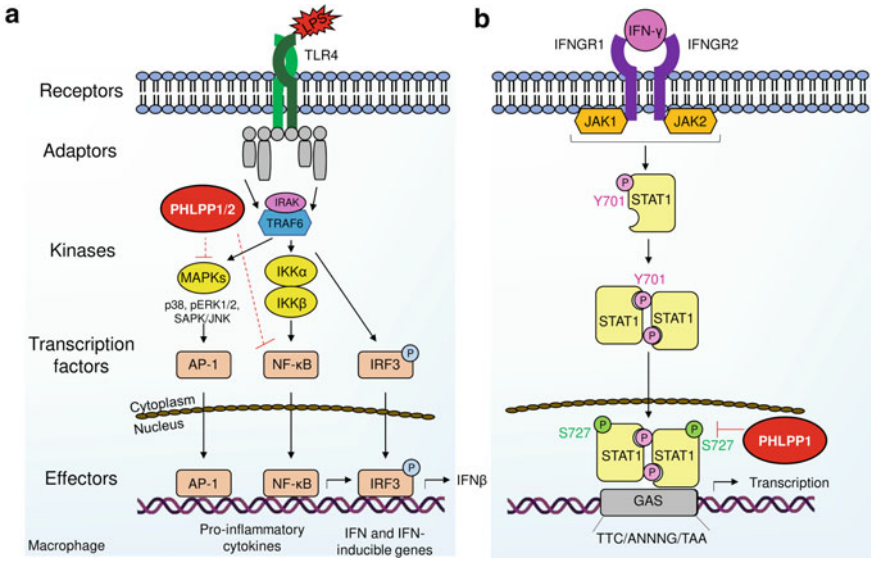

Fig. 3 PHLPP regulation of TLR and IFN-γ signaling. **a** TLR4 engagement activates MAPKs and IKKs through various adaptor proteins; in turn, these kinases promote the nuclear translocation of the transcription factors AP-1 and NF-κB to regulate transcription. TLR4-activated TRAF6 also activates and translocates IRF3 to the nucleus. Both PHLPP1 and PHLPP2 may inhibit TLR4 signaling by dephosphorylating and inactivating p38 (observed in RAW 264.7 cells), Erk1/2 (MAPKs), and NF-κB. **b** IFN-γ acts through its receptor to activate JAK-STAT1 signaling. Phosphorylated STAT1 (Tyr 701) homodimerizes and translocates to the nucleus, where it is further phosphorylated at Ser 727 for full transcriptional activity at promoter elements such as GAS (IFN-γ-activated sequences). In macrophages, PHLPP1 dephosphorylates STAT1 at Ser 727 to oppose its full activation

such as EGFR (Reyes et al. 2014). Thus, PHLPP1 controls two key oncogenic signaling pathways downstream of RTKs, PI3K and Erk1/2, in turn suppressing cell proliferation. Furthermore, the recent finding that PHLPP1 dephosphorylates the transcription factor STAT1 on Ser 727 to suppress its transcriptional activity (Figs. 2 and 3) cements a new role for PHLPP1 in innate immune control (see Sect. 2) (Cohen Katsenelson et al. 2019).

1.3 PHLPP Relevance in Disease

Given its cell cycle-centric regulatory role, it is unsurprising that PHLPP deregulation is associated with multiple pathologies. PHLPP1 mRNA or protein loss is associated with prostate cancer (Chen et al. 2011), chronic lymphocytic leukemia (CLL) (Ouillette et al. 2008), colorectal cancer (Liu et al. 2009; Li et al. 2013), glioblastoma (Warfel et al. 2011), and breast cancer (Qiao et al. 2007), among others. Interestingly,

PHLPP2 expression is elevated during prostate cancer metastasis (Nowak et al. 2015), a paradox resolved by the finding that PHLPP2 can dephosphorylate and stabilize Myc in advanced stages of disease (Fig. 2) (Nowak et al. 2019). Conversely, PHLPP upregulation is also associated with disease: diabetic patients have higher levels of PHLPP1 in skeletal and adipose tissue (Andreozzi et al. 2011; Cozzone et al. 2008). Moreover, PHLPP has other documented functions with potential disease relevance, including circadian regulation (Masubuchi et al. 2010), regulation of chondrocyte proliferation and bone morphogenesis (Hwang et al. 2018; Bradley et al. 2013, 2015), and cardiomyocyte homeostasis (Miyamoto et al. 2010). Finally, PHLPP controls regulatory T cell function (see Sect. 3.1), and its modulation of cell survival in CLL suggests potential involvement in B cell homeostasis as well (see Sect. 3.2).

2 Role of PHLPP in Innate Immunity

Macrophages are key innate immune effector cells. Their expression of PRRs and cytokine receptors enables a critical function in immune homeostasis: the collective detection of danger. Upon exposure either to bacterial products such as lipopolysaccharide (LPS) via the PRR Toll-like receptor 4 (TLR4) or to proinflammatory cytokine signals such as interferon (IFN)-γ via its receptor IFNGR, macrophages activate signaling cascades to aid immune activation and pathogen clearance (Lu et al. 2008). Signal transduction via mitogen-activated protein kinases (MAPKs) and inhibitor of nuclear factor κB (IκB) kinases lead to activation of the transcription factors activator protein 1 (AP)-1, nuclear factor-κB (NF-κB), and interferon regulatory factor (IRF) 3. This ultimately results in the production of prototypical inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12, and type I IFNs (Fig. 3a), among other functions (Lu et al. 2008). To drive the resolution of inflammation, these classically activated macrophages switch to an alternatively activated phenotype characterized by expression of IL-4, IL-13, IL-10, and transforming growth factor (TGF)-β (Nathan and Ding 2010; Spite et al. 2014; Sugimoto et al. 2019). Excessive classical macrophage activity can lead to chronic inflammation and tissue damage, whereas protracted reprogramming of alternatively activated macrophages can favor fibrosis or tumor development (Mantovani et al. 2002; Gieseck et al. 2018). Perhaps the best characterized function of PHLPP1 in macrophages is its regulation of LPS-mediated activation (Cohen Katsenelson et al. 2019; Alamuru et al. 2014), though analogous research in the macrophage response to IFN-γ has begun to shed light on a broader role for PHLPP1 in innate immune control (Alamuru et al. 2014).

2.1 Transcriptional Regulation of PHLPP During Inflammation

Macrophages express both PHLPP1 and PHLPP2, and mounting evidence supports a key role for the PHLPP1 isozyme in these cells. Parsa and colleagues first described that macrophage exposure to LPS decreased PHLPP1 at both the mRNA and protein levels (Alamuru et al. 2014). This may be a more global phenomenon, as this effect has also been observed in non-immune cells such as the Caco-2 intestinal epithelial cell line (Wen et al. 2015). Although the molecular mechanism underlying LPS-driven PHLPP1 downregulation is still not fully understood, a recent study implicated a role for the transcription factor specificity protein 1 (SP1), previously been shown to be depleted during LPS stimulation (Alamuru-Yellapragada et al. 2017; Ye and Liu 2001, 2007, 2002). Analysis of the *PHLPP1* promoter region revealed potential binding sites for various inflammation-related transcription factors, including SP1, STAT1, STAT3, STAT5, and NF-κB (Alamuru-Yellapragada et al. 2017). In support of these in silico findings, SP1 overexpression in the RAW 264.7 macrophage-like cell line results in LPS-dependent recruitment of SP1 to the *PHLPP1* promoter and its transcriptional activation. This effect is synergistically enhanced when SP1 is co-expressed with its coactivators p300 and CREB-binding protein (Tsai et al. 2000; Du et al. 2014), which are also involved in LPS-regulated gene transcription. In contrast, depletion of SP1 reduces *PHLPP1* promoter activity and hence transcription and translation, which is reversed by reintroducing SP1 in LPS-treated macrophages. Taken together, PHLPP1 expression is driven transcriptionally by SP1, which is downregulated by LPS. Of note, this regulatory pathway is conserved in non-immune cells, including several human melanoma cell lines: methylation of the *PHLPP1* promoter in these cells prevents SP1 binding and decreases PHLPP1 expression (Dong et al. 2014).

In addition to macrophages, PHLPP1 is transcriptionally regulated in chondrocytes during inflammation. Histone deacetylase 3 (HDAC3) expressed by mouse chondrocytes binds the *Phlpp1* promoter to reduce its transcription and thus protein expression, ultimately facilitating AKT signaling, matrix production, and chondrocyte proliferation (Bradley et al. 2013). Conversely, human chondrocytes from patients with osteoarthritis, characterized by matrix loss, upregulate *PHLPP1* transcription as a result of epigenetic regulation. Chondrocyte expression of PHLPP1 mRNA and protein is inducible by the inflammatory cytokines IL-6 and TNF-α as well as osteoarthritis-associated reactive oxygen species via demethylation (Bradley et al. 2016).

Overall, PHLPP is transcriptionally regulated by multiple inflammatory stimuli, including bacterial components and cytokines, but the outcomes and mechanisms underpinning these changes are cell- and context-dependent. A more thorough characterization of PHLPP expression in inflammation and homeostasis and the resulting downstream effects in different cell types is needed.

2.2 PHLPP Regulation of TLR4- and IFN-γ-Mediated Signaling

As discussed above, inflammation regulates PHLPP expression to alter cellular outcomes, but emerging evidence suggests that PHLPP phosphatase activity also regulates inflammatory signaling. This regulatory function of PHLPP is particularly evident in signaling cascades downstream of TLR4, which recognizes components of Gram-negative bacteria such as LPS and the inflammatory cytokine IFN-γ.

Normal signal transduction in macrophages downstream of TLR4 and the IFN-γ receptor (IFNGR) involves multiple players. Following TLR4 engagement by LPS, the transcription factor IRF3 induces the expression and secretion of type I IFNs (Gao et al. 1998; Sakaguchi et al. 2003; de Weerd et al. 2007). Once released, IFNs bind to type I IFN receptor complexes to mediate their biological activities in an autocrine and paracrine manner. Additionally, IFN-γ binding to its receptor recruits JAK and STAT1, resulting in the phosphorylation of STAT1 at Tyr 701. This phosphorylation event induces STAT1 homodimerization and nuclear translocation, enabling STAT1 recruitment to IFN-γ-activated sequence (GAS) elements in the promoters of a subset of IFN-γ-responsive genes. Once bound to DNA, STAT1 is further phosphorylated at Ser 727 to enhance its transcriptional activity (Fig. 3b) (Stark et al. 1998; Muller et al. 1994). In addition to inflammatory cytokine secretion, macrophages activated by either LPS or IFN-γ ultimately also produce nitric oxide (NO) in an iNOS (inducible NO synthase)-dependent manner to mediate a wide range of important functions (Bogdan 2001). PHLPP1 has recently been shown to oppose STAT1 function by specifically dephosphorylating Ser 727, unveiling a key role in negatively regulating innate immunity otherwise driven by LPS and IFN-γ (Cohen Katsenelson et al. 2019; Alamuru et al. 2014).

In the RAW 264.7 macrophage-like cell line, Alamuru *et al.* found that PHLPP1 restrained LPS- and IFN-γ-mediated responses (Alamuru et al. 2014). Upon activation by either LPS or IFN-γ, PHLPP1 suppresses iNOS induction by reducing both IFN-β production and STAT1 phosphorylation at Ser 727 (Alamuru et al. 2014). The mechanism by which this occurs varies by stimulus: PHLPP1 opposes LPS-induced iNOS by inhibiting p38 phosphorylation and IFN-γ-activated iNOS by inhibiting Erk1/2 phosphorylation (Alamuru et al. 2014). The finding that PHLPP1 loss results in increased Erk1/2 phosphorylation at its activation loop sites (Cohen Katsenelson et al. 2019; Reyes et al. 2014) supports a negative regulatory role for PHLPP in Erk1/2 signaling.

Studies in primary macrophages confirmed PHLPP1 as a key regulator of STAT1 activity downstream of inflammatory signaling (Cohen Katsenelson et al. 2019). Specifically, upon in vitro activation with the LPS component Kdo2-lipid A (KLA), PHLPP1-deficient macrophages exhibited an increase in STAT1 phosphorylation at Ser 727 but not at Tyr 701. In addition, biochemical analysis revealed that PHLPP1 directly binds to STAT1 and selectively dephosphorylates Ser 727 without affecting the Tyr 701 site. This regulation of STAT1 depends on both PHLPP1's catalytic activity and nuclear localization, the latter driven by the NLS within its

NTE region (Cohen Katsenelson et al. 2019). Transcriptome analysis of PHLPP1-deficient bone marrow-derived macrophages after KLA challenge revealed increased induction of genes involved in the innate immune response. Nearly half of the upregulated genes in these cells contained promoters with a consensus STAT binding motif, and the absence of PHLPP1 enhances STAT1 binding and transactivation of its target gene promoters, such as *Cd69*, *Ifit2*, and *Gbp5* (guanylate-binding protein 5). PHLPP1 also suppresses expression of the STAT1-dependent genes *Socs1*, *Socs3*, *Ccl4*, and *Cxcl10* (Ohmori and Hamilton 2001; Ramana et al. 2001). Furthermore, gene ontology analysis revealed that PHLPP1 also dampens the NF-κB branch of TLR4 signaling. Other gene ontology terms altered by PHLPP1 in TLR4-stimulated bone marrow-derived macrophages encompass many cellular processes, including: cellular receptors, kinases/phosphatases, G-protein-related genes, lipid metabolism, cellular import/export, transcription, protein folding modification, cell adhesion, nucleotide metabolism, extracellular matrix, response to toxins, and immune response. Taken together, PHLPP1 negatively regulates STAT1 transcriptional activity and restrains inflammatory processes in macrophages. It will be interesting to determine whether PHLPP1 is involved in the differentiation of any of the documented alternatively activated macrophage phenotypes and whether PHLPP1 acts similarly in other innate immune cells in response to LPS or IFN-γ.

TLR4 signaling can also be activated by free fatty acids, triggering inflammatory pathways that play a critical role in the development of obesity-associated insulin resistance and type 2 diabetes (Hirosumi et al. 2002; Shi et al. 2006). Genetic studies have identified PHLPP polymorphisms associated with type 2 diabetes (Andreozzi et al. 2011; Turki et al. 2013; Meigs et al. 2007). Obese and diabetic patients have also been found to have increased levels of PHLPP1, but not PHLPP2, in skeletal muscle and adipose tissue (Andreozzi et al. 2011; Cozzone et al. 2008). The finding that PHLPP1 suppresses inflammation upon TLR4 stimulation in macrophages (Alamuru et al. 2014; Cohen Katsenelson et al. 2019) is a new concept, suggesting that these two discoveries may be related. Understanding the molecular and cellular mechanisms of how PHLPP1 regulates insulin signaling through AKT versus inflammatory signaling should be addressed. This research may open new avenues for controlling the low-grade inflammation that constitutes the *sine qua non* of type 2 diabetes, obesity, and other associated metabolic disorders.

2.3 *PHLPP Regulation of the Immune Response upon Bacterial Infection*

Antimicrobial resistance is escalating worldwide as a result of increased antibiotic use and the consequential emergence of competitively advantageous mutations in bacteria (Laxminarayan et al. 2013). Gram-negative pathogens such as *Escherichia*

coli, Salmonella typhimurium, and *Klebsiella pneumoniae* are responsible for potentially fatal infections and have developed various strategies to enable host persistence and immune escape (Laxminarayan et al. 2013; Monack et al. 2004). Uncovering potential therapeutic targets for these bacterial infections and the ensuing host immune response are thus of prime importance. In this context, several reports suggest PHLPP may regulate the antimicrobial immune response.

In macrophages, PHLPP1 elevates lysosomal activity during phagocytosis, suggesting an important role for this phosphatase in bacterial clearance (Fischer et al. 2019) as phagocytosis is a primary mechanism by which macrophages eliminate invading pathogens. Microbes recognized by PRRs are engulfed in phagosomes, which fuse with lysosomes to form a phagolysosome; the acidity and degradative enzymes within the lysosomes destroy the contents of the phagolysosome (Uribe-Querol and Rosales 2017). The circulating adipokine leptin, which activates the mTORC2-AKT signaling axis, has been shown to control phagocytosis via PHLPP1 in macrophages infected by *S. typhimurium.* Genetic and pharmacological inhibition of leptin signaling improves bacterial clearance by augmenting macrophage lysosomal function, and this process is mediated by PHLPP1-dependent AKT dephosphorylation (Fischer et al. 2019). Notably, PHLPP1 can increase lysosomal activity in other contexts, such as in the maintenance of cellular homeostasis: PHLPP1 controls chaperone-mediated autophagy by its association to the lysosomal membrane and enables lysosome-dependent clearance of protein aggregates (Arias et al. 2015). In light of the link between PHLPP1 and mTORC2-AKT-mediated microbial phagocytosis, it will be of particular interest to explore the outcomes of PHLPP1 function in bacterial infection in vivo.

The relevance of PHLPP1 in the immune response in vivo has also been investigated in a mouse model of sepsis. Sepsis is a systemic inflammatory response to bacterial infection characterized by a massive production of cytokines that triggers tissue injury and life-threatening multiorgan failure (Salomao et al. 2012; Glauser et al. 1991; Parrillo 1993; Engelberts et al. 1991). Currently, sepsis is one of the leading causes of death, but the factors contributing to dysregulation of the immune system and poor clinical outcomes remain elusive. A recent study found that PHLPP1-deficient mice were protected from endotoxin-driven (LPS) and bacterial (*E. coli*) sepsis. Specifically, mice lacking PHLPP1 exhibited reduced serum levels of the proinflammatory cytokines IL-6 and IL-1β following LPS injection (Cohen Katsenelson et al. 2019). These findings are in apparent contrast to the aforementioned in vitro work in which PHLPP1 was found to dampen the inflammatory phenotype of macrophages via STAT1 inhibition (Cohen Katsenelson et al. 2019; Alamuru et al. 2014). This discrepancy may be attributed to cell type-specific functions mediated by PHLPP1 in septic shock induced by Gram-negative bacteria. For instance, in another study examining the role of PHLPP in LPS-stimulated neutrophils, neutrophils lacking PHLPP exhibited improved migratory function in vivo and in vitro (Ran et al. 2019). This unique effect of PHLPP1 loss in neutrophils could fine-tune the immune response at a systemic level and contribute to the effect observed in the in vivo model of sepsis.

To date, our mechanistic understanding of the protective role of PHLPP1 deficiency during bacterial or endotoxin-induced septic shock is incomplete. Further experimentation in *Phlpp1*$^{-/-}$ animals, as well as work in animals with myeloid cell-specific PHLPP1 deficiency, will help characterize the effects of this phosphatase. Additionally, given the cell type-specific functions of PHLPP1, a detailed examination of other innate immune cells such as neutrophils, dendritic cells, and natural killer cells may clarify in vivo outcomes. Finally, based on the findings in mice, PHLPP1 may be a promising potential therapeutic target for sepsis by alleviating excessive inflammation and thus mitigating the multiorgan failure associated with septic shock.

3 Role of PHLPP in Adaptive Immunity

The adaptive immune system, comprising B cells and T cells, amplifies innate immune responses in an antigen-specific manner. Following the induction of innate immunity, antigen-presenting cells, such as macrophages, B cells, and dendritic cells, process and present antigens in the form of peptides to T cells. Mounting evidence supports a key role of PHLPP in regulating adaptative immunity.

3.1 PHLPP Signaling in CD4$^+$ T Cells and Regulatory T Cells

T cell activation requires both cognate antigen recognition via their TCR and a costimulatory signal, such as engagement of CD80 or CD86 to CD28 on T cells. The nature of these stimuli as well as the microenvironmental context determine the effector functions of CD4$^+$ and CD8$^+$ T cells following their activation (Lever et al. 2014; Geginat et al. 2014; Arens and Schoenberger 2010). T cell subsets exhibit a certain degree of functional plasticity and primarily contribute to adaptive immunity by lysing infected or cancerous cells, activating and recruiting other immune cells, and establishing antigen-specific memory with potent recall responses.

PI3K-AKT-mTOR signaling in T cells can be activated by TCR engagement, co-stimulation (such as CD80/CD86 ligation to CD28), and IL-2 signaling (Han et al. 2012). PI3K stimulates AKT phosphorylation at Thr 308 via PDK-1, and mTORC2 promotes phosphorylation of AKT at Ser 473; as previously discussed, phosphorylation at both sites is required for full AKT activation. AKT drives proliferation, metabolic reprogramming, and T cell effector function through a variety of targets, including activation of mTORC1-mediated biosynthesis and inhibition of the quiescence-associated Foxo transcription factors (Manning and Toker 2017).

CD4$^+$CD25$^+$ conventional T cells (Tconvs) from *Phlpp1*-deficient mice exhibit exaggerated TCR-induced AKT phosphorylation at Ser 473 (Patterson et al. 2011).

Phlpp1$^{-/-}$ Tconvs are also refractory to suppression by wild-type CD4^{+}Foxp3^{+} regulatory T cells (Tregs; discussed below) (Patterson et al. 2011). As PI3K-AKT-mTOR signaling drives the differentiation of both Tconvs and CD8^{+} T cells (Han et al. 2012; Kim and Suresh 2013), PHLPP deficiency may lead to their overactivation. To date, however, no loss-of-function polymorphisms in *PHLPP1* or *PHLPP2* have been associated with autoimmunity (Chen et al. 2017).

T cell responses are controlled at least in part by a subset of CD4^{+} T cells known as Tregs, which constitutively express the IL-2 receptor alpha chain CD25 and the master transcription factor FOXP3. Tregs use a variety of mechanisms to suppress unwanted immune activity and are required for the establishment and maintenance of immune homeostasis (Vignali et al. 2008; Sakaguchi et al. 2009; Ferreira et al. 2019). As such, their potential as an adoptive cell therapy to induce tolerance in autoimmune or transplant settings is under active preclinical and clinical investigation (Ferreira et al. 2019; Raffin et al. 2020; MacDonald et al. 2019). Of particular interest is uncovering the mechanisms controlling Treg function, adaptability, and stability in inflammation for the development of next-generation Treg therapies. PHLPP has emerged as a potential regulator of Treg identity, but its specific role is not well defined.

A hallmark of both murine and human Tregs is dampened AKT signaling, specifically reduced phosphorylation at Ser 473 upon TCR- or IL-2-mediated activation, as well as reduced phosphorylation of the AKT targets S6 and Foxo1/3a (Bensinger et al. 2004; Crellin et al. 2007). Pharmacological inhibition of PI3K, AKT, or its downstream target mTORC1 in Tconvs promotes de novo expression of Foxp3 and a Treg-like expression profile in vitro (Sauer et al. 2008); Retroviral overexpression of a constitutively active AKT impairs Treg differentiation in vitro and in vivo (Haxhinasto et al. 2008). Furthermore, T cells genetically deficient in mTOR (both mTORC1 and mTORC2) preferentially differentiate into Tregs in vitro (Delgoffe et al. 2009), though some degree of PI3K and mTORC1 activation is required for Treg function in vivo (Patton et al. 2006; Zeng et al. 2013; Soond et al. 2012; Zeng and Chi 2017). Taken together, attenuated activation of PI3K-AKT-mTOR signaling is essential for the development and function of Tregs.

As PHLPP specifically dephosphorylates AKT at Ser 473, its role as a potential negative regulator of AKT signaling in Tregs became of great interest. Indeed, murine and human Tregs have elevated expression of both PHLPP1 and PHLPP2 at the transcript and protein levels compared to Tconvs (Patterson et al. 2011). Consequently, siRNA-mediated or genetic ablation of *Phlpp1* in murine Tregs restores TCR-induced AKT phosphorylation at Ser 473 to levels similar to those of Tconvs. In human Tregs, however, only the combined siRNA knockdown of *PHLPP1* and *PHLPP2* is sufficient to restore phosphorylation of AKT at Ser 473 (Patterson et al. 2011). This may reflect a species-specific compensatory effect by PHLPP2 for PHLPP1 or a gene dose-dependent phenotype as siRNA only reduced *PHLPP1* and *PHLPP2* expression by half in this context (Patterson et al. 2011).

As a negative regulator of AKT activation, PHLPP1 plays an important role in Treg function. Tregs from *Phlpp1*$^{-/-}$ mice have a reduced capacity to inhibit TCR-activated T cell proliferation in a classical in vitro suppression assay and are unable

to protect mice from colitis induced by adoptive transfer of Tconvs (Patterson et al. 2011). The molecular mechanism underlying this functional impairment is an open question. Additionally, whether PHLPP1 deficiency primarily impairs Treg identity during development or Treg function in the periphery is unknown. *Phlpp1*$^{-/-}$ Tregs do not exhibit abnormal proliferation in response to TCR or IL-2 signals (Patterson et al. 2011), suggesting that other aspects of Treg biology are deregulated with PHLPP1 ablation. Many facets of Treg activation, suppressive function, and lineage stability are under metabolic and transcriptional control by AKT targets such as mTOR (Shi and Chi 2019) and Foxo1/3a (Kerdiles et al. 2010; Ouyang et al. 2010, 2012; Luo et al. 2016). These pathways may also be involved in the defective suppressive capacity of PHLPP1-deficient Tregs. Accordingly, human Tregs treated with a small-molecule pan-PHLPP inhibitor (Sierecki et al. 2010) exhibit reduced mitochondrial membrane potential, implying impaired mitochondrial fitness, though this effect is more pronounced with combined inhibition of PHLPP and the lipid phosphatase PTEN (Chen et al. 2017) (discussed below).

Although PHLPP1 and PHLPP2 are important for in vitro Treg differentiation, their role in Treg development in vivo is less clear. Murine Tconvs treated with TGF-β upregulate Foxp3 expression and acquire Treg suppressive properties; however, this in vitro system is less physiologically relevant in human T cells as neither TGF-β- nor activation-induced FOXP3 expression in human Tconvs is sufficient to confer Treg functions or phenotypic features (Allan et al. 2007; Wang et al. 2007; Rossetti et al. 2015). Tconvs from *Phlpp1*$^{-/-}$ mice are less able to differentiate into Tregs in vitro, and this inhibitory effect is potentiated by *Phlpp2* siRNA knockdown, suggesting functional complementation by PHLPP2 for PHLPP1 loss in this setting (Patterson et al. 2011). Similarly, pharmacological inhibition of PHLPP (both PHLPP1 and PHLPP2) in human naive T cells reduces their propensity to upregulate FOXP3 in the presence of TGF-β (Chen et al. 2017). Mechanistically, TGF-β-activated Smad3 binds the *Phlpp1* promoter to induce its expression (Patterson et al. 2011). As TGF-β has a demonstrated role in the development of both thymus-derived and peripherally derived Tregs in vivo (Chen and Konkel 2015; Savage et al. 2020), it is tempting to speculate that PHLPP1 is required for Treg development in vivo. *Phlpp1*$^{-/-}$ mice, however, exhibit normal frequencies of $CD4^+$, $CD8^+$, and $Foxp3^+$ T cell numbers in the thymus and lymph nodes, suggesting unimpaired development for at least thymus-derived Tregs in a global murine PHLPP1 knockout setting (Patterson et al. 2011). Nevertheless, PHLPP2 may functionally compensate in the absence of PHLPP1, and any T cell-extrinsic effects of PHLPP1 ablation cannot be ruled out.

Multiple phosphatases regulating PI3K-AKT activation may function in conjunction in a redundant manner. As alluded above, PHLPP2 may functionally compensate in part for PHLPP1 loss in T cells. Other phosphatases may also cooperate to control PI3K-AKT signaling. The lipid phosphatase PTEN is another major regulator of PI3K-AKT: PTEN dephosphorylates $PI(3,4,5)P_3$ into $PI(4,5)P_2$ to inhibit downstream PI3K signaling (Lee et al. 2018). Like PHLPP, PTEN is also highly expressed by murine and human Tregs (Chen et al. 2017; Bensinger et al. 2004). Curiously, contrary Treg phenotypes are seen in mice and humans with genetic PTEN deficiency.

Mice with a Treg-specific loss of *Pten* develop systemic lymphoproliferative autoimmunity; *Pten*$^{-/-}$ Tregs sequentially lose CD25 and Foxp3, are more glycolytic, and have impaired mitochondrial fitness (Huynh et al. 2015; Shrestha et al. 2015). In contrast, whereas patients with germline heterozygous loss-of-function mutations in *PTEN* (PTEN hamartoma tumor syndrome (PHTS)) also develop autoimmunity, their Tregs do not downregulate CD25 or other Treg-associated markers and maintain normal S6 phosphorylation (mTORC1 target) and mitochondrial membrane potential (Chen et al. 2017). One explanation may be functional compensation for PTEN loss by PHLPP1/2. In support of this notion, PHLPP1 and PTEN are co-recruited to the immunological synapse upon TCR activation of healthy human Tregs (Chen et al. 2017). It remains to be determined whether a similar phenomenon occurs in Tregs from patients with PHTS and whether PHLPP1 potentially compensates by increased expression, increased recruitment to the synapse, elevated phosphatase function, or some combination therein.

Alternatively, *Pten* in Tregs may be partially haplosufficient and not require functional compensation by PHLPP1/2: the aforementioned mouse models lack both copies of *Pten* in Tregs, whereas patients with PHTS maintain one functional copy. Indeed, mice with a less penetrant Treg-specific deletion of *Pten* (deletion in ~ 95% of Foxp3^{+} Tregs) maintain TCR-dependent Treg suppressive activity in vitro (Sharma et al. 2015). Moreover, the stronger autoimmune phenotype in more penetrant strains of Treg-specific *Pten* deletion suggests that, despite unperturbed expression of PHLPP1/2, PTEN has a nonredundant function in maintaining Treg stability (Huynh et al. 2015). These hypotheses are not mutually exclusive. Overall, further investigation is needed to clarify whether PHLPP1/2 indeed functionally compensates for partial or complete loss of PTEN to dampen PI3K-AKT signaling.

Other PI3K-AKT-targeting phosphatases, such as SHIP-1, SHIP-2, and PP2A, may also cooperate with PHLPP1/2 or PTEN in a phosphatase network at the immunological synapse of Tregs. SHIP-1/2 dephosphorylates PI(3,4,5)P_3 into PI(3,4)P_2 (Pauls and Marshall 2017), whereas PP2A dephosphorylates AKT at Thr 308 as well as other targets, including MAPK, NF-κB, and mTOR, in a context- and cell type-dependent manner (Wlodarchak and Xing 2016). Earlier work suggested that the effects of SHIP-1/2 are largely Treg-extrinsic in mice (Tarasenko et al. 2007), but recent studies have implicated a role for SHIP-1 in human Treg function (Cuadrado et al. 2018; Lucca et al. 2019). Additionally, PP2A plays an important role in Treg development and function in mice and humans, particularly in potentiating IL-2 signals (Apostolidis et al. 2016; Sharabi et al. 2019; Ding et al. 2019). Delineating the contexts and relative contributions of PHLPP1/2 and other phosphatases in Treg function as well as in other T cells will be important for rationally targeting these pathways to treat autoimmunity and cancer.

3.2 PHLPP Signaling in B Cells and Chronic Lymphocytic Leukemia

As with T cells, B cells integrate antigenic signals through their BCR and microenvironmental stimuli to orchestrate their development, activation, proliferation, function, and differentiation (Cyster and Allen 2019). In addition to acting as antigen-presenting cells to activate T cells, B cells can differentiate into plasma cells to secrete antibodies, which neutralize pathogens and toxins, facilitate complement activation and pathogen lysis, and promote phagocytosis. Modulation of PI3K-AKT-mTOR signaling in B cells governs nutrient sensing, metabolic reprogramming, and cell fate (Limon and Fruman 2012; Woyach et al. 2012; Jellusova and Rickert 2016). Aberrant AKT activation downstream of BCR engagement contributes to the survival and expansion of malignant B cells, including CLL (Woyach et al. 2012).

A hint that PHLPP1 may be involved in B cell homeostasis and CLL pathogenesis comes from gene profiling studies finding that *PHLPP1* transcript expression is substantially reduced or undetectable in CLL (Basso et al. 2005; Haslinger et al. 2004; Ouillette et al. 2008; O'Neill et al. 2013). At the protein level, blood and tonsillar B cells from healthy individuals express PHLPP1, whereas CLL cells express tenfold less PHLPP1 (Suljagic et al. 2010). In contrast, PHLPP2 protein expression is unchanged between normal B cells, CLL cells, and B lymphoma cell lines. The primary mechanism for PHLPP1 protein downregulation appears to be transcriptional repression as the majority of CLL samples with low PHLPP1 protein expression have a concomitant reduction in *PHLPP1* transcript (Suljagic et al. 2010). Consistent with its phosphatase activity, reintroduction of PHLPP1 in CLL cells by in vitro-transcribed mRNA reduces BCR-induced AKT phosphorylation at Ser 473 as well as Erk1/2 activation, though basal AKT signaling remains undetectable. Other microenvironmental signals, including CD40L, CpG oligodeoxynucleotides, and CXCL12, also increase Erk1/2 activation in the presence of PHLPP1, but AKT phosphorylation at Ser 473 remains limited (Suljagic et al. 2010). Thus, PHLPP1 may differentially regulate signaling pathways downstream of BCR engagement versus other stimuli. Mechanistically, loss of PHLPP1 in CLL promotes cellular survival by upregulating the anti-apoptotic factor MCL-1 (Suljagic et al. 2010), which is normally controlled by AKT (Longo et al. 2008). It will be interesting to determine whether PHLPP1 protein downregulation is a sufficient initiating factor for the survival and propagation of a malignant B cell clone. Finally, given that PHLPP1 loss contributes to CLL pathogenesis, PHLPP1 activity may also plausibly regulate normal B cell homeostasis.

4 Role of PHLPP in Inflammatory Bowel Disease and Colorectal Cancer

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract arising from a combination of genetic and environmental factors, and it is generally classified as Crohn's disease or ulcerative colitis depending on the location of inflammation (Wallace et al. 2014). In a mouse model of dextran sodium sulfate (DSS)-induced colitis, combined deficiency of PHLPP1 and PHLPP2 had a protective effect by increasing AKT activity and consequently reducing epithelial cell apoptosis (Wen et al. 2015). In intestinal epithelial organoids, the absence of PHLPP1/2 upregulates AKT and prevents TNF-α-induced apoptosis (Wen et al. 2015); TNF-α is a major cytokine involved in the development of IBD (Wang and Fu 2005). Although unhindered AKT activity maintains intestinal epithelial cell homeostasis in the context of acute colitis, protracted inflammation and prolonged PI3K-AKT signaling can result in pathology. Indeed, both PHLPP1 and PHLPP2 are downregulated upon inflammatory stimuli via proteasomal degradation, and both isozymes are similarly reduced in colon samples from patients with IBD (Wen et al. 2015). Therefore, although inflammation-driven PHLPP1/2 downregulation initially protects from intestinal epithelial cell apoptosis, its prolonged absence and concomitantly sustained AKT activity may drive the progression of IBD (Wen et al. 2015).

In addition to epithelial cell damage and compromised intestinal barrier integrity, alterations in innate and adaptive immunity have been implicated in the pathogenesis of IBD (Wallace et al. 2014). As mentioned previously, PHLPP-deficient neutrophils exhibit augmented migration in vivo and in vitro and decreased expression of the proinflammatory factors IL-6, IL-1β, and TNF-α. This phenotype is accompanied by enhanced phagosome-lysosome fusion and increased phosphorylation of known PHLPP1 substrates, namely AKT and STAT1, as well as increased Erk1/2 phosphorylation (Ran et al. 2019). The adoptive transfer of neutrophils lacking PHLPP into wild-type mice is sufficient to improve mucosal homeostasis and alleviate symptoms associated with DSS-induced colitis (Ran et al. 2019). Thus, PHLPP is an important player in IBD by modulating not only intraepithelial cell homeostasis but also neutrophil function. Exploring the role that PHLPP plays in other immune cell types in models of colitis may shed light on the molecular mechanisms underlying IBD pathogenesis.

IBD is one of the critical risk factors contributing to colon cancer in humans (Beaugerie and Itzkowitz 2015). Immunohistochemical staining of colorectal tumor samples reveals that both PHLPP1 and PHLPP2 expression are frequently lost, suggesting a tumor-suppressive role for PHLPP isozymes (Liu et al. 2009; Li et al. 2013). This role was further characterized by studies in which knockdown of endogenous PHLPP in cancer cells significantly increased their proliferation rate, and ectopic expression of either PHLPP isozyme interfered with important checkpoints of the cell cycle and reduced cellular growth (Liu et al. 2009). PHLPP-mediated suppression of cellular proliferation is mainly attributed to its negative regulation of AKT signaling. In addition, tumorigenesis induced by subcutaneous injection of colon cancer cells

into nude mice is suppressed with overexpression of PHLPP (Liu et al. 2009). The maintenance of normal PHLPP levels in the epithelial cells of colonic mucosa is thus essential to suppress tumor development. In this context, the deubiquitinase USP46 stabilizes PHLPP expression by reducing its degradation, and downregulation of PHLPP1 expression correlates with decreased levels of USP46 in colorectal cancer (Li et al. 2013).

Whether inflammatory factors produced by immune cells in a PHLPP1-dependent manner could also contribute to tumor development in the colon is still an open question. Given the strong association between chronic inflammation and tumor development, the repression of PHLPP1 expression may amplify proinflammatory signaling in macrophages, exacerbating aggressive tumor growth in colorectal cancer. In other cancer cells, it has been observed that the presence of PHLPP suppresses the production of proinflammatory cytokines such as TNF-α, IL-17, and IL-1β (Teng et al. 2016). IL-1β and IL-17 in particular can promote tumor progression by recruiting macrophages and myeloid-derived suppressor cells and driving angiogenesis (Yang et al. 2014; Iida et al. 2011; Mantovani et al. 2018). These data indicate that the loss of PHLPP isozymes not only promotes proliferation/survival events by increasing AKT phosphorylation (Brognard et al. 2007; Gao et al. 2005) but may also generate an immune microenvironment to favor tumor growth. Exploring the inflammatory state of colonic macrophages and the relevance of PHLPP in immune cell infiltration in the colon during colitis development may shed light on the role of these phosphatases in the pathogenesis of colorectal cancer.

5 Concluding Remarks

PHLPP isozymes are relatively new players in the immune system, yet several roles have already been established for these phosphatases in inflammation and immunoregulation. Thus far, research in this area has focused primarily on the involvement of PHLPP1, with little known about the functional relevance of PHLPP2. In macrophages, PHLPP1 inhibits the adoption of a proinflammatory phenotype by suppressing TLR4 signaling as well as IFN-γ-driven STAT1 activation and increases phagocytosis via AKT dephosphorylation. PHLPP also regulates the migratory and inflammatory capacity of neutrophils in vivo. Interestingly, whole-body deficiency in PHLPP1 protects mice from sepsis, suggesting that PHLPP1 has differential roles dependent on cell type and context. In adaptive immunity, dampened AKT signaling mediated by PHLPP1 is required for immunosuppression by Tregs, though the underlying mechanisms remain unclear. In these cells, PHLPP1 may operate in conjunction with other phosphatases to maintain Treg stability and function, but further investigation is needed to elucidate these effects. PHLPP expression is tightly modulated by inflammation, and aberrant regulation can drive the pathogenesis of diseases such as B cell CLL and IBD. How the effects of PHLPP on TLR4 and STAT1 signaling affect adaptive immunity is an unexplored area of research. Future work in this rapidly

evolving field to dissect the roles of these isozymes may pave the way for novel therapeutics seeking to control a range of immune-mediated diseases, including sepsis, diabetes, autoimmunity, and cancer.

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